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Bioluminescence imaging of nuclear calcium oscillations in intact pancreatic islets of Langerhans from the mouse

Carlos Villalobos [a,1], Angel Nadal [b,1], Lucía Núñez [a], Iván Quesada [b], Pablo Chamero [a], María T. Alonso [a], Javier García-Sancho [a,*]

[a] Instituto de Biología y Genética Molecular (IBGM), Facultad de Medicina, Universidad de Valladolid and CSIC, Facultad de Medicina, Avda Ramón y Cajal 7. 47005 Valladolid, Spain

[b] Instituto de Bioingeniería, Universidad Miguel Hernández, Sant Joan d'Alacant, Alicante, Spain

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Abstract

The stimulus-secretion coupling for insulin secretion by pancreatic β cells in response to high glucose involves synchronic cytosolic calcium oscillations driven by bursting electrical activity. Calcium inside organelles can regulate additional functions, but analysis of subcellular calcium signals, specially at the single cell level, has been hampered for technical constrains. Here we have monitored nuclear calcium oscillations by bioluminescence imaging of targeted aequorin in individual cells within intact islets of Langerhans as well as in the whole islet. We find that glucose generates a pattern of nuclear calcium oscillations resembling those found in the cytosol. Some cells showed synchronous nuclear calcium oscillations suggesting that the islet of Langerhans may also regulate the activation of Ca^{2+}-responsive nuclear processes, such as gene transcription, in a coordinated, synchronic manner. The nuclear Ca^{2+} oscillations are due to bursting electrical activity and activation of plasma membrane voltage-gated Ca^{2+} channels with little or no contribution of calcium release from the intracellular Ca^{2+} stores. Irregularities in consumption of aequorins suggests that depolarization may generate formation of steep Ca^{2+} gradients in both the cytosol and the nucleus, but further research is required to investigate the role of such high $[Ca^{2+}]$ microdomains.
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1. Introduction

Pancreatic β-cells within the islet of Langerhans are responsible for insulin secretion after an increase of blood glucose and their dysfunction causes diabetes mellitus. The stimulus-secretion coupling process involves glucose metabolism, which provokes the closure of ATP-dependent potassium channels (K_{ATP}), responsible for the resting membrane potential [1]. As a result, the plasma membrane depolarizes and causes the opening of voltage dependent Ca^{2+} channels and a rise of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) [2]. Glucose-induced Ca^{2+} signals in all the β-cells within the same islet of Langerhans have a synchronous and homogeneous $[Ca^{2+}]_{cyt}$ oscillatory pattern. This is a consequence of the bursting pattern of electrical activity characteristic of pancreatic β-cells [3,4] which drives Ca^{2+} oscillations, and the characteristic gap junctional-mediated coupling among the β-cell population [5]. Consequently, insulin secretion is pulsatile, coinciding with the peaks of $[Ca^{2+}]_{cyt}$ [6–8].

In addition to its role in insulin secretion, Ca^{2+} mediates other processes, such as gene transcription, proliferation and apoptosis. The ability of Ca^{2+} to elicit different cell responses depends on its route of entry, localization and code of amplitude or frequency of Ca^{2+} oscillations. The role of local subplasmalemmal gradients in the control of exocytosis is well known [9]. In contrast, information on the role of local Ca^{2+} signals in subcellular organella is scarce. It is well estab-

* Corresponding author. Tel.: +34 983 423085; fax: +34 983 423588.
E-mail address: jgsancho@ibgm.uva.es (J. García-Sancho).
[1] Both contributed equally to this work.

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lished that the nucleoplasmic concentration of free calcium ($[Ca^{2+}]_{nuc}$) regulates gene transcription [10], but how nucleoplasmic Ca^{2+} signals are generated is still unclear.

In pancreatic β-cells, nutrients, such as glucose and certain fatty acids, modulate the expression of several candidate immediate early genes (*IEGs*), most of them being involved in many aspects of β-cell function including differentiation, proliferation and apoptosis. The action of these nutrients is specific for some IEGs and occurs at the transcriptional level in a Ca^{2+}-dependent manner [11–13]. Additionally, Ca^{2+} ions are thought to participate in the transcriptional control of insulin [14,15]. Thus, regulation of Ca^{2+} dynamics in the nucleus might be important for β-cell function. In pancreatic β-cells there seems to be no Ca^{2+} barrier between the cytosol and the nucleoplasm [16], but the nuclear envelope contains functional ATP-dependent K^+ channels that may link glucose metabolism, nuclear Ca^{2+} signaling and gene expression [17]. Therefore Ca^{2+} signals in nucleus and cytosol may be differentially regulated. Current evidence suggests independent regulation of nuclear calcium in some cell types [18–22].

The use of targeted aequorins has enabled unambiguous monitoring of subcellular calcium changes in several organella including the nucleus [23]. We have reported recently that single-cell imaging of targeted aequorin can be used to monitor $[Ca^{2+}]_{nuc}$ oscillations in excitable cells [24]. We also reported that sustained $[Ca^{2+}]_{cyt}$ increases were faithfully transmitted into the nucleus, whereas $[Ca^{2+}]_{cyt}$ oscillations driven by electric activity were dampened by the nuclear envelope [20].

The aim of the present work is to study nuclear Ca^{2+} signals in β-cells within intact islets by bioluminescence imaging of targeted aequorins. We find that $[Ca^{2+}]_{nuc}$ was increased in response to stimulation with high glucose. Some individual cells of the islet showed synchronous $[Ca^{2+}]_{nuc}$ oscillations which resemble those triggered by electrical activity in cytosol. $[Ca^{2+}]_{nuc}$ oscillations were dependent of Ca^{2+} entry through L-type Ca^{2+} channels at the plasma membrane but were not affected by emptying of intracellular Ca^{2+} stores. The existence of this nuclear synchronization indicates that β-cells within each intact islet may also behave as a syncitium in terms of nuclear Ca^{2+} signals and the regulation of nuclear Ca^{2+}-responsive processes. This is suggested by the fact that gap-junction-mediated coupling between β-cells is a requirement for an appropriate gene expression [25].

2. Material and methods

2.1. Materials

Twelve-weeks old, male Balb/c mice were kept at the university animal facilities under 12 h light–12 h dark conditions and fed ad libitum. Culture medium, sera and antibiotics were obtained from GIBCO. Fura-2 and fluo-3 were purchased from Molecular Probes Europe (Leiden, The Netherlands). Nuclear and cytosolic aequorin cDNAs were obtained from Molecular Probes and cloned in the pHSVpUC plasmid as previously reported [26].

2.2. Pancreatic islet culture and expression of aequorins

Experiments were conducted under the guidelines of the Commission d'Ethique d'Expérimentation Animale of the University of Louvain School of Medicine. Islets were isolated by collagenase digestion of the pancreas followed by selection by hand [27]. Islets were infected with herpes simplex virus type 1 (HSV1) delivering the targeted aequorins ($1–3 \times 10^3$ infectious virus particles per islet) [26]. Infected islets were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics for 24 h. Packaging and titration of the pHSVnuAEQ (nuclear) and pHSVcytAEQ (cytosolic) viruses was performed as reported previously [26].

2.3. Fura-2 imaging of cytosolic calcium

Cultured pancreatic islets were incubated with fura-2/AM (4 μM) for about 1 h at room temperature in standard medium of the following composition (mM): NaCl, 145; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; HEPES, 10; pH, 7.4; glucose, 10. Then, islets were washed with the same medium and placed in a chamber thermostated at 37 °C in the stage of an inverted microscope (Nikon Diaphot). Islets were then perifused with prewarmed (37 °C) bicarbonate-buffered solution of the following composition (in mM): NaCl, 120; KCl, 4.8; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 24; glucose, 3. The solution was continuously gassed with 94% O_2–6% CO_2 to maintain pH at 7.4. Islets were epi-illuminated alternately at 340 and 380 nm, and light emitted above 520 nm was measured using a Magical Image Processor (Applied Imaging, Newcastle, UK). Pixel by pixel ratios of consecutive frames were produced and $[Ca^{2+}]_i$ was estimated from these ratios by comparison with fura-2 standards. Other details have been reported earlier [4,28].

2.4. Confocal microscopy of cytosolic calcium

Swiss albino OF1 male mice (8–10 weeks old) were killed by cervical dislocation according to national guidelines. Pancreatic islets of Langerhans were isolated by collagenase digestion as previously described [27] and loaded with 5 μM Fluo-3 AM for at least 1 h at room temperature. Loaded islets were kept in a medium containing (mM): NaCl, 115; $NaHCO_3$, 25; KCl, 5; $MgCl_2$, 1.1; NaH_2PO_4, 1.2; $CaCl_2$, 2.5; HEPES; 2,5; bovine serum albumin, 1%; D-glucose, 5 mM. This solution was kept at pH 7.35 by continuous gassing with 95% O_2–5% CO_2. Islets were perfused with a modified Ringer solution containing (mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 1.1 $MgCl_2$ and 2.5 $CaCl_2$; pH 7.35, when gassed with 95% O_2 and 5% CO_2. Experiments were performed at 34 °C. Calcium measurements were performed in individual cells with a Zeiss Pascal 5 confocal microscope equipped with

a ×40 oil immersion objective (N.A., 1.3). Images were collected at 2 s intervals and treated with the Zeiss LSM software package. Results are expressed as the change in fluorescence ΔF expressed as the percentage of the basal fluorescence (F_0) observed in absence of stimulus. α and β-cells within the islets were identified by their $[Ca^{2+}]_i$ oscillatory pattern in 3 and 11 mM glucose, respectively [29].

2.5. Bioluminescence imaging of islets expressing targeted aequorin

Islets expressing apoaequorins were incubated for 1–2 h at room temperature in standard medium with 1 μM coelenterazine. Then the islets were placed into a incubation chamber thermostated to 37 °C mounted in a Zeiss Axiovert 100 TV microscope and perfused at 5–10 ml/min with the same bicarbonate-buffered detailed above with low (3 mM) glucose. At the times indicated, islets were perfused with the same medium containing high glucose (11 mM), high K^+ (30 mM, replacing isosmotically Na^+) or other test solutions, prewarmed at 37 °C. At the end of each experiment, cells were permeabilized with 0.1 mM digitonin in 10 mM $CaCl_2$ to release all the residual aequorin counts. Bioluminescence images were taken with a Hamamatsu VIM photon counting camera handled with an Argus-20 image processor and integrated for 10 s periods. Photons/cell in each image were quantified using the Hamamatsu Aquacosmos software. Data were first quantified as rates of photoluminescence emission/total c.p.s remaining at each time and divided by the integration period (L/L_{TOTAL} in s^{-1}). Emission values of less than 4 c.p.s or 40 c.p.s for individual cells and whole islets respectively, were not used for calculations. Calibrations for $[Ca^{2+}]$ are shown with the Figures. More details about bioluminescence imaging of aequorin have been reported previously [24]. For calculation of oscillation indexes the differences (in absolute value) between each L/L_{TOTAL} value and the following one were added and divided by the total number of measurements during the integration period. This parameter is sensitive to both the amplitude and the frequency of oscillations [20,24,30]

2.6. Batch luminescence measurements

In some experiments batch measurements using 2–5 islets were performed in order to increase sensitivity. For these purposes, islets expressing nuclear aequorin were incubated with coelenterazine for 2 h at room temperature. Then 2–5 islets were placed into the incubation chamber of a luminometer constructed by Cairn Research. The islets were covered with a 70 μm mesh nylon net to keep them in place and they were perfused with prewarmed (37 °C) standard medium and photoluminescence emission measured using a photon-counting phototube. Counts were integrated at 1 s intervals and $[Ca^{2+}]_{nuc}$ or $[Ca^{2+}]_{cyt}$ was estimated as previously reported [20,31]. For $[Ca^{2+}]_{cyt}$ measurements, islets were infected with a virus delivering an aequorin construct fused with the luciferase structural gene. The resulting fusion protein locates at the cytosol, but is excluded from the nucleus (data not shown).

3. Results

Adequate expression of the targeted aequorins requires 24 h of culture. Therefore, we investigated first whether the infection and the subsequent culture for 1 day in vitro (DIV) influences the normal cytosolic calcium responses of pancreatic islets. For this end, islets were infected with the virus delivering targeted probes and cultured for 24 h. Then, they were loaded with fura-2 or fluo-3 and subjected to either conventional fluorescence microscopy (Fig. 1A) or confocal microscopy (Fig. 1B). Fig. 1A shows the cytosolic calcium responses imaged in a single fura-2 loaded islet. Increasing the glucose (GLU) concentration from 3 to 11 mM (GLU) induced, after a 2–4 min lag, a rapid increase of $[Ca^{2+}]_{cyt}$ followed by a plateau that was sometimes associated with oscillations occurring over the plateau. When imaged with confocal microscopy, these $[Ca^{2+}]_{cyt}$ oscillations were more apparent in some individual cells (Fig. 1B), with clear signs

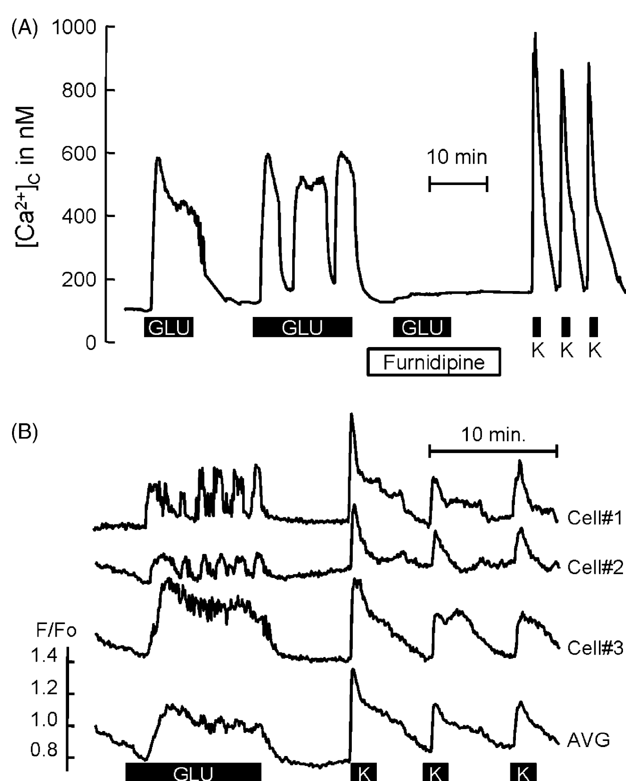

Fig. 1. Cytosolic calcium responses in cultured pancreatic islets. Freshly isolated mouse pancreatic islets were infected with herpes virus delivering nuclear aequorin and cultured for 24 h. Then, the islets were loaded with fura-2 (A) or fluo-3 (B) and $[Ca^{2+}]_{cyt}$ was monitored by conventional fluorescence (A) or confocal (B) microscopy. A. The effects of increasing glucose concentration from 3 to 11 mM (GLU) and of high K^+ (K) was studied. Furnidipine was used at 1 μM. The results shown are representative of 3 (A) and 4 (B) similar experiments.

of synchronization among them, as previously documented [4,29]. Return to low glucose concentration restored resting $[Ca^{2+}]_{cyt}$ values. A second stimulation with glucose provoked a second, $[Ca^{2+}]_{cyt}$ increase, which was often associated with slow $[Ca^{2+}]_{cyt}$ oscillations (Fig. 1A). The dihydropyridine VOCC blocker furnidipine prevented the effects of stimulation with high glucose (Fig. 1A). Removal of extracellular calcium had the same effect (not shown). Depolarization with high K^+ provoked larger $[Ca^{2+}]_{cyt}$ increases (Fig. 1A and B), which were also prevented by Ca^{2+}-free medium or furnidipine (not shown). Taken together, these data indicate that islets cultured for 1 DIV show the same behavior in terms of $[Ca^{2+}]_{cyt}$ responses to glucose and depolarization than freshly obtained pancreatic islets.

We asked next whether bioluminescence imaging of targeted aequorins would enable monitoring dynamics of $[Ca^{2+}]_{nuc}$ in pancreatic islets. Fig. 2A shows the changes of $[Ca^{2+}]_{nuc}$ in an islet stimulated first with high glucose and then with high K^+. Four representative images of photonic emissions superimposed to the bright field image of the pancreatic islet are also shown. In the resting condition the rate of photon emission was very low (*Resting* in Fig. 2A). Increasing glucose concentration from 3 to 11 mM induced, after a 2–3 min lag, a rise in the rate of photonic emissions (*Glucose*) that returned to resting values when glucose was again decreased to 3 mM. Depolarization with high K^+ induced a much larger increase in the rate of photonic emissions (*High* K^+). Finally, all the remaining aequorin was burned by permeabilization of the islet with digitonin and exposure to excess (10 mM) Ca^{2+} (*Digitonin*). Integration of the whole aequorin counts is required for calibration of the signal [31]. This maneuver also revealed all the (infected) individual cells showing large levels of photonic emissions, thus enabling analysis of individual cells within the islet (see supplementary movie). Fig. 2B compares the average changes of $[Ca^{2+}]_{nuc}$ in the whole islet and in four representative individual cells within the same islet. At the single cell level the response to high glucose include $[Ca^{2+}]_{nuc}$ oscillations that were not apparent either before increasing glucose concentration or after returning to low glucose concentration. The oscillatory activity remained as long as perfusion with high glucose was maintained. In Fig. 2C the oscillatory activity has been quantified using the oscillation index, a parameter which reflects both the amplitude and frequency of oscillations [24,30]. The oscillation index increased about 20 fold on stimulation with high glucose and returned back near the resting levels when glucose was decreased to the low concentration (Fig. 2C).

It has been established previously that high glucose induces $[Ca^{2+}]_{cyt}$ oscillations that are synchronized among pancreatic β-cells of the same islet [4,29], and we have shown here that this behavior is preserved in islets cultured during 1 DIV (Fig. 1). We asked then whether the glucose-induced $[Ca^{2+}]_{nuc}$ oscillations could also be synchronized. Fig. 3 shows an experiment in which $[Ca^{2+}]_{nuc}$ was monitored simultaneously in two individual islets (a and b) that become attached during culture. The effects of two consecutive stim-

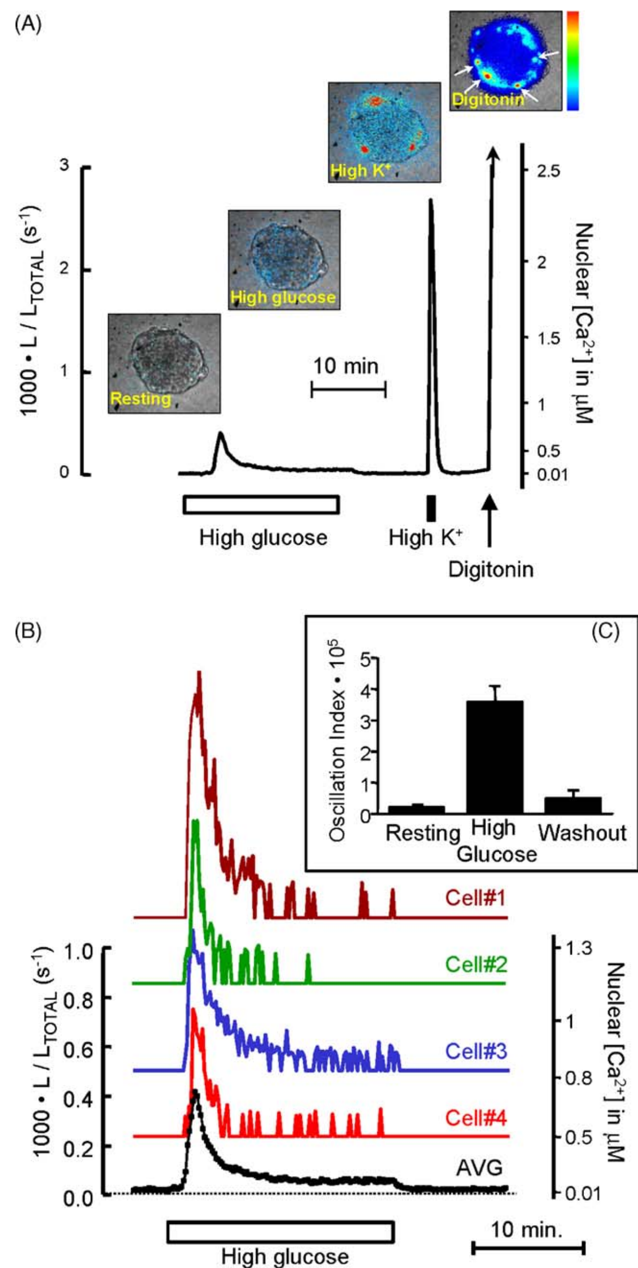

Fig. 2. Nuclear calcium responses of cultured pancreatic islets monitored by bioluminescence imaging of targeted aequorin. Islets were infected with nuclear aequorin as in Fig. 1, cultured for 24 h and reconstituted for 2 h at 20 °C with 1 μM coelenterazine h. (A) Nuclear calcium dynamics of a single pancreatic islet stimulated sequentially with high glucose (11 mM) and high K^+ medium (75 mM). The pictures on top show photon 10 s-counting images superimposed over a bright field image in resting conditions, during high glucose stimulation and during stimulation with high potassium. The pseudocolor scale is shown at right. The experiment was terminated by permeabilization with digitonin (100 mg/ml) in medium containing high calcium (10 mM). (B) Response to high glucose in four individual cells (shown by arrows in A) of the same islet. (C) Comparison of the oscillation indexes (OI; computed for 5 min-periods) before, during stimulation with high glucose and after returning to control medium. Mean ± S.E. of 4 values is shown. The OI during stimulation was computed 5 min after beginning perfusion with 11 mM glucose. Data are representative of 53 individual cells studied in four independent experiments.

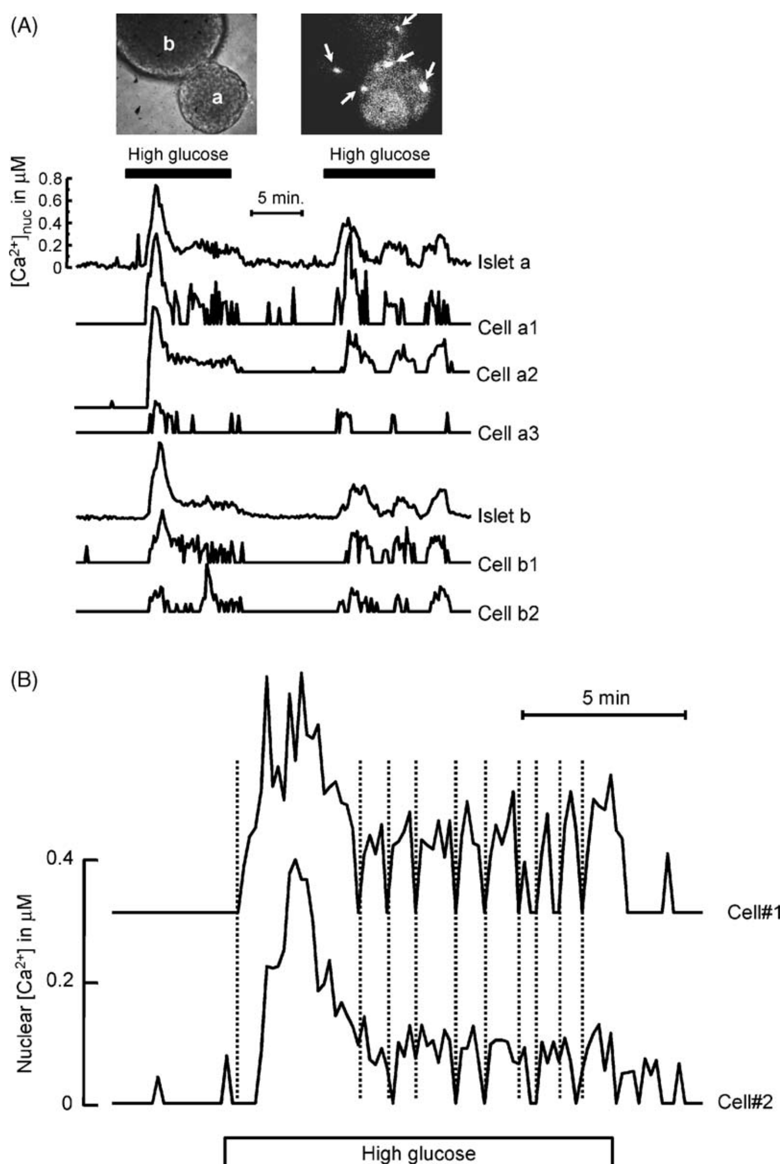

Fig. 3. Synchronization of nuclear calcium oscillations. (A) Effects of two sequential stimulation with high glucose on nuclear calcium dynamics in two islets (a and b). The traces corresponding to the average of each islet and to five individual cells is shown. (B) Detailed comparison of $[Ca^{2+}]_{nuc}$ oscillations induced by high glucose in two individual cells. Results are representative of three independent experiments.

ulations with glucose in both islets and in several individual cells within them (a1–a3 and b1–b2) are shown. The bright field (left) and photonic emissions (right) images are shown on top. The first stimulation with glucose induced a biphasic increase of $[Ca^{2+}]_{nuc}$ in both islets whereas the second stimulation induced a $[Ca^{2+}]_{nuc}$ increase composed of three slow waves. The behavior of the individual cells illustrates further the synchronization among cells of the same islet. Notice that the slow oscillations occurring during second glucose stimulation were also observed at the level of the cytosol (Fig. 1A). Fig. 3B illustrates further a quite extensive (though not absolute) synchronization among two cells within the same islet.

It has been established that high glucose-induced cytosolic calcium oscillations are due to electrical activity and opening of voltage-gated L-type Ca^{2+} channels (VOCC) [2–4]. We next investigated the source of Ca^{2+} for the $[Ca^{2+}]_{nuc}$ changes induced by high glucose. Fig. 4 shows that removal of extracellular Ca^{2+} abolished the effects of high glucose on $[Ca^{2+}]_{nuc}$ and that these effects resumed on re-addition of Ca^{2+}. The effects of glucose on $[Ca^{2+}]_{nuc}$ were also inhibited by furnidipine but not by emptying the intracellular calcium stores with thapsigargin (Fig. 4), suggesting that the intracellular Ca^{2+} stores do not participate in the generation of the $[Ca^{2+}]$ responses.

Fig. 5 compares the $[Ca^{2+}]_{nuc}$ responses to sequential stimulation with high glucose and with high K^{+}. Two consecutive applications of high glucose provoked a reproducible aequorin consumption of about 5% (Fig. 5A). In contrast, the first high K^{+} stimulus consumed much more aequorin than the following ones (25% versus 5%; Fig. 5A). As a consequence,

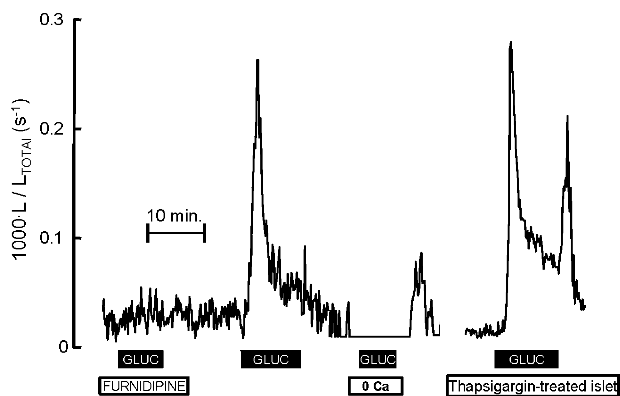

Fig. 4. The high-glucose-induced $[Ca^{2+}]_{nuc}$ increase is due to activation of voltage-operated calcium channel and not to release of calcium from intracellular stores. Pancreatic islets were infected, cultured and handled as in Fig. 2. The effects of furnidipine (1 μM), Ca^{2+} removal and thapsigargin pre-treatment (1 μM during 1 h) are shown.

the estimate of the $[Ca^{2+}]_{nuc}$ response (shown as L/L_{TOTAL} in Fig. 5B) was much larger for the first high-K^+ stimulus than for the two following. Conversely, both fura-2 (Fig. 1A) and fluo-3 (Fig. 1B) reported reproducible $[Ca^{2+}]_{cyt}$ responses to repetitive stimulations with high K^+. Fig. 5C compares the estimates of $[Ca^{2+}]_{cyt}$ and of $[Ca^{2+}]_{nuc}$ for the first and the second high K^+ stimulus obtained in several experiments. $[Ca^{2+}]_{cyt}$ was measured using both approaches, fura-2 and aequorin. Measurements using aequorins reported that the first response to K^+ was about four times larger than the following ones. Despite these differences between consecutive K^+ pulses, Ca^{2+} values in the cytosol and in the nucleus were similar for each stimuli, suggesting that changes of $[Ca^{2+}]_{cyt}$ are faithfully transmitted to the nucleus (see Section 4).

4. Discussion

Calcium signals within cells are sculpted in time and space in the form of calcium oscillations, waves and microdomains. This allows specific regulation of different cell functions, which may be located in different cell compartments, for example, regulated exocytosis in the subplasmalemmal region, respiration in mitochondria or gene transcription in nucleus. Measurements of subcellular calcium concentration inside discrete organelles or functional domains has been hampered by technical constrains, but development of targeted (protein) probes is paving the way for this analysis. For example, the bioluminescent probe aequorin has been used to monitor calcium changes in different organelles including the nucleus [23], even though single-cell imaging of targeted aequorin in discrete organelles has been a very difficult task achieved only in a few cases [20,24,32]. Here we have used this novel approach to gain new insights into the homeostasis of nuclear calcium in whole pancreatic islets and its regulation by glucose. As demonstrated by in vivo studies [33], intact whole islets constitute a preparation closer to the physiological situation than isolated single pancreatic cells or islet-derived cell lines, specially when considering glucose-induced Ca^{2+} signals.

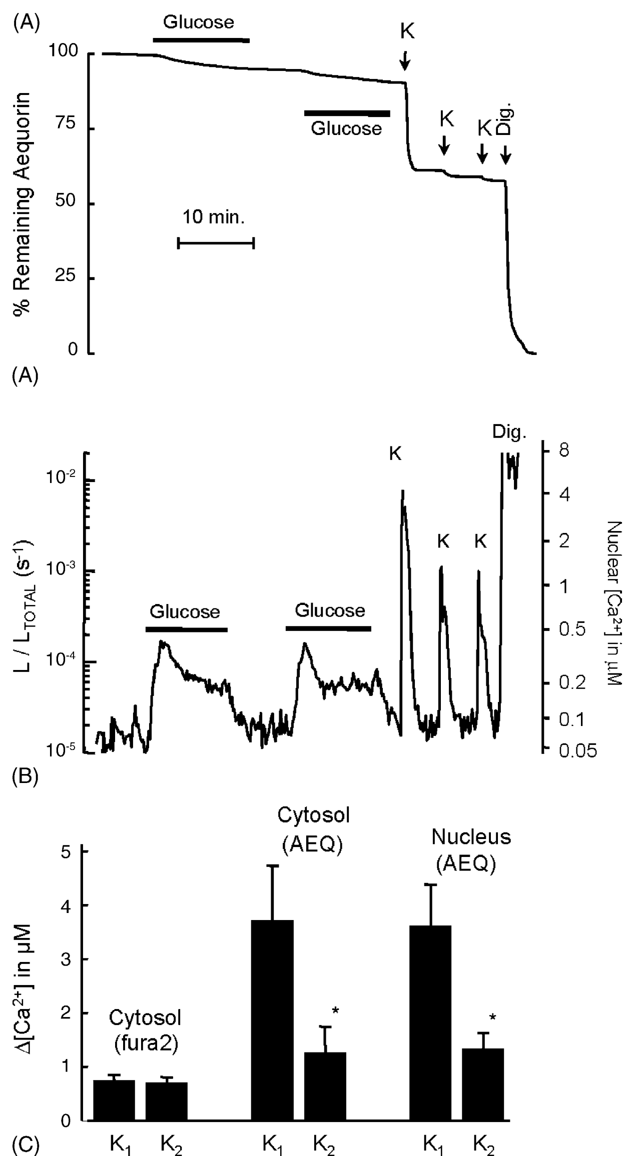

Fig. 5. Comparison of the $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{nuc}$ responses to repetitive stimulation with high glucose or high K^+. Pancreatic islets were infected, cultured and handled as in Fig. 2. (A and B) Time course of the effects of the stimuli on aequorin content (A; remaining cps) or the calibrated signal (B, L/L_{TOTAL}; note logarithmic scale). Data are representative of four similar experiments. (C) Comparison of the $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{nuc}$ responses to repetitive stimulation. The values (mean ± S.E., $n = 7$) reported by fura-2, cytosolic aequorin and nuclear aequorin are shown. $^{*}p < 0.05$ (Student's t-test). The values for $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{nuc}$ were not significantly different.

We find that stimulation by high glucose produced an increase of $[Ca^{2+}]_{nuc}$, which often took the form of oscillations that were synchronized in numerous single cells within the islet of Langerhans. This $[Ca^{2+}]_{nuc}$ response pattern resembles the $[Ca^{2+}]_{cyt}$ response, synchronous and oscillatory, responsible for the pulsatile secretion of insulin [6–8]. Thus, our results suggest that the cytosolic calcium

oscillations propagate into the nucleus to generate a similar synchronic pattern of nuclear calcium oscillations. Signal synchronization is mainly the result of the gap-junction-mediated coupling network among the β-cell population. Interestingly, the lack of coupling, which leads to synchronicity malfunction, can affect not only insulin secretion [34] but also insulin gene expression [25]. Thus, synchronization of nuclear function could be critical for β-cell function.

It has been reported that Ca^{2+} signals participate in the regulation of some transcription factors [35,36] as well as IEGs and insulin gene expression [12–15,37] in the pancreatic β-cell. This regulation occurs at the transcriptional level and can be modified or abolished by pharmacological interference with the electrical activity and Ca^{2+} signals in the pancreatic β-cell [12–15]. Thus, the nuclear calcium oscillatory pattern reported here could lead to a pulsatile pattern of insulin gene expression, or any other genes involved in β-cell function. This is not entirely new, as we have shown a pulsatile pattern for the transcription of genes coding for other hormones or neuropeptides that are secreted in a pulsatile manner [38,39]. In addition, in both cases, gene transcription dynamics may be secondary to an oscillatory calcium pattern driven by electrical activity [38–42]. Thus, the bursting pattern of electrical activity could be translated to a pattern of transcriptional activity by means of the nuclear calcium oscillatory pattern found here.

Whether nuclear and cytosolic calcium signaling are differentially regulated is a matter of controversy. Depending on the cell type studied or the technical approach employed, evidences supporting either one view or the other have accumulated [18–22]. In the case of the pancreatic β-cell, a previous study concluded that glucose induced parallel changes in both $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{nuc}$ of isolated individual cells from the ob/ob mouse [16]. However, it has been reported recently that activation of K_{ATP} channels located at the nuclear envelope could lead to changes of $[Ca^{2+}]_{nuc}$ that may modulate nuclear function [17]. In addition, some nutrients may induce Ca^{2+} signals of different amplitude in the cytosol and the nucleus of isolated pancreatic β-cells [43]. Here we have found in intact islets that high glucose induces a similar pattern of $[Ca^{2+}]$ oscillations in the cytosol and in the nucleus. In addition, the response to glucose was dependent on Ca^{2+} entry through VOCCs in both the cytosol and the nucleus and not affected by emptying intracellular calcium stores with thapsigargin. Thus, our observations using targeted aequorins support the view that glucose-induced $[Ca^{2+}]_{nuc}$ changes are primarily relayed by cytosolic Ca^{2+} signals, and mainly depend on extracellular Ca^{2+} entry rather than intracellular inputs. Therefore, it is likely that the contribution of intracellular stores during glucose signaling, represents a minor fraction of the total Ca^{2+} change in the nucleus. The discrepancies with previous reports indicating a involvement of a nuclear Ca^{2+} store in $[Ca^{2+}]_{nuc}$ changes [17] may arise from different reasons. First, while the technique used in these previous studies, spot confocal microscopy, excels in measuring local Ca^{2+} signals and intracellular Ca^{2+} release sites in spatial domains at the submicron level, this approach may fail to give measurements of average changes in large intracellular compartments such as the nucleoplasm [44]. Thus, the Ca^{2+} signals previously reported in the β-cell nucleus could have a particular local and restricted function in these microdomains but would not probably represent a significant change for the nucleoplasm Ca^{2+} homeostasis, specially when considering massive Ca^{2+} entries from the cytosol. Second, these previous studies have used isolated cultured cells rather than intact islets. It is known that important differences exist between both preparations in terms of Ca^{2+} signals, being the latter model closer to the physiological situation as evidenced by in vivo studies [29,33,45].

Repetitive stimulation by depolarization with high K^+ showed that both the cytosolic and the nuclear aequorins reported Ca^{2+} signals of the same magnitude, but in both cases the response to the first stimulus was larger than the response to the following ones. This behaviour is at variance with the findings in rat pituitary cells and bovine chromaffin cells, where repeated stimulations yielded reproducible $[Ca^{2+}]_{nuc}$ increases [24,28]. As proposed before to explain a similar output in mitochondria of chromaffin cells [24,28,31], these results could be interpreted as the result of the generation of high Ca^{2+} microdomains in β-cells during stimulation with high glucose. This interpretation is complicated, however, by the observation of the same behavior with both the cytosolic and the nuclear aequorins, which would require generation of high Ca^{2+} domains both in the cytosol and in the nucleoplasm. Whether such microdomains may be important for insulin secretion and gene expression remains to be established.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ceca.2005.06.029.

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