

Celična fiziologija v tkivnih rezinah - Preučevanje celic beta v Langerhansovih otočkih

Cell Physiology in Tissue Slices Studying Beta Cells in the Islets of Langerhans

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Izvleček

Uporaba izoliranih endokrinih celic je omogočila razvoj številnih metod za oceno osnovne celične arhitekture in opredelitev pomembnega dela molekularnih mehanizmov, ki sodelujejo pri celičnih procesih, kot sta vzdražnost celic in izločanje hormonov v procesu eksocitoze po porastu znotrajcelične koncentracije kalcijevih ionov. Ta prizadevanja so vodila v oblikovanje dogovornih modelov, ki razlagajo aktivacijo in splošen način delovanja določenega celičnega tipa. Z uporabo svežih tkivnih rezin lahko presežemo dosedanje raziskave in razkrijemo celo vrsto porajajočih se lastnosti, ki jih dogovorni modeli ne morejo zlahka predvideti niti razložiti. V tem članku predstavljamo izbor naših najpomembnejših eksperimentalnih ugotovitev na celicah beta v tkivnih rezinah trebušne slinavke.

Abstract

Isolated endocrine cells have enabled the development of numerous methods to assess basic cellular architecture and define a significant part of the molecular machinery involved in cellular processes, such as excitability or the exocytotic release of hormones following cytosolic calcium increase. These efforts have generated consensus models explaining the activation or general operation of a particular cell type. The use of fresh tissue slices can go even further and uncover a series of emergent properties never explained or predicted by consensus models. In this paper, we present a selection of our most important experimental findings in the beta cells of pancreatic tissue slices.

INTRODUCTION

Reductionism, a scientific paradigm that focuses on single cells, organelles, or even molecules, is necessary but not sufficient for the advancement of knowledge (1). Complex multicellular organs and organisms exhibit emergent properties that cannot be explained or predicted by studying their individual constituents (2). For example, the function of the heart cannot be understood by extrapolating findings from isolated cardiomyocytes (3) and the behavior of neuronal networks cannot be predicted solely from the function of individual neurons (which neglects interconnectivity and glia cells) (4). In addition, cell dissociation has been shown to alter the endocrine function of adrenal chromaffin cells (5) and pituitary melanotrophs (6).

Endocrine cells from the islets of Langerhans, particularly beta cells, were initially studied as dispersed individual cells. Upon the introduction of the isolated islet as an experimental method to study endocrine cells within their normal cellular context, it became clear that many pancreatic functions depend on undisturbed interactions between individual beta cells, including sensitivity to secretagogues, electrical activity patterns, changes in intracellular calcium, and insulin secretion. The coupling of beta cells into a functional syncytium is mediated by connexins (Cx36) and other intercellular contacts (7–9). Unfortunately, isolated whole islets typically have to be cultured for a few days; the culture conditions and duration have been shown to negatively influence various parameters of beta cell physiology (10–12).

To enable physiological experiments on beta cells within their normal environment, without the need for their culture, we have introduced the pancreatic tissue slice method (13), successfully combined it with morphological (14) and electrophysiological methods (15–20), and recently combined it with live cell calcium imaging (21–23). In this paper, we summarize the main findings of studies on beta cells in the Islets of Langerhans from acute mouse pancreatic tissue slices.

Beta cell physiology

The islets of Langerhans represent approximately 1% of the pancreas in mass and volume. These microorgans contain different types of endocrine cells, the majority (approximately two-thirds) being beta cells (24). A beta cell adapts the rate of insulin release to: (i) basic metabolic requirements, preventing the excess catabolism of bodily energy stores, and (ii) postprandial stimulation by various secretagogues, primarily glucose, to fine-tune the length and amplitude of the postprandial rise in plasma nutrient concentrations. This is achieved by a process termed stimulus–secretion coupling (Figure 1A). At rest, the beta cell has a high input conductance, due to open ATP-sensitive potassium channels (K_{ATP}), that hold a negative membrane potential close to the Nernst potential for potassium ions. Glucose (or other nutrient secretagogues) enters the beta cell and is metabolized to yield ATP, which decreases the probability of open K_{ATP} channels and depolarizes the plasma membrane. This activates voltage-activated calcium channels (VACCs), triggering the influx of calcium ions and a rise in intracellular calcium concentration, thereby setting into motion the process of exocytosis. During this process, insulin-containing granules fuse with the plasma membrane, releasing their hormonal contents into the extracellular space (25). Insulin exocytosis can also be influenced by nutrient and non-nutrient secretagogues (including various neurohormonal factors, such as acetylcholine, catecholamines, and incretins) through altering the sensitivity of the exocytotic machinery to calcium ions (17, 26). The process of stimulus–secretion coupling is presently studied at several levels: (i) by changes in the concentration of intracellular calcium ($[Ca^{2+}]_i$, Figure 1B), (ii) by changes in electrical activity (Figure 1C), and (iii) by changes in capacitance due to the fusion of exocytotic vesicles with the plasma membrane (Figures 1D and 1E).

The tissue slice method

To prepare tissue slices, we sacrificed mice, accessed the abdomen via laparotomy, and injected fluid low-melting point agarose into the proximal common bile duct clamped distally at the major duodenal papilla. After injection, the pancreas was cooled, the agarose-permeated pancreas extracted, and small

Figure 1. Stimulus–secretion coupling in the beta cell – methods overview. **A.** Beta cell stimulus–secretion coupling. Physiological stimuli (glucose, FA – free fatty acids, AA – amino acids) increase intracellular ATP concentration, which decreases open probability of K_{ATP} channels, depolarizing cell membrane, activating voltage activated calcium channels (VACCs), and increasing intracellular calcium concentration ($[Ca^{2+}]_i$), which can also be released from and taken up into intracellular calcium stores in endoplasmic reticulum (ER). Increased $[Ca^{2+}]_i$ sets into motion fusion of exocytotic vesicles with the plasma membrane, releasing vesicle content into the extracellular space. This so called triggering pathway is indicated in red. Hormones also act as secretagogues, however they act via the so called amplifying pathway indicated in blue. Hormones that have a stimulatory (CCK – cholecystokinin, Ach – acetylcholine, GIP – gastric inhibitory polypeptide, GLP-1 – glucagon-like peptide-1) or an inhibitory (somatostatin, A – adrenaline, NA – noradrenaline) effect on the insulin secretion from the beta cells act either via adenylate cyclase (AC), increasing cAMP concentration, or via phospholipase C (PLC), increasing concentration of diacylglycerol (DAG). **B.** Calcium-sensitive dyes emit $[Ca^{2+}]_i$ -dependent fluorescent light. **C.** The whole-cell configuration of the patch-clamp technique enables studying the exocytotic activity of the beta cells directly. **D.** Depolarization of the plasma membrane via the patch pipette triggers stimulated exocytosis via VACC-dependent influx of Ca^{2+} . A vesicle fused with the plasma membrane increases the total surface area of the membrane. Since the surface area of plasma membrane is directly proportional to membrane capacitance (C_m), this increment can be detected as an increase in C_m as shown in A. Inversely, endocytosis is followed by a decrease in C_m . **E.** UV light can serve as a stimulus to increase $[Ca^{2+}]_i$ directly, releasing Ca^{2+} from molecular cages. With this method we can trigger regulated exocytosis directly, bypassing activation of VACCs and release from internal stores.

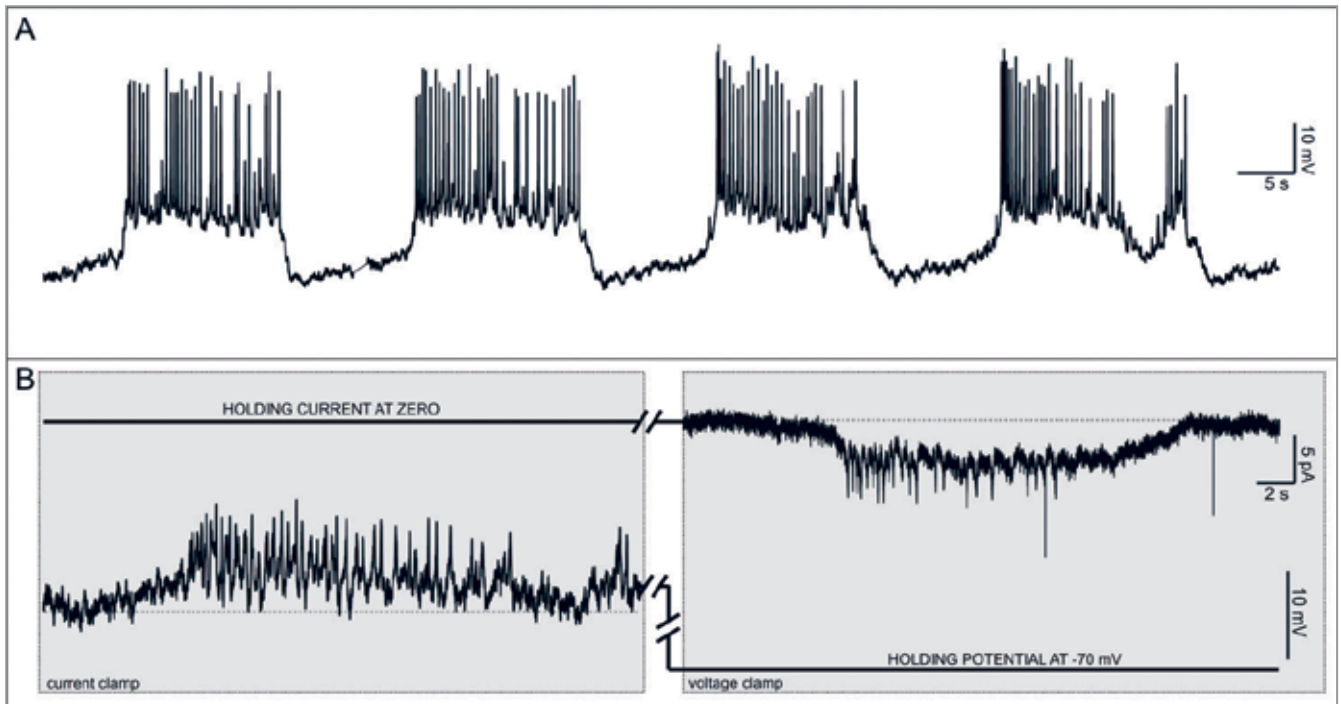


Figure 2. Beta cells in an islet of Langerhans are electrically coupled. A Beta cells show intermittent membrane potential depolarizations during stimulation with 12 mM glucose. **A.** A 'burst' consists of a depolarizing deflection of the potential, the plateau phase of which bears superimposed spikes formed by fast oscillations of the potential. **B.** In a coupled cell, electrical activity is formed by inward currents from neighboring cells. Left: current clamp of a beta cell during stimulation with 12 mM glucose. Right: voltage clamp of the same cell during the same stimulation. Note inward currents during the burst.

tissue blocks were cut from the organ. Tissue blocks were subsequently cut into 140- μm thick slices with a surface area of approximately 20–100 mm^2 . The slices were either immediately used in electrophysiological experiments or loaded with a membrane-permeable and calcium-sensitive fluorescent indicator (for example, Oregon Green 488 BAPTA-1, Invitrogen, Eugene, Oregon, USA). All procedures for tissue isolation and animal handling were in strict accordance with local and EU regulations. Further technical details for slice preparation, dye loading, and experimentation can be found in previously published work (13, 17, 22).

MAIN FINDINGS

Membrane potential and currents

Pancreatic beta cells are electrically excited by physiological stimuli and electrically coupled through Cx36, functioning as a syncytium (9, 27). Typical changes in the membrane potential upon stimulation were used to discriminate beta cells from other cell types in the islets of Langerhans. At sub-stimulatory glucose concentrations (for example, 6 mM or lower), no spontaneous electrical activity was observed in beta cells. During high glucose stimulation (for example, 12 mM or higher), the probability of open K_{ATP} channels decreased, depolarizing the plasma membrane (Figure 1A). Electrical activity in a beta cell during high glucose stimulation consisted of “bursts” – transient membrane potential depolarizations, on which high-frequency oscillations of the potential were superimposed (Figure 2A). With the whole-cell patch-clamp in current-clamp mode, changes in membrane potential could be recorded (Figure 2B, left trace). Switching to the voltage-clamp mode (in our case at -70 mV), we could observe current via gap junctions from active neighboring cells (Figure 2B, right trace). From the amplitude of this injected current and the membrane potential excursions, the gap junction conductance was estimated as:

$$G_j = \frac{\Delta I}{\Delta V} \approx \frac{5 \text{ pA}}{10 \text{ mV}} = 0.5 \text{ nS}$$

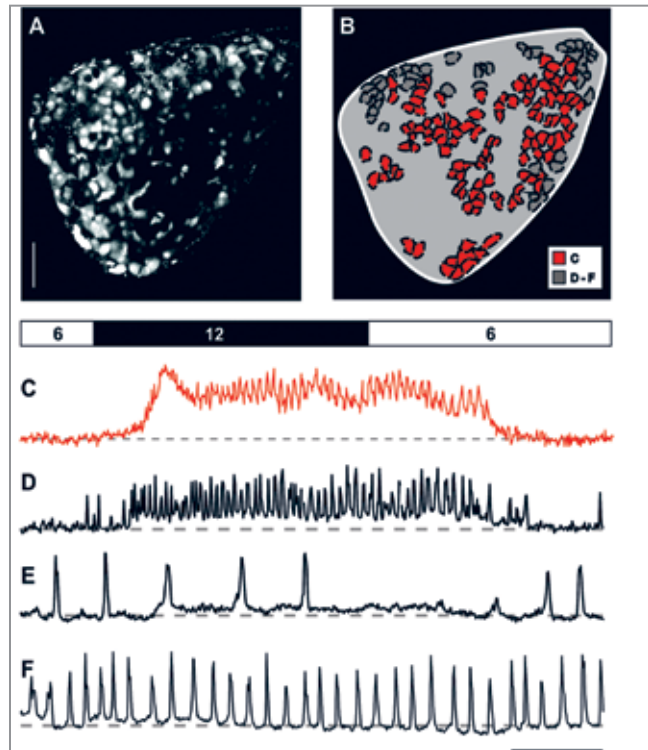


Figure 3. Calcium oscillations in cells from an islet of Langerhans upon stimulation with glucose. 6 mM glucose is non-stimulatory for beta cells, whereas 12 mM concentration was used as a stimulus. **A.** Cells from an islet of Langerhans were loaded with the calcium sensitive indicator Oregon Green® 488 BAPTA-1. Scale bar indicates 50 micrometers. **B.** A color-coded schematic representation of cells from A that responded to stimulation with 12 mM glucose as shown in C–F. The light grey area indicates cells and parts of islet that did not respond. **C.** A typical beta cell response to stimulation with glucose. Note an increase in $[\text{Ca}^{2+}]_i$ from the basal level, followed by a plateau phase with superimposed high frequency oscillations. **D.** A typical delta cell response. The cell is already active in 6 mM glucose; stimulation with glucose increases the frequency of oscillations as well as the baseline. **E.** A typical alpha cell response. The cell is active in 6 mM glucose but its activity is reduced after increasing the concentration of glucose. **F.** Some cells display glucose-independent activity. Likely, these cells are already maximally active delta cells. Scale bar indicates 200 s.

Non-beta cells did not seem to be electrically coupled to beta cells. In addition, non-beta cells in the islets

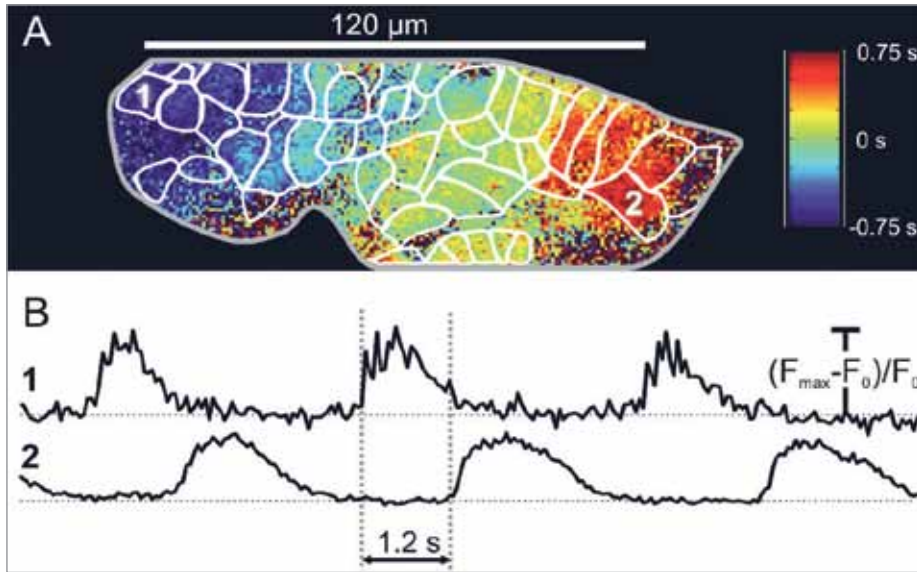


Figure 4. $[Ca^{2+}]_i$ oscillations in individual beta cells are synchronized by means of $[Ca^{2+}]_i$ waves regularly spreading within an islet. **A.** Cross-correlation determined color-coded time delays for each pixel demonstrating the average direction of spreading of $[Ca^{2+}]_i$ waves for 6 subsequent oscillations in 45 cells from this islet, outlined in white. **B.** 3 consecutive $[Ca^{2+}]_i$ oscillations in 2 cells indicated in A. For the second oscillation in B, the velocity of the $[Ca^{2+}]_i$ wave between cell 1 and 2 was 100 $\mu\text{m}/\text{s}$. In 4 different islets, for 6 consecutive oscillations in 10 cells from each islet, the average speed of waves was approximately 100 $\mu\text{m}/\text{s}$ as well (data not shown).

of Langerhans were not electrically coupled to other types of non-beta cells.

Calcium oscillations and waves

In beta cells, the glucose-stimulated Ca^{2+} influx through VACCs and the accompanying changes in $[Ca^{2+}]_i$ occur rhythmically, which is likely due to the rhythmic nature of the underlying electrical activity. In isolated islets, a considerable overlap has been demonstrated between glucose-stimulated electrical activity occurring in the form of membrane potential bursts and $[Ca^{2+}]_i$ oscillations of comparable frequency, regularity, and duration (28, 29). Also, oscillations in secreted insulin are well correlated temporally with electrical activity and Ca^{2+} signaling (11, 30). In islets *in vivo* and cultured islets studied within 24 hours after enzyme-assisted isolation, fast $[Ca^{2+}]_i$ oscillations (frequency = 2–7 min^{-1}) corresponding to bursts of membrane potential are the predominant response. However, in islets cultured over longer periods of

time and stimulated with glucose, slow $[Ca^{2+}]_i$ oscillations (frequency = 0.2–1 min^{-1}) prevail (10, 11, 28, 29, 31–33). In beta cells from a single islet, electrical activity and $[Ca^{2+}]_i$ oscillations in different cells are well synchronized, with only short phase lags (up to 1–2 seconds) detected between oscillations in different islet regions (28, 29, 34). In isolated islets cultured over longer periods of time, $[Ca^{2+}]_i$ waves originating from the most sensitive cells, spreading across or along the periphery of islets at a speed of 20–200 $\mu\text{m}/\text{s}$, have been demonstrated (35–37). However, it has been suggested that in islets *in vivo* $[Ca^{2+}]_i$ waves are absent and appear only after several days of culture. Moreover, the use of isolated islets in $[Ca^{2+}]_i$

imaging experiments has an important technical drawback. Due to their compact spherical structure, the uptake of fluorescent indicators into the core of isolated islets is limited by diffusion and dye trapping in the outermost cells (38–41). Thus, characterizing responses and assessing synchronization in a large number of cells from all layers of an islet have not been feasible to date.

By combining the acute pancreatic tissue slice technique with live cell $[Ca^{2+}]_i$ imaging employing confocal laser scanning microscopy (Figure 1B), we gained access to cells from all layers of an islet and characterized their responses (Figure 3A) (22). Beta cells were located at the core of the islet and typically responded to glucose stimulation with fast $[Ca^{2+}]_i$ oscillations superimposed on a sustained plateau (Figures 3B and 3C). Non-beta cells predominated at the periphery of islets (Figure 3B). Delta cells responded to glucose stimulation with an increase in frequency or amplitude of calcium oscillations (Figure

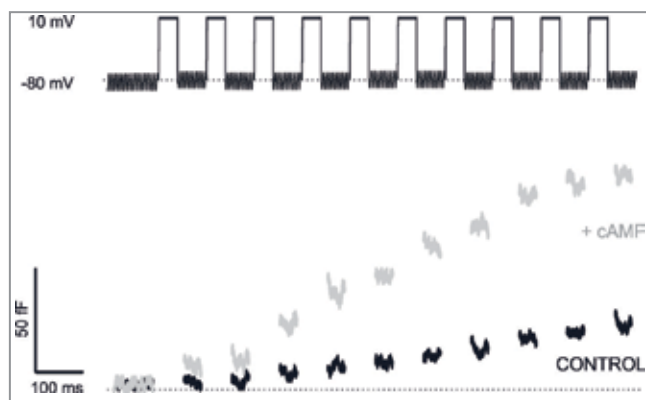


Figure 5. Exocytosis during stimulation with repetitive depolarization pulses A. voltage protocol with 40 ms long depolarization pulses from -80 mV to $+10$ mV at a frequency of 10 Hz (upper panel) was used. Increase in $[Ca^{2+}]_i$ by means of voltage activated calcium channels (VACC) triggered Ca^{2+} dependent exocytosis which was detected as a change in membrane capacitance (C_m) (lower panel) in the control cell (black trace) and the cell with an increased concentration of cAMP (grey trace).

3D). In alpha cells, oscillations subsided upon high glucose exposure (Figure 3E). In some cells at the periphery, no changes in frequency or amplitude were detected (Figure 3F). These cells were likely delta cells that had already been maximally activated. In all types of cells, the effect of glucose was reversible.

Calcium oscillations were not synchronized between heterotypic or homotypic non-beta cells. Calcium oscillations in individual beta cells were synchronized by $[Ca^{2+}]_i$ waves originating from a group of cells and repeatedly spreading across islets with a velocity of approximately $100 \mu\text{m/s}$ (Figure 4).

Capacitance

Exocytosis in most neuronal and endocrine tissues, including pancreatic beta cells, is a Ca^{2+} -dependent process. Exocytosis involves multiple stages (42); it starts with vesicle biogenesis, continues with the loading of secretory granules, translocation of granules to the plasma membrane, vesicle docking, and ends with a Ca^{2+} -dependent fusion of vesicles with the plasma membrane by the SNARE (soluble N ethylmaleimide-sensitive factor attachment protein receptor) fusion machinery (43, 44). Ca^{2+} is

a key signal for activating the secretory machinery and has multiple roles in regulating exocytosis. It is required at several distinct granule-maturation steps prior to fusion (45) and is directly involved in the fusion of insulin-containing vesicles with the plasma membrane (46).

The exocytotic activity in pancreatic beta cells can be modulated through second messengers like cAMP and phospholipase C (PLC), and a number of physiological signals (47–49) (Figure 1A). Various nutrient and non-nutrient secretagogues can amplify or inhibit glucose-induced insulin secretion. Cholecystokinin and acetylcholine stimulate insulin secretion via PLC and increase the concentration of diacylglycerol, whilst incretins, like the gastric inhibitory polypeptide or glucagon-like peptide-1, amplify glucose-induced insulin secretion by activating adenylate cyclase (AC), thereby increasing cAMP concentration in the beta cell (50). On the other hand, inhibition of insulin secretion via AC can be caused by somatostatin, adrenaline, or noradrenaline (51, 52). One of the central signaling molecules that regulate exocytosis in different cell types, including pancreatic beta cells, is cAMP. Two cAMP-dependent pathways have been reported to directly control Ca^{2+} -dependent exocytosis, either through activation of protein kinase A (PKA) or via a cAMP-guanidine nucleotide exchange factor 2 (GEFII)/Epac2-dependent pathway (Figure 1A) (53).

We aimed to clarify the role of cAMP in beta cell physiology. To mimic glucose-dependent depolarization in beta cells through opening inward Ca^{2+} currents and increasing $[Ca^{2+}]_i$, we used a voltage pulse protocol (Figure 1D). Repetitive 40 ms long depolarization pulses from -80 mV to $+10$ mV (Figure 5, upper panel) opened VACCs and the subsequent influx of Ca^{2+} through these channels increased $[Ca^{2+}]_i$ and triggered Ca^{2+} -dependent exocytosis. This Ca^{2+} -dependent fusion of secretory vesicles with the plasma membrane was measured through increases in membrane capacitance (C_m) (Figure 5, lower panel). After the first few depolarization pulses, a large increase in C_m was already observed in cells with a high cytosolic cAMP concentration (Figure 5, lower panel, grey trace) compared to the control cells (Figure 5, lower panel, black trace), despite the unchanged Ca^{2+}

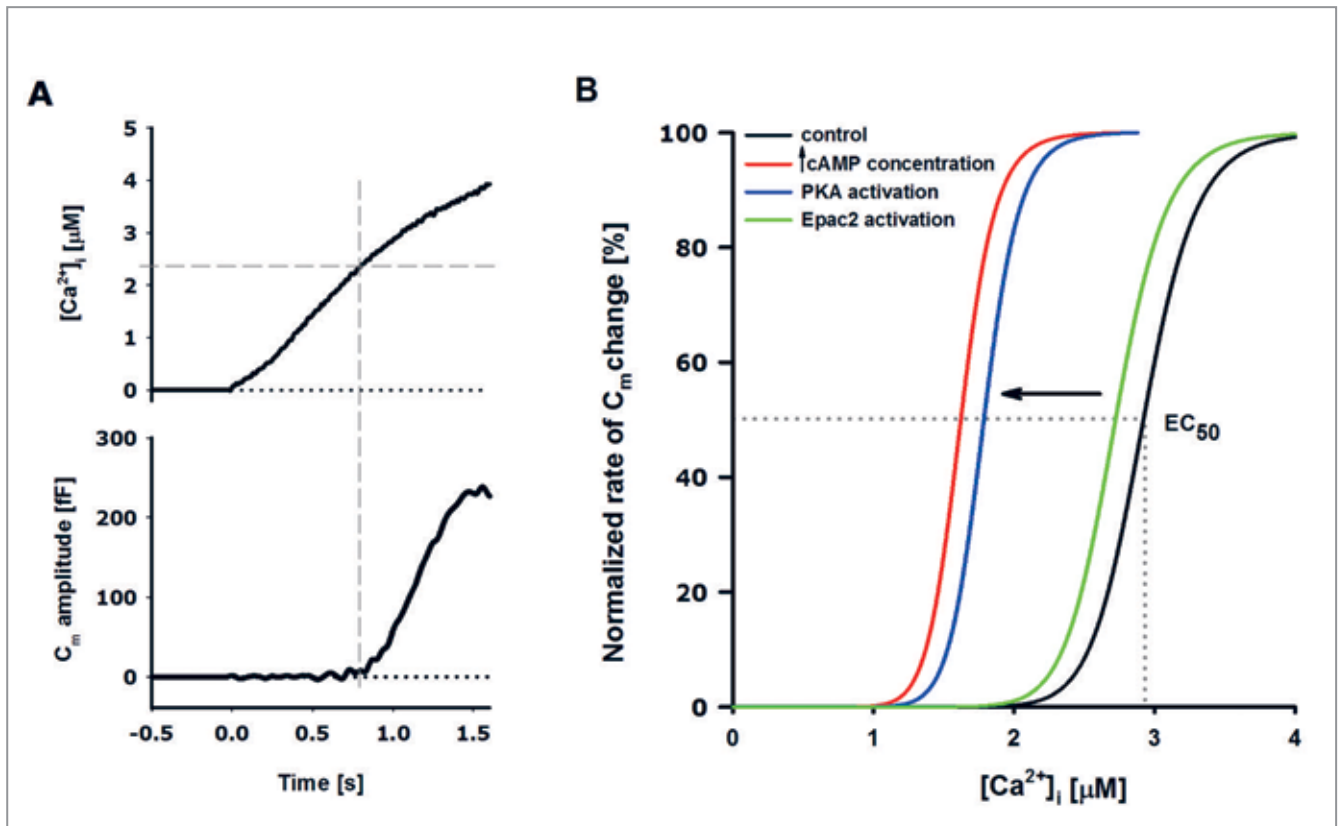


Figure 6. cAMP increases the sensitivity of exocytosis to Ca^{2+} primarily through PKA. **A.** Slow photolysis of caged Ca^{2+} triggers a ramp-like increase in $[Ca^{2+}]_i$ (upper panel). When $[Ca^{2+}]_i$ reaches a threshold level (dashed line) exocytosis is triggered and an increase in membrane capacitance (C_m) is observed (lower panel). **B.** The rate of C_m change expressed as a function of $[Ca^{2+}]_i$ shows saturation kinetics with high cooperativity. In the control, half-maximal rate of C_m change is achieved at $\sim 3 \mu M$ $[Ca^{2+}]_i$ (EC_{50}), while in cells with a high level of cAMP, a shift to lower EC_{50} can be observed. When PKA is activated, the half-effective Ca^{2+} concentration again decreases, while activation of Epac2 does not change the half-effective Ca^{2+} concentration. The arrow is indicating the described shift in EC_{50} .

currents (data not shown). These data suggest that an elevated cytosolic cAMP concentration effectively sensitizes the secretory machinery to Ca^{2+} .

To dodge the issue of high variability in Ca^{2+} current densities between different beta cells, we employed the slow photo-release of caged Ca^{2+} (Figure 1E). The slow photo-release of Ca^{2+} ions caged to NP-EGTA buffer released $[Ca^{2+}]_i$ in a range of several μM (Figure 6A, upper panel). Increased $[Ca^{2+}]_i$ stimulated exocytosis was detected again as an increase in C_m using high-resolution whole-cell patch-clamp recordings (Figure 6A, lower panel). Exocytosis was triggered when $[Ca^{2+}]_i$ reached a threshold level (Figure 6A, upper and lower panel, dashed line). A detailed analysis revealed that the rate of C_m change expressed for the corresponding $[Ca^{2+}]_i$ showed saturation kinetics (17). After fitting the

Hill function, it was established that a half-maximal rate of C_m change in control animals was achieved at $\sim 3 \mu M$ $[Ca^{2+}]_i$ (Figure 6B, black trace, dotted line). When the same experiment was done with 200 μM cAMP applied intracellularly via a pipette solution, the half-maximal rate of C_m change was achieved at a significantly lower $[Ca^{2+}]_i$ concentration (Figure 6B, red trace), suggesting that cAMP sensitizes the secretory machinery to Ca^{2+} and therefore triggers exocytosis at a lower $[Ca^{2+}]_i$. To assess which of the previously described cAMP-dependent pathways is responsible for this shift in Ca^{2+} sensitivity, we separately activated the PKA-dependent pathway and the Epac2-dependent pathway. In PKA-activated cells (Figure 6B, blue line), a similar shift in the half-effective Ca^{2+} concentration (EC_{50}) was observed, while Epac2 activation did not

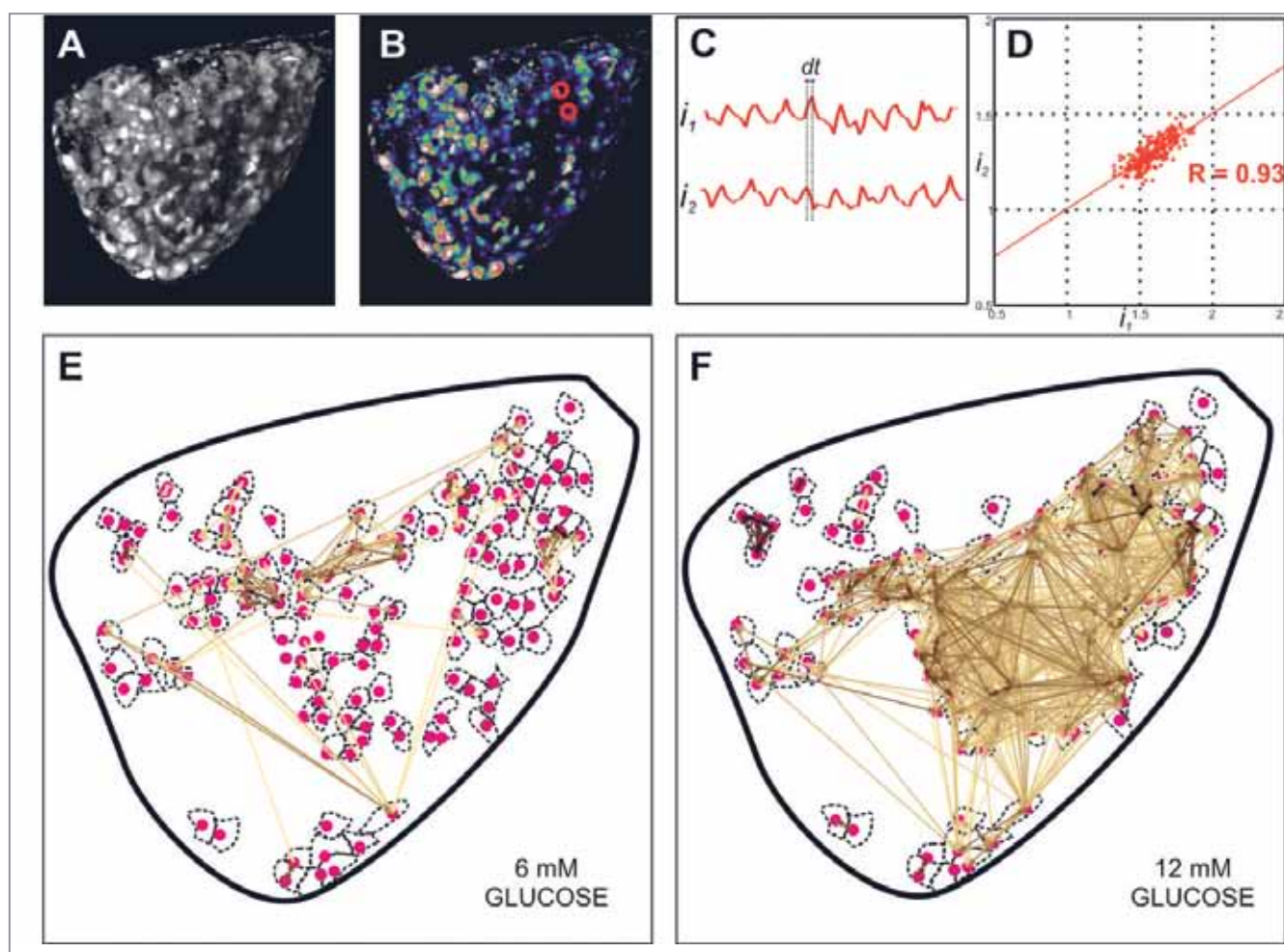


Figure 7. Graph analysis of functional networks of beta cells reveals that intercellular connectivity is modulated by physiological stimuli. **A.** Beta cells from acute pancreatic tissue slice were loaded with the calcium indicator dye Oregon Green® 488 BAPTA-1. **B.** Calcium concentration is pseudo colored for easier visualization. **C.** Oscillatory changes in $[Ca^{2+}]_i$ from two cells indicated in panel B. $[Ca^{2+}]_i$ signals of individual beta cells (cells i_1 and i_2) are well synchronized but not completely in phase. **D.** Signals of every two beta cells are compared such that the Pearson product moment correlation is calculated over a given time interval for signals of the two cells being considered ($r=0.93$ for cells i_1 and i_2 and the time interval during stimulation with glucose). Setting a threshold value at $r=0.75$, every two cells whose signals show a correlation above this value can be connected by a link. **E.** In nonstimulatory concentration of glucose (6 mM), the connections between beta cells are the consequence of chance similarity between two noisy signals, thus sparse and not showing any dependence on physical distance. **F.** In stimulatory glucose (12 mM), physically close cells are connected such that the neighbors of a cell are also connected with each other. In addition, a few longer-range connections exist as well. This is characteristic of small worlds. In panels E and F, links are color-coded, such that light yellow depicts a correlation close to the threshold value and dark brown a correlation close to 1.

significantly change the Ca^{2+} sensitivity (Figure 6B, green line). Since the effect of incretins is exerted via the PKA-dependent signaling pathway, we suggest that they increase the sensitivity of the secretory machinery in pancreatic beta cells to Ca^{2+} , thereby enhancing the beta cell response to glucose (17).

Understanding the beta cell syncytium as a graph

Successfully combining pancreatic tissue slice preparation with confocal laser-scanning calcium imaging (Figure 1B) has enabled us to practically simultaneously record the activity of a large number of beta cells and to study beta cell physiology

from a systems biology perspective. Over the last decade, network science has unraveled the common properties of real-world systems, including biological, computer, technical, communication, and social networks (54–56). The most important functional properties in these networks are their so called small-world topology (57) and scale-free nature (58). The presence of a small-world topology implies particular dynamic properties of the observed network, such as a high signal propagation speed and a high degree of synchronizability (57, 59). Considering the advantages conferred by small-world properties and emerging evidence on their presence in real biological systems, we set out to apply the basic ideas of graph theory and to seek evidence of small-world properties in beta cells from the islets of Langerhans. We extracted beta cell functional networks through a pairwise comparison of intracellular $[Ca^{2+}]_i$ signals from beta cells and analyzed them with diagnostic tools from graph theory. The methodology to represent $[Ca^{2+}]_i$ signals from a large number of cells in the form of a graph consisting of nodes connected if their signals are sufficiently similar is briefly summarized in Figure 7. Analyzing characteristic parameters, such as the average clustering coefficient, global efficiency, and the probability distribution for the number of links in each cell, we found that beta cells in the islets of Langerhans display properties of broad-scale small worlds. The observed properties might crucially support the rapid activation of beta cells after high glucose exposure and their synchronized activity during stimulation. In addition, the high clustering is expected to make the syncytium of beta cells resilient to the random dysfunction of individual cells (23). In contrast, the presence of highly-connected cells (hubs) also suggests that islets might be particularly vulnerable to failure in one of these cells. Since the decrease in beta-cell mass before the onset of a clinically overt disease is typically two-fold higher in type 1 diabetes than in type 2 diabetes (60), we hypothesize that beta cell failures occur in a more random manner in type 1 diabetes, possibly mediated by attacks on omnipresent antigens. In type 2 diabetes, beta cell death may be a hierarchical

process in which the functionally more important cells are destroyed first.

CONCLUSIONS

Endocrine tissue slices were initially developed to overcome limitations of cell isolation and culture in addressing the physiology of perinatal and compensated tissues. This approach offered a fast preparation, employing limited mechanical stress and no enzymatic stress, with sufficient yields to apply control and test conditions on tissues from the same donor. The methods and previous results from experiments on single cells in culture proved convenient, but often failed to predict important properties that emerge in the intact socio-cellular environment of the islets of Langerhans. Methods often had to be innovatively evolved to tackle higher demands, like the simultaneous observation of a large number of different cell types within tissue. The ultimate reward is the taming of the islets of Langerhans into a systems biology framework to address completely novel diagnostic and therapeutic challenges in the future.

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