Pancreatic islet cells: a model for calcium-dependent peptide release

Bernat Soria,^{1,3} Eva Tudurí,^{2,3} Alejandro González,^{2,3}
Abdelkrim Hmadcha,^{1,3} Franz Martin,^{1,3} Angel Nadal,^{2,3} and
Ivan Quesada^{2,3}

¹Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), 41092 Sevilla, Spain

²Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, 03202 Elche, Alicante, Spain

³CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)

(Received 22 January 2010; accepted 25 February 2010; published online 30 March 2010)

In mammals the concentration of blood glucose is kept close to 5 mmol/l. Different cell types in the islet of Langerhans participate in the control of glucose homeostasis. β -cells, the most frequent type in pancreatic islets, are responsible for the synthesis, storage, and release of insulin. Insulin, released with increases in blood glucose promotes glucose uptake into the cells. In response to glucose changes, pancreatic α -, β -, and δ -cells regulate their electrical activity and Ca^{2+} signals to release glucagon, insulin, and somatostatin, respectively. While all these signaling steps are stimulated in hypoglycemic conditions in α -cells, the activation of these events require higher glucose concentrations in β and also in δ -cells. The stimulus-secretion coupling process and intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) dynamics that allow β -cells to secrete is well-accepted. Conversely, the mechanisms that regulate α - and δ -cell secretion are still under study. Here, we will consider the glucose-induced signaling mechanisms in each cell type and the mathematical models that explain Ca^{2+} dynamics. [DOI: 10.2976/1.3364560]

CORRESPONDENCE

Bernat Soria:
bernat.soria@cabimer.es

THE HETEROGENEOUS COMPOSITION AND FUNCTIONAL ARCHITECTURE OF THE ISLET OF LANGERHANS

The regulation of glycemia depends on the individual action of the different populations that form the islet and their functional interaction. Each islet is composed of a heterogeneous group of 1000–3000 cells, where the predominant cell type is the insulin-releasing β -cell, which coexists with the population of α -cells, responsible for glucagon secretion, and δ -cells, responsible for somatostatin release. While β - and α -cell populations represent, respectively, about 70–80% and 20% of the islet in mice, recent studies in human have demonstrated an increased presence of the latter population (~55% of β -cells versus ~40% of α -cells) (Cabrera *et al.*, 2006). In the case of δ -cells, they can represent a 10% of the

islet. Other cell types such as pancreatic polypeptide-producing cells constitute less than 1%. As we will discuss later, the interaction among these cell types depends not only on their proportions within the islet but also on their spatial distribution. The control of glycemia as a result of islet function depends mainly on the direct action of α - and β -cell populations. While β -cells release insulin with increased glucose concentrations, glucagon secretion from α -cells takes place in hypoglycemic conditions (Quesada *et al.*, 2008). However, δ -cells exert an indirect action on glycemia by paracrine mechanisms given that the release of somatostatin inhibits α - and β -cell function (Nadal *et al.*, 1999; Hauge-Evans *et al.*, 2009).

β -cells of pancreatic islets are responsible for the biosynthesis, storage, and release of insulin. Insulin is a peptide hormone that controls blood glucose concentration. A decrease in either

insulin release or its peripheral effects (insulin resistance) results in diabetes, a devastating condition suffered by more than 150×10^6 people worldwide. When nutrients (glucose, amino acids) increase in the blood, insulin is released to the portal system then acting first in the liver and subsequently in peripheral tissues (muscle, adipose tissue). The effect of insulin on specific receptors, activate glucose uptake into the cell and promotes energy storage (glycogen, fat). Under physiological conditions, the blood glucose concentration in fasted mammals, including humans, is maintained at around 5 m mol/l. While lower glucose concentrations (hypoglycaemia) may be lethal for certain cell types (nerve cells), higher concentrations (hyperglycaemia) damage cellular structures and cause long-term complications (retinopathy, nephropathy, neuropathy, etc.). Blood glucose is continuously and precisely controlled by an integrated group of agents. A broad group of agents (glucagon, catecholamine) increases blood glucose but only insulin decreases it. Thus, no physiological substitutes of the hypoglycaemic factor may act when insulin is not present.

A better knowledge of β -cells is instrumental in order to find new treatments for diabetes or in the attempt to generate β -cell surrogates, one of the central aims of regenerative medicine (Soria *et al.*, 2000; León-Quinto *et al.*, 2004; Vaca *et al.*, 2006). Pancreatic islet cells are also a model for calcium-dependent peptide release. As compared with neurotransmitter release, which last for periods measured in milliseconds, hormone peptide release is a slow process lasting for seconds or even minutes. However, both secretory systems share common mechanisms (Martín *et al.*, 1995a, 1996). The aim of this review is to discuss the experimental and mathematical models that explain how this system works under physiological conditions.

NUTRIENT INDUCED ELECTRICAL ACTIVITY, CYTOSOLIC CALCIUM, AND INSULIN RELEASE

Pancreatic β -cells are blood glucose sensors, which adapt release insulin to the amount of glucose detected. Glucose sensor mechanisms include: (i) a high capacity and low-affinity transporter (Glut-2) acting as an open door that equalizes glucose both sides of the membrane, (ii) a low-affinity isoform of hexokinase, hexokinase IV is also called as glucokinase, (iii) glucose metabolism producing intermediates such as adenosine triphosphate (ATP) and diadenosine polyphosphates (DPs), (iv) a potassium channel (K_{ATP}) sensitive to the increase in ATP and DPs, and (v) a voltage-dependent Ca^{2+} channel (Ca_v) (Fig. 1). In the case of the β -cell, there is a consensus about the stimulus-secretion coupling model. Electrical activity, $[Ca^{2+}]_i$ signals, and consequently, insulin release are all stimulated at high-glucose concentrations. The metabolism of glucose and other nutrients (Bolea *et al.*, 1997), especially at the oxidative level in the mitochondria, leads to a rise of the ATP/adenosine diphosphate (ADP) ratio and diadenosine polyphosphates (Martín *et al.*, 1998), inhibiting ATP-dependent K^+ channels (K_{ATP}) and depolarizing the β -cell plasma membrane.

Figure 1. Stimulus-secretion coupling in the pancreatic β -cell. Glut-2 is a low-affinity, high capacity glucose transporter that equilibrates glucose concentration both sides of the membrane. Hexokinase IV (glucokinase: GK) phosphorylates glucose at physiological concentrations (5–7 mM). Nutrient metabolism increase ATP/ADP ratio and diadenosine polyphosphates (DPs) in the cytosol that in turn block ATP-regulated K-channels (K_{ATP}), depolarize cell membrane, and open voltage-dependent Ca channels (Ca_v) increasing cytosolic Ca^{2+} . An increase in cytosolic calcium triggers exocytosis and activate gene expression. Both mitochondria and endoplasmic reticulum buffer cytosolic Ca^{2+} changes.

As a consequence, voltage-dependent Ca^{2+} channels (Ca_v) activate, driving extracellular Ca^{2+} entry (Quesada *et al.*, 2000; Sanchez-Andrés *et al.*, 1988; Santos *et al.*, 1991; Valdeolmillos *et al.*, 1992). Although there are evidences of $[Ca^{2+}]_i$ nondependent release (Martín *et al.*, 1995c), it is broadly accepted that this increase in cytosolic Ca^{2+} is the signal that triggers the exocytotic process and insulin release.

Glucose-induced electrical activity consists in a depolarization followed by repetitive action potentials (calcium spikes) organized in bursts. The duration of these bursts membrane depends on glucose concentration. In order to keep blood glucose at 5 m mol/l, EC50 of the dose-secretion curve has to be close to this figure. In-vitro recorded glucose-induced electrical activity and insulin release have a threshold at about 7 m mol/l and an EC50 value of around 12 m mol/l glucose, far outside the physiological range (5 m mol/l). Only when other nutrients (amino acids) are present the electrical response to glucose is close to the normal physiological level of glucose (Bolea *et al.*, 1997). Previous studies from our group showed that intracellular calcium (in the whole islet) and cell bursting electrical activity (in one single β -cell) are synchronized (Santos *et al.*, 1991). This observation suggest that each calcium wave is due to Ca^{2+} entering the cells during a depolarized phase of electrical activity and that calcium oscillations occur synchronously across the whole islet tissue. Simul-

taneous measurements of $[Ca^{2+}]_i$ and insulin release resolved pulsatile insulin secretion that paralleled slow $[Ca^{2+}]_i$ oscillations (Martin *et al.*, 1995b).

GLUCOSE-INDUCED $[Ca^{2+}]_i$ DYNAMICS IN THE PANCREATIC β -CELL

In contrast with neurotransmitter release, in endocrine cells secretion is relatively slow and continues for tens of milliseconds after Ca^{2+} entry through the voltage-dependent Ca^{2+} channels has ceased, there is a latency between $[Ca^{2+}]_i$ and exocytosis and since calcium affinity of the sensor mechanism is lower, a single action potential may not be sufficient to trigger significant secretion and trains of action potentials may be more effective. The properties of the calcium-sensor for glucose-induced insulin secretion may be explored using cell-permeant $[Ca^{2+}]_i$ buffers with different kinetics and affinities (Pertusa *et al.*, 1999). Slow Ca^{2+} buffers [ethylene glycol tetraacetic acid (EGTA) and calcium orange-SN] did not affect glucose-induced insulin release while fast Ca^{2+} buffers (BAPTA and calcium green-5N) caused a 50% inhibition of the early phase and completely blocked late phase of insulin release. These data are consistent with: (i) the existence of a calcium-sensor with higher affinity than that of neurons and (ii) the existence of two pools of granules: “primed” vesicles colocalized with Ca^{2+} -channels responsible for the first phase of insulin release and “reserve pool” vesicles not colocalized and responsible for the second phase.

Glucose exerts three basic actions on the intracellular calcium activity $[Ca^{2+}]_i$: an increase in extracellular glucose induces an increase beneath the membrane, a decrease in the rest of the cytosol, and an increase in the intranuclear space (Fig. 2). Glucose-induced $[Ca^{2+}]_i$ changes not only trigger exocytosis when increases in a restricted space beneath the membrane (Quesada *et al.*, 2000) but also controls gene expression at the nuclear level (Quesada *et al.*, 2002). Furthermore, since high $[Ca^{2+}]_i$ levels are toxic for any cell, both mitochondria (than in addition provides energy) and endoplasmic reticulum are responsible for the nutrient-induced Ca^{2+} sequestration (Valdeolmillos *et al.*, 1992). Mathematical models explaining Ca^{2+} dynamics should take in account the existence of these pools.

$[Ca^{2+}]_i$ changes beneath the cell membrane are those directly responsible for exocytosis. To monitor those changes we developed a confocal spot microscope able to follow an optical signal in a region of $0.6 \mu m$ of diameter (Quesada *et al.*, 2000). Glucose metabolism generates $[Ca^{2+}]_i$ microgradients that reach the values of $8-10 \mu mol/l$ beneath the membrane that rapidly decayed to $0.27 \mu mol/l$ at a distance of $2 \mu m$ (Fig. 2). Mitochondrial $[Ca^{2+}]_i$ uptake and endoplasmic stores are involved in its regulation. The computer simulation of the effect of Ca^{2+} chelators on $[Ca^{2+}]_i$ gradients resulted in similar figures (Pertusa *et al.*, 1999). Conversely, this increase in $[Ca^{2+}]_i$ beneath the membrane nutrients decreases it in the cytosol. This second effect is usually masked by the increase when the system

Figure 2. Glucose-induced Ca^{2+} dynamics in the pancreatic β -cell. Glucose induces three main changes in cytosolic Ca^{2+} : (i) increases Ca^{2+} beneath the cell membrane by opening voltage-dependent Ca^{2+} channels (Ca_V), (ii) reduces Ca^{2+} in the rest of the cytosol by increasing Ca^{2+} uptake into mitochondria and endoplasmic reticulum, and (iii) increases Ca^{2+} in the nucleoplasm (from Quesada *et al.*, 2000, 2002). Lower part depicts the spatial course of $[Ca^{2+}]_i$ from cell membrane (left) to nucleus. Black lines represents the basal $[Ca^{2+}]_i$ under resting conditions (3–5 mM glucose) while red curve depicts $[Ca^{2+}]_i$ changes with glucose stimulatory concentrations (glucose 10–15 mM). Values of $[Ca^{2+}]_i$ and distance are estimates (from Pertusa *et al.*, 1999; Quesada *et al.*, 2000, 2002)

has not spatial resolution. Before using spot confocal microscopy we visualized this effect by adding diazoxide (see Fig. 6 of Valdeolmillos *et al.*, 1992). Diazoxide opens K_{ATP} channels and keeps the membrane potential clamped at the potassium equilibrium potential. Under these circumstances, addition of glucose decreases $[Ca^{2+}]_i$. Both mitochondria and endoplasmic reticulum participate in the sequestration of cytosolic calcium. The third effect of glucose on $[Ca^{2+}]_i$ is an increase in the nucleoplasmic space (Quesada *et al.*, 2002). Patch-clamp recording of the nuclear envelope allowed us to identify an ATP-sensitive K-channel (K_{ATP}) with similar properties to that found in the plasma membrane. The blockade of K_{ATP} channel with tolbutamide or diadenosine polyphosphates triggers nuclear $[Ca^{2+}]$ transients and induce phosphorylation of the transcription factor CREB (camp response element). Then K_{ATP} channels in the nuclear envelope link glucose metabolism, nuclear $[Ca^{2+}]$ signals and nuclear function.

MATHEMATICAL MODELS

Ca^{2+} triggered secretion in pancreatic β -cells is known to be a relatively slow process with longer latencies when compared with neurotransmitter release. A plausible explanation for such

slow mechanism is the existence of cytosolic barriers (endogenous buffers) that delay the response by stepping down the free calcium transient. The effect of exogenous chelators (Pertusa *et al.*, 1999) supports this possibility. Furthermore, not only nutrients control these cells but blood glucose regulation also relies on multiple levels of hormonal and neuronal control (Quesada *et al.*, 2008). Both inhibitory sympathetic and stimulatory parasympathetic inputs control endocrine pancreas. Cholinergic effects on membrane potential and cytosolic calcium acts as potentiators of glucose (initiator of insulin release). We have previously shown that cholinergic potentiation of insulin release is initiated by an early calcium entry (Sanchez-Andrés *et al.*, 1988). Biphasic responses of β -cells to changes in glucose concentration and the depolarizing effects of the sarcoplasmic reticulum calcium ATP-ase pump poison thapsigargin are accounted in a model, which incorporates I_{CRAC} in β -cells (Bertram *et al.*, 1995).

To explain cell behavior physiologists use structures (cell membranes, channels, mitochondrias, etc.) and concentrations of free particles. The structures also have concentrations and densities (n channels per μm^2 or 1,000 mitochondria/cell). Since physiological functions need to be quantified we use chemical parameters (affinity, concentrations, etc.) to explore the interactions between the free particles or between the free particles and structures. Mathematical models describing such processes could be classified in continuous models and stochastic models. Stochastic models are, in general, suitable for discrete and/or fast processes or for systems presenting elements with irregular geometry such as, for instance, the distribution of channels and vesicles on the membrane surface of neuroendocrine cells (Gil *et al.* 2000; Segura *et al.* 2000). On the other hand, continuous models, based on the solution of systems of differential equations, are more appropriated for slow and/or regular systems. This is the case of the secretory response in pancreatic cells.

A significant number of papers covering different aspects of modeling of pancreatic β -cells can be found in scientific literature: Diederichs (2006, 2008), Fridlyand *et al.* (2003, 2005), Meyer-Hermann (2007), Zhan *et al.* (2007), Boutayeb and Chetouani (2006), and Bertram *et al.* (2007, 2009) were just some of the examples of recent papers on modeling electrical activity, glucose metabolism, and insulin secretion in β -cells. Specifically, Diederichs (2006) focused on the study of the glucose metabolism of the pancreatic β -cell, proposing a mathematical model that includes membrane fluxes and currents, as well as metabolic processes of the beta-cell during rest and activation, including oscillations. Fridlyand *et al.* (2003) presented a model for β -cells that include an extended variety of ionic channels and pumps as well as other calcium sequestration mechanisms in order to investigate the possible role of K^+ channels, Na^+ , and IP3 in regulating beta-cell Ca^{2+} oscillations. In Fridlyand *et al.* (2005), the authors used the model presented in Fridlyand *et al.* (2003) to

relate glucose consumption, nucleotide pool concentration, respiration, Ca^{2+} fluxes, and K_{ATP} channel activity to study high-glucose-induced $[Ca^{2+}]_i$ oscillations. In Meyer-Hermann (2007), a model based on a bottom-up approach, which includes modeling mechanisms using the data from single protein level or conductance measurements, is presented. Zhan *et al.* (2007) considered a mathematical model for beta-cells describing the effects of both glucose and IP3 concentrations on membrane potentials. Boutayeb and Chetouani (2006) presented a global overview of mathematical models dealing with many aspects of diabetes and using various tools, including glucose and insulin dynamics, management and complications prevention, cost and cost effectiveness of strategies, and epidemiology of diabetes in general. Finally, Bertram *et al.* (2007) and Bertram *et al.* (2009) were just a two of the recent examples of many publications about this theoretical group on beta-cell modeling and application to different aspects of beta-cell regulation. The group also has an open-source web page for the software: www.math.fsu.edu/~bertram/software/islet. In contrast with the large number of papers on the modeling of beta-cells, there are very few theoretical studies on alpha cells, probably due to the limited number of experimental data available (Diderichsen and Göpel 2006; González Velez *et al.* 2009). The scarcity of models is even more noticeable in the case of pancreatic delta-cells for which theoretical models are not available at the level of single cell but limited to pancreatic islet studies (Jo *et al.* 2009).

STIMULUS-SECRETION COUPLING IN α - AND δ -CELLS

Changes in the intracellular Ca^{2+} concentration are a key element in the signaling pathway that results in hormone release not only in β -cells but also in α - and δ -cells. These Ca^{2+} signals are a consequence of the characteristic set of channels and electrical activity of each cell type (Kanno *et al.*, 2002). Although there is not an established model for α - and δ -cell function, it is accepted in both cases that glucose concentrations modulate their electrical activity, Ca^{2+} signals, and hormone secretion (Gromada *et al.*, 2004). In the light of evidences accumulated in the last years, it is assumed that δ -cells operate in a very similar scheme as β -cells (Kanno *et al.*, 2002) increases in extracellular glucose levels enhance electrical and Ca^{2+} signaling activity, and thus, somatostatin release. Since these cells possess K_{ATP} channels as well, it has been proposed that they link metabolic changes to an electrical and Ca^{2+} response by producing membrane depolarization, which leads to the generation of action potentials mediated by Ca^{2+} and Na^+ channels (Göpel *et al.*, 2000a; Kanno *et al.*, 2002). This activation promotes Ca^{2+} entry and granule exocytosis. These cells are activated at glucose concentrations as low as 3 m mol/l (Nadal *et al.*, 1999). This increased response to glucose compared to β -cells has been explained by the smaller density of K_{ATP} channels in the δ -cells since in this case, the membrane depolarization required to reach the threshold to generate action potentials would take place before a complete inhibition of K_{ATP} channels occurs

(Kanno *et al.*, 2002). Thus, glucose seems to exert a direct effect on the regulation of somatostatin secretion. Actually, glucose modulates Ca^{2+} signals in isolated δ -cells (Berts *et al.*, 1996). In these conditions, they are not exposed to the influence of paracrine factors. In addition, recent studies using redox confocal microscopy in intact islets have shown that glucose enhances the mitochondrial metabolism in δ -cells (Quesada *et al.*, 2006b), further supporting the link between glucose metabolism and somatostatin release by means of K_{ATP} channels. Thus, the stimulus-secretion coupling mechanisms in response to glucose in δ -cells should be very similar as those working in β -cells. Recently, it has been reported that a K_{ATP} channel independent pathway is also operating in δ -cells for glucose concentrations higher than 10 m mol/l (Zhang *et al.*, 2007). This pathway involves a process of Ca^{2+} -induced Ca^{2+} -release. Additionally, the control of δ -cell secretion is further subjected to paracrine and neural communication. However, little is known about this kind of regulation at the cellular level.

In contrast to the case of β - and δ -cells, electrical activity, Ca^{2+} signals, and hormone release are stimulated at low glucose levels in the α -cell population while all these events are inhibited when glucose concentrations are elevated (Gromada *et al.*, 2004; Quesada *et al.*, 2008). Thus, they have an opposite physiological response to that of β - and δ -cells. So far, there is not a consensus model to explain glucagon secretion in response to glucose changes. Moreover, α -cells are notably regulated by other key levels of control, particularly paracrine mechanisms (Ishihara *et al.*, 2003), which complicates the discrimination of a direct action of glucose in the inhibition of glucagon release at high-glucose concentrations. In any case, a model describing the stimulus-secretion coupling for α -cells has been proposed (Ashcroft, 2000; Gopel *et al.*, 2000b; Gromada *et al.*, 2004). This model reconciles recent findings in intact mouse islets and isolated cells but leaves unanswered some other aspects. According to this model, K_{ATP} channels, which are present in α -cells as well, are also responsible for changes in the membrane potential. At low glucose concentrations, the negative membrane potential in these cells is situated within a range that allows an electrical activity based on action potentials of T-type voltage-dependent Ca^{2+} channels. These channels drive the membrane potential to more positive values, allowing the activation of Na^{+} and N-type voltage-dependent Ca^{2+} channels. Repolarization would take place because of A-type K^{+} channels activation. Ca^{2+} entering through Ca^{2+} channels would be responsible for triggering granule exocytosis. An increase in the extracellular glucose levels would produce a rise in the ATP/ADP ratio as a consequence of glucose intracellular metabolism, blocking K_{ATP} channels. This inhibition would depolarize the plasma membrane to potential values that inactivate all the abovementioned currents and channels that sustain action potentials, consequently decreasing electrical activity, Ca^{2+} signaling, and glucagon release (Gromada *et al.*, 2004). Later studies have demonstrated that glucagon secretion is stimulated within a window of intermediate K_{ATP} channel activity, which

leads to an appropriate range of membrane potential that drives a sustained action potential firing (MacDonald *et al.*, 2007). Thus, this model propose a direct action of glucose and a key role of K_{ATP} channels to couple metabolic changes to ionic currents and Ca^{2+} signals that control secretion.

However, several studies have reported important differences between α - and β -cells that do not fit in the abovementioned model. Although both cell types possess different glucose transporters, it has been demonstrated that glucose transport is not a limiting factor in the metabolism of this sugar (Gorus *et al.*, 1984; Heimberg *et al.*, 1995, 1996). It seems that the main differences take place at the mitochondrial level. Using redox confocal microscopy to monitor metabolic changes and immunochemistry to identify the different islet cell types, it has been reported that α -cells exhibit a much lower activation of the mitochondrial aerobic metabolism in response to glucose (Fig. 3) (Quesada *et al.*, 2006b). Actually, several biochemical studies in islet cells separated by cell sorting indicate a higher level of anaerobic metabolism in α -cells in contrast to the elevated mitochondrial metabolic activity of β -cells (Schuit *et al.*, 1997). This idea is also supported by investigations that indicate that the ratio of lactate dehydrogenase/mitochondrial glycerol phosphate dehydrogenase is low in the β -cell, favoring the mitochondrial oxidation of glucose while this ratio is higher in non- β -cells (Sekine *et al.*, 1994). Thus, it seems that α -cells exhibit a very low coupling between the glycolytic events in the cytosol and ATP synthesis in the mitochondrial respiration. All these information about the α -cell glucose metabolism agrees with the fact that ATP/ADP changes in response to glucose are almost invariable in α -cells compared to the situation of β -cells (Detimary *et al.*, 1998). Therefore, there are some questionable aspects in the mentioned model for the α -cell, especially those concerning the regulation of the K_{ATP} channel activity by an increase in the ATP/ADP ratio. There are probably some other additional mechanisms regulating K_{ATP} channels, and thus, this model will probably require

Figure 3. During the activation of the Krebs cycle, FAD molecules are reduced to FADH_2 , which are less fluorescent. Changes in this fluorescence can be used as an indicator of the cell metabolic state and the activation of the mitochondrial metabolism. The record illustrates a typical glucose-induced decrease in FAD fluorescence in β -cells. This response does not take place in α -cells. The picture illustrates an islet optical section (2 μm) showing the autofluorescence from FAD. Scale bar is 50 μm .

the incorporation of new elements. In this regard, in addition to a direct effect of glucose, several paracrine mechanisms have an important effect in the inhibition of glucagon release such as insulin, zinc, somatostatin, and γ -aminobutyric acid, mainly by interaction with electrical activity (Ishihara *et al.*, 2003; Ravier and Rutter, 2005; Wendt *et al.*, 2004; Quoix *et al.*, 2009). Moreover, it has been proposed that a store-operated membrane conductance regulates α -cell electrical activity in response to glucose independently of K_{ATP} channels (Liu *et al.*, 2004; Vieira *et al.*, 2007). Recent studies also demonstrate that the hyperpolarization-activated cyclic nucleotide-gated channels are present in α -cells and play a role in the regulation of glucagon secretion by modulating the activity of Na^+ and Ca^{2+} channels (Zhang *et al.*, 2008). All these different mechanisms are most likely operating in the regulation of glucagon release. In the light of new evidences, more studies will be required to establish a model for the stimulus-secretion coupling in the α -cell.

LIMITATIONS OF CONVENTIONAL TECHNIQUES TO STUDY INDIVIDUAL Ca^{2+} RESPONSES WITHIN THE ISLET

The majority of studies about islet function have been focused on β -cell and insulin release. However, little is known about the physiology of other islet cell populations despite their relevance in glucose homeostasis. This lack of information is the result of several factors (Quesada *et al.*, 2008). One of them is the scarcity of the different cell types versus the β -cell predominance in the islet. Additionally, because of a limited characterization of these non- β populations, individual and specific physiological patterns for each cell type were absent. Finally, most of the available approaches to investigate islet physiology presented technical limitations to study individually each cell type. Although the information about non- β -cells from studies with cell lines or isolated cells are relevant, several physiological differences have been found when these cells are within the islet. Actually, several “*in vivo*” studies have evidenced that the intact islet as a study model is closer to the physiological scenario (Sanchez-Andres *et al.*, 1995). These discrepancies result from the critical importance of the intercellular communication and paracrine regulations within the islet an aspect that should be included in future mathematical models. These reasons have motivated the exploration of the physiological behavior of the different cell types in the intact islet. In the last years, several groups have characterized the electrophysiological properties and Ca^{2+} signals in response to glucose of the main cell types in the intact islet (Gopel *et al.*, 2000a; Nadal *et al.*, 1999; Quesada *et al.*, 2006a).

The study of individual Ca^{2+} responses with conventional microscopy is impractical as a result of the out-of-focus information. With this technique, the fluorescence emitted from Ca^{2+} -sensitive probes arrive to the detector from the focal plane but also from adjacent planes. Since a large fraction of the islet is composed by the β -cell population, the fluorescence from

multiple planes will be dominated by the β -cell signal, and thus, the response of α - and δ -cells, which are less abundant, will not be discriminated. This limitation can be overcome using confocal microscopy (Fig. 4) (Nadal *et al.*, 1999). With this technique, fluorescence is only monitored from a thin optical section and thus, light mainly comes from the focal plane without the interference of information from out-of-focus planes (Quesada *et al.*, 2000). In the three cell types α , β , and δ , intracellular Ca^{2+} increases are the signal that triggers exocytosis and hormone secretion. For a long time, the detection of the characteristic Ca^{2+} response of each cell type within the islet was not feasible. Confocal microscopy allowed the detection of different cell-specific Ca^{2+} signals in response to glucose (Fig. 2), which were correlated with cell types by immunocytochemistry (Nadal *et al.*, 1999; Quesada *et al.*, 2006a). Using these methods, it was reported that there is a cell population that is silent at 3 mM glucose and exhibits an intracellular Ca^{2+} increase followed by a train of oscillations when glucose is elevated to 11 mM. This is the typical response of the β -cell. However, two groups of cells have an oscillatory Ca^{2+} signal in 3 mM glucose. When glucose is elevated to 11 mM, a group

Figure 4. (a) On the left, a picture acquired by conventional fluorescence microscopy illustrates an islet loaded with the Ca^{2+} -sensitive fluorescent probe Fura-2. On the right, a picture captured by confocal microscopy illustrates an optical section (8 μm) of an islet loaded with the Ca^{2+} -sensitive fluorescent probe Fluo-3. Scale bar is 50 μm . (b) Characteristic Ca^{2+} signal from an islet that was monitored by conventional microscopy when glucose (g) concentration was changed from 3 mM to 11 mM. (c) Typical Ca^{2+} signals of α -, β -, and δ -cells within the islet in response to glucose (g) using confocal microscopy.

corresponding to the α -cell population becomes silent while another group identified as δ -cells remains without significant changes in frequency (Fig. 4). At 0.5 m mol/l glucose, only α -cells display Ca^{2+} signaling. The behavior of all these Ca^{2+} signals correlates with the patterns of electrical and secretory activity in response to glucose for each cell type (Kanno *et al.*, 2002). Those signaling patterns were demonstrated in mice and, more recently, in humans (Nadal *et al.*, 1999; Quesada *et al.*, 2006a).

ORGANIZATION OF GLUCOSE-INDUCED Ca^{2+} SIGNALS WITHIN THE ISLET

In mice, all the oscillatory Ca^{2+} signals from individual β -cells are synchronized throughout the islet (Nadal *et al.*, 1999; Santos *et al.*, 1991). This communication also occurs at the electrical level and is a consequence of the high degree of coupling among β -cells, which is mainly mediated by gap-junctions of connexin 36 (Quesada *et al.*, 2003; Serre-Beinier *et al.*, 2000). This synchronization of individual Ca^{2+} signals would explain that Ca^{2+} oscillations can be recorded from the islet with conventional fluorescence microscopy. With nonsynchronous individual responses, the oscillations would be cancelled out. It has been indicated that this signal synchronization would lead to a more vigorous insulin secretion (Vozzi *et al.*, 1995). In humans, however, coupling among individual Ca^{2+} signals can only be found in small clusters of cells but not in the whole islet (Quesada *et al.*, 2006a). The significance of this is still unknown. In any case, this functional organization agrees with the spatial cell organization in the islet. While in mice the majority of β -cells are close together in the islet center and are surrounded by a peripheral layer of non- β -cells, in humans, β and non- β -cells are distributed individually or in small clusters (Cabrera *et al.*, 2006). In the case of α - and δ -cells, they work as individual units in terms of signaling in both mice and humans. Electrophysiological techniques as well as studies of their Ca^{2+} signals demonstrated no functional coupling in these cells. So far, mathematical models provided plausible explanations for glucose-induced Ca^{2+} signaling and insulin release. However, pancreatic β -cells are integrated in islets interacting with other cell types. In addition, both in rodents and in humans β -cells are connected through gap-junctions, which allow fluxes of Ca^{2+} between cells, as demonstrated in the recovery after bleaching experiments (Quesada *et al.*, 2003). More than 20 years ago Sherman *et al.* (1988) suggested an attractive explanation by which “channel sharing” in clusters of cells tightly coupled by gap junctions could convert chaotic behavior of single cells into regular bursting in islets. Then, high conductance gap junction coupling, which converts the cluster into a single “super-cell” determines the minimal size for the cluster in 10–50 cells (Sherman and Rinzel, 1991). Since depending of their size or species involved islets may contain more than one synchronized cluster, a more recent model (De Vries *et al.*, 1998) analyzed this behavior. Gap junctions not only modulate electrical coupling but also functional fluxes of calcium diffusion con-

cluding that is not necessary for islet synchronization but not excluding other physiological roles, e.g., glycolytic oscillations. More recent mathematical models have analyzed the beneficial effects of intercellular interactions between pancreatic islet cells in blood glucose regulation (Jo *et al.*, 2009) or the role of intrapancreatic ganglia islet synchronization mechanisms (Fendler *et al.*, 2009). Kang *et al.* (2008) presents a simple islet model consisting of alpha-, beta-, and delta-cells, where clusters of beta-cells dominate glucose regulation. They connect the microscopic bursting mechanism and the macroscopic blood glucose regulation of the body. Finally, in Jo *et al.* (2009) a mathematical model for an islet consisting of two-state alpha-, beta-, and delta-cells is presented in order to analyze cell-to-cell variations in response to external glucose concentrations as well as glucose dynamics, which depends on insulin and glucagon. The combination of transgenic technology (connexin 36 knock-out mice) with high-speed imaging and theoretical modeling (Benninger *et al.*, 2008) uncovered the mechanisms underlying emergent properties of islets such as electrical bursting. Future mathematical models should incorporate all these observations then giving a more appropriate description of the behavior of cells at the single cell level and at the islet (micro organ) level.

ACKNOWLEDGMENTS

The authors are supported by the Fundación Progreso y Salud, Consejería de Salud, Junta de Andalucía (Grant No. PI-0022/2008), the Spanish Ministry of Science and Innovation (Grant Nos. BFU2007-67607 and BFU2008-01492), and the Red TERCEL (Grant No. RD06/0010/0025). CIBERDEM is an initiative of Instituto de Salud Carlos III.

REFERENCES

- Ashcroft, FM (2000). “The yin and yang of the K_{ATP} channel.” *J. Physiol.* **528**, 405.
- Benninger, RK, Zhang, M, Head, WS, Satin, LS, and Piston, DW (2008). “Gap junction coupling and calcium waves in the pancreatic islet.” *Biophys. J.* **95**, 5048–5061.
- Bertram, R, Budu-Grajeanu, P, and Jafri, MS (2009). “Using phase relations to identify potential mechanisms for metabolic oscillations in isolated beta-cell mitochondria.” *Islets* **2**, 87–94.
- Bertram, R, Sherman, A, and Satin, L (2007). “Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion.” *Am. J. Physiol. Endocrinol. Metab.* **293**, E890–E900.
- Bertram, R, Smolen, P, Sherman, A, Mears, D, Atwater, I, Martin, F, and Soria, B (1995). “A role for calcium release activated current (CRAC) in cholinergic modulation of insulin secretion.” *Biophys. J.* **68**, 2323–2332.
- Berts, A, Ball, A, Dryselius, G, Gylfe, E, and Hellman, B (1996). “Glucose stimulation of somatostatin-producing islet cells involves oscillatory Ca^{2+} signalling.” *Endocrinology* **137**, 693–697.
- Bolea, S, Pertusa, JAG, Martin, F, Sanchez-Andrés, JV, and Soria, B (1997). “Regulation of pancreatic β -cell electrical activity and insulin release by physiological amino acid concentrations.” *J. Physiol.* **43**, 699–704.
- Boutayeb, A, and Chetouani, A (2006). “A critical review of mathematical models and data used in diabetology.” *Biomed. Eng. Online* **5**, 43.
- Cabrera, O, Berman, DM, Kenyon, NS, Ricordi, C, Berggren, PO, and Caicedo, A (2006). “The unique cytoarchitecture of human pancreatic islets has implications for islet cell function.” *Proc. Natl. Acad. Sci. U.S.A.* **103**, 2334–2339.
- De Vries, G, Sherman, A, and Zhu, H-R (1998). “Diffusively coupled bursters: effects of cell heterogeneity.” *Bull. Math. Biol.* **60**,

- 1167–1200.
- Detimary, P, Dejonghe, S, Ling, Z, Pipeleers, D, Schuit, F, and Henquin, JC (1998). “The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in alpha cells and are also observed in human islets.” *J. Biol. Chem.* **273**, 33905–33908.
- Diderichsen, PM, and Göpel, SO (2006). “Modelling the electrical activity of pancreatic α -cells based on experimental data from intact mouse islets.” *J. Biol. Phys.* **32**, 209–229.
- Diederichs, F (2006). “Mathematical simulation of membrane processes and metabolic fluxes of the pancreatic β -cell.” *Bull. Math. Biol.* **68**, 1779–1818.
- Diederichs, F (2008). “Ion homeostasis and the functional roles of SERCA reactions in stimulus secretion coupling of the pancreatic beta-cell A mathematical simulation.” *Biophys. Chem.* **134**, 119–143.
- Fendler, B, Zhang, M, Satin, L, and Bertram, R (2009). “Synchronization of pancreatic islet oscillations by intrapancreatic ganglia: a modelling study.” *Biophys. J.* **97**, 722–729.
- Fridlyand, LE, Ma, L, and Philipson, LH (2005). “Adenine nucleotide regulation in pancreatic beta-cells: modeling of ATP/ADPCa²⁺ interactions.” *Am. J. Physiol. Endocrinol. Metab.* **289**, E839–E848.
- Fridlyand, LE, Tamarina, N, and Philipson, LH (2003). “Modeling of Ca²⁺ flux in pancreatic β -cells: role of the plasma membrane and intracellular stores.” *Am. J. Physiol. Endocrinol. Metab.* **285**, E138–E154.
- Gil, A, Segura, J, Pertusa, JAG, and Soria, B (2000). “Monte Carlo simulation of 3-D buffered Ca²⁺ diffusion in neuroendocrine cells.” *Biophys. J.* **78**, 13–33.
- González Velez, V, Gil, A, and Quesada, I (2009). “Modelling electrical currents and ionic channels in pancreatic alpha cells.” Submitted.
- Gopel, SO, Kanno, T, Barg, S, and Rorsman, P (2000a). “Patch-clamp characterisation of somatostatin-secreting-cells in intact mouse pancreatic islets.” *J. Physiol. (London)* **528**, 497–507.
- Gopel, SO, Kanno, T, Barg, S, Weng, XG, Gromada, J, and Rorsman, P (2000b). “Regulation of glucagon release in mouse alpha-cells by K_{ATP} channels and inactivation of TTX-sensitive Na⁺ channels.” *J. Physiol. (London)* **528**, 509–520.
- Gorus, FK, Malaisse, WJ, and Pipeleers, DG (1984). “Differences in glucose handling by pancreatic α - and β -cells.” *J. Biol. Chem.* **259**, 1196–1200.
- Gromada, J, Ma, X, Hoy, M, Bokvist, K, Salehi, A, Berggren, PO, and Rorsman, P (2004). “ATP-sensitive K⁺ channel-dependent regulation of glucagon release and electrical activity by glucose in wild-type and SUR1^{-/-} mouse alpha-cells.” *Diabetes* **53**, S181–S189.
- Hauge-Evans, AC, King, AJ, Carmignac, D, Richardson, CC, Robinson, IC, Low, MJ, Christie, MR, Persaud, SJ, and Jones, PM (2009). “Somatostatin secreted by islet delta-cells fulfills multiple roles as a paracrine regulator of islet function.” *Diabetes* **58**(2), 299–301.
- Heimberg, H, De Vos, A, Moens, K, Quartier, E, Bouwens, L, Pipeleers, D, Van Schaftingen, E, Madsen, O, and Schuit, F (1996). “The glucose sensor protein glucokinase is expressed in glucagon-producing alpha-cells.” *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7036–7041.
- Heimberg, H, De Vos, A, Pipeleers, D, Thorens, B, and Schuit, F (1995). “Differences in glucose transporter gene expression between rat pancreatic alpha- and beta-cells are correlated to differences in glucose transport but not in glucose utilization.” *J. Biol. Chem.* **270**, 8971–8975.
- Ishihara, H, Maechler, P, Gjinovci, A, Herrera, PL, and Wollheim, CB (2003). “Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells.” *Nat. Cell Biol.* **5**, 330–335.
- Jo, J, Choi, MY, and Koh, DS (2009). “Beneficial effects of intercellular interactions between pancreatic islet cells in blood glucose regulation.” *J. Theor. Biol.* **257**, 312–319.
- Kang, H, Han, K, Jo, J, Kim, J, and Choi, MY (2008). “Systems of pancreatic beta-cells and glucose regulation.” *Front. Biosci.* **13**, 6421–6431.
- Kanno, T, Gopel, SO, Rorsman, P, and Wakui, M (2002). “Cellular function in multicellular system for hormone-secretion: electrophysiological aspect of studies on alpha-, beta- and delta-cells of the pancreatic islet.” *Neurosci. Res.* **42**, 79–90.
- León-Quinto, T, Jones, J, Skoudy, A, Burcin, M, and Soria, B (2004). “*In vitro* directed differentiation of mouse embryonic stem cells into insulin-producing cells.” *Diabetologia* **47**(8), 1442–51.
- Liu, YJ, Vieira, E, and Gylfe, E (2004). “A store-operated mechanism determines the activity of the electrically excitable glucagon-secreting pancreatic alpha-cell.” *Cell Calcium* **35**, 357–365.
- MacDonald, PE, De Marinis, YZ, Ramracheya, R, Salehi, A, Ma, X, Johnson, PR, Cox, R, Eliasson, L, and Rorsman, P (2007). “A K_{ATP} channel-dependent pathway within α -cells regulates glucagon release from both rodent and human islets of Langerhans.” *PLoS Biol.* **5**, e143.
- Martín, F, Moya, F, Gutierrez, LM, Reig, JA, and Soria, B (1995a). “Role of syntaxin in mouse pancreatic beta cell.” *Diabetologia* **38**, 860–863.
- Martín, F, Pintor, J, Rovira, JM, Ripoll, C, Miras-Portugal, MT, and Soria, B (1998). “Intracellular diadenosine polyphosphates: a novel second messenger in stimulus-secretion coupling.” *FASEB J.* **12**, 1499–1506.
- Martín, F, Reig, JA, and Soria, B (1995b). “Secretagogue induced [Ca²⁺]_i changes in single rat pancreatic islets and its correlation with simultaneously measured insulin release.” *J. Mol. Endocrinol.* **15**, 177–185.
- Martín, F, Salinas, E, Vazquez, J, Soria, B, and Reig, JA (1996). “Inhibition of insulin release by synthetic peptides shows that the H3 region at the C-terminal domain of syntaxin-1 is crucial for calcium but not for GTP- γ -S induced secretion.” *Biochem. J.* **320**, 201–205.
- Martín, F, Sanchez-Andrés, JV, and Soria, B (1995c). “Slow [Ca²⁺]_i oscillations induced by ketoisocaproate in mouse pancreatic islets.” *Diabetes* **44**, 300–305.
- Meyer-Hermann, ME (2007). “The electrophysiology of the beta-cell based on single transmembrane protein characteristics.” *Biophys. J.* **93**, 2952–2968.
- Nadal, A, Quesada, I, and Soria, B (1999). “Homologous and heterologous asynchronicity between identified alpha-, beta- and delta-cells within intact islets of Langerhans in the mouse.” *J. Physiol. (London)* **517**, 85–93.
- Pertusa, JAG, Sanchez-Andrés, JV, Martín, F, and Soria, B (1999). “Effect of alien intracellular calcium chelators on nutrient induced insulin secretion: an approximation to the calcium sensor.” *J. Physiol. (London)* **520**, 473–483.
- Quesada, I, Fuentes, E, Andreu, E, Meda, P, Nadal, A, and Soria, B (2003). “On-line analysis of gap junctions reveals more efficient electrical than dye coupling between islet cells.” *Am. J. Physiol. Endocrinol. Metab.* **284**, E980–E987.
- Quesada, I, Martín, F, and Soria, B (2000). “Nutrient modulation of polarized and sustained submembrane Ca²⁺ microgradients in mouse pancreatic islet cells.” *J. Physiol. (London)* **525**, 159–167.
- Quesada, I, Rovira, JM, Martín, F, Roche, E, Nadal, A, and Soria, B (2002). “Nuclear KATP channels trigger nuclear Ca²⁺ transients and gene expression.” *Proc. Natl. Acad. Sci. U.S.A.* **99**(14), 9544–9549.
- Quesada, I, Todorova, MG, Alonso-Magdalena, P, Beltrá, M, Carneiro, EM, Martín, F, Nadal, A, and Soria, B (2006a). “Glucose induces opposite [Ca²⁺]_i oscillatory patterns in identified α - and β -cells within intact human islets of Langerhans.” *Diabetes* **55**, 2463–2469.
- Quesada, I, Todorova, MG, and Soria, B (2006b). “Different metabolic responses of α , β and δ -cells monitored by redox confocal microscopy within the intact islet of Langerhans.” *Biophys. J.* **90**, 2641–2650.
- Quesada, I, Tudurí, E, Ripoll, C, and Nadal, A (2008). “Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes.” *J. Endocrinol.* **199**, 5–19.
- Quoix, N, Cheng-Xue, R, Mattart, L, Zeinoun, Z, Guiot, Y, Beauvois, MC, Henquin, JC, and Gilon, P (2009). “Glucose and pharmacological modulators of ATP-sensitive K⁺ channels control [Ca²⁺]_i by different mechanisms in isolated mouse alpha-cells.” *Diabetes* **58**, 412–421.
- Ravier, MA, and Rutter, GA (2005). “Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells.” *Diabetes* **54**, 1789–1797.
- Sanchez-Andres, JV, Gomis, A, and Valdeolmillos, M (1995). “The electrical activity of mouse pancreatic beta-cells recorded *in*

- vivo* shows glucose-dependent oscillations." *J. Physiol. (London)* **486**, 223–228.
- Sanchez-Andrés, JV, Ripoll, C, and Soria, B (1988). "Evidence that muscarinic potentiation of insulin release is initiated by an early calcium entry." *FEBS Lett.* **231**, 144–147.
- Santos, RM, Rosario, LM, Nadal, A, Garcia-Sancho, J, Soria, B, and Valdeolmillos, M (1991). "Widespread synchronous $[Ca^{2+}]_i$ oscillations due to bursting electrical activity in single pancreatic islets." *Pflugers Arch. Gesamte Physiol Menschen Tiere* **418**, 417–422.
- Schuit, F, De Vos, A, Farfari, S, Moens, K, Pipeleers, D, Brun, T, and Prentki, M (1997). "Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells." *J. Biol. Chem.* **272**, 18572–18579.
- Segura, J, Gil, A, and Soria, B (2000). "Modelling study of exocytosis in neuroendocrine cells: influence of the geometrical parameters." *Biophys. J.* **79**, 1771–1786.
- Sekine, N, *et al.* (1994). "Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing." *J. Biol. Chem.* **269**, 4895–4902.
- Serre-Beinier, V, Le Gurun, S, Belluardo, N, Trovato-Salinaro, A, Charollais, A, Haefliger, JA, Condorelli, DF, and Meda, P (2000). "Cx36 preferentially connects beta-cells within pancreatic islets." *Diabetes* **49**, 727–734.
- Sherman, A, and Rinzel, J (1991). "Model for synchronization of pancreatic beta-cells by gap junction coupling." *Biophys. J.* **59**, 547–559.
- Sherman, A, Rinzel, J, and Keizer, J (1988). "Emergence of organized bursting in clusters of pancreatic beta-cells by channel sharing." *Biophys. J.* **54**, 411–425.
- Soria, B, Roche, E, Berná, G, León-Quinto, T, Reig, JA, and Martin, F (2000). "Insulin-secreting cells derived from embryonic stem-cells normalize glycaemia in streptozotocin-induced diabetic mice." *Diabetes* **49**, 157–162.
- Vaca, P, Martin, F, Vegara, JM, Rovira, JM, Berná, G, and Soria, B (2006). "Induction of differentiation of ES cells into islet cells by fetal soluble factors." *Stem Cells* **24**(2), 258–265.
- Valdeolmillos, M, Nadal, A, Contreras, D, and Soria, B (1992). "The relationship between glucose-induced K(ATP) channel closure and the rise in $[Ca^{2+}]_i$ in single mouse pancreatic islet cells." *J. Physiol. (London)* **455**, 173–186.
- Vieira, E, Salehi, A, and Gylfe, E (2007). "Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic alpha cells." *Diabetologia* **50**, 370–379.
- Vozzi, C, Ullrich, S, Charollais, A, Philippe, J, Orci, L, and Meda, P (1995). "Adequate connexin-mediated coupling is required for proper insulin production." *J. Cell Biol.* **131**, 1561–1572.
- Wendt, A, Birnir, B, Buschard, K, Gromada, J, Salehi, A, Sewing, S, Rorsman, P, and Braun, M (2004). "Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring beta-cells." *Diabetes* **53**, 1038–1045.
- Zhan, X, Wu, D, Yang, L, Liu, Q, and Jia, Y (2007). "Effects of both glucose and IP3 concentrations on action potentials in pancreatic β -cells." *Eur. Biophys. J.* **36**, 187–197.
- Zhang, Q, *et al.* (2007). "R-type Ca^{2+} -channel-evoked CICR regulates glucose-induced somatostatin secretion." *Nat. Cell Biol.* **9**, 453–460.
- Zhang, Y, Zhang, N, Gyulkhanyan, AV, Xu, E, Gaisano, HY, Wheeler, MB, and Wang, Q (2008). "Presence of functional hyperpolarisation-activated cyclic nucleotide-gated channels in clonal alpha cell lines and rat islet alpha cells." *Diabetologia* **51**, 2290–2298.