Nutrients Induce Different Ca^{2+} Signals in Cytosol and Nucleus in Pancreatic β-Cells

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Specific activation of Ca^{2+}-dependent functions is achieved by the particular dynamics and local restriction of Ca^{2+} signals. It has been shown that changes in amplitude, duration, or frequency of Ca^{2+} signals modulate gene transcription. Thus, Ca^{2+} variations should be finely controlled within the nucleus. Although a variety of mechanisms in the nuclear membrane have been demonstrated to regulate nuclear Ca^{2+}, the existence of an autonomous Ca^{2+} homeostasis within the nucleus is still questioned. In the pancreatic β-cell, besides their effect on insulin secretion, Ca^{2+} messages generated by nutrients also exert their action on gene expression. However, the dynamics of these Ca^{2+} signals in relation to nuclear function have been explored little in islet cells. In the current study, Ca^{2+} changes both in the nucleoplasm and in the cytosol of INS-1 and pancreatic β-cells were monitored using spot confocal microscopy. We show that nutrients trigger Ca^{2+} signals of higher amplitude in the nucleus than in the cytosol. These amplitude-modulated Ca^{2+} signals transmitted to the nucleus might play an important role in the control of gene expression in the pancreatic β-cell. *Diabetes* 53 (Suppl. 1):S92–S95, 2004

The enormous versatility and specificity of Ca^{2+} to relay information in the cell can be sustained by a complex system that ensures precise codification and spatial restriction of Ca^{2+} signals (1). Constraint of Ca^{2+} transmission generates local domains, which prevent undesired activation of Ca^{2+}-dependent processes, while amplitude, duration, and/or frequency regulation permit a code of Ca^{2+} signals, improving the specificity of cell messages (1). Gene expression is a cell function that is remarkably sensitive to the complex signaling attributes of this second messenger. Amplitude, frequency, and duration of Ca^{2+} signals can control the magnitude of gene expression (1–3). Furthermore, differential transcriptional response has been reported depending on the source location of these signals—namely the nucleus or the cytoplasm (2,4). Actually, nuclear Ca^{2+} alone without cytosolic inputs can activate specific transcription factors in neurons (5). Because nuclear Ca^{2+} plays an important role in controlling nuclear function, it is expected that an autonomous and precise Ca^{2+} homeostasis exists within the nucleus.

Although the regulation of nuclear Ca^{2+} is an issue that has attracted the attention of many investigators, data have not been conclusive (6). Some studies have demonstrated the presence of functional channels and mechanisms in the nuclear membrane of isolated nuclei such as inositol 1,4,5-trisphosphate and ryanodine receptors and Ca^{2+}-ATPases, which regulate the release and uptake of Ca^{2+} in the nucleoplasm and nuclear envelope (7,8). These observations have led several groups to postulate that the nucleus is a dynamic Ca^{2+} pool, which could account for the cytosolic-nuclear Ca^{2+} gradients observed in some systems (6). However, independent Ca^{2+} homeostasis within the nucleus different from that in the cytosol has been disputed by the presence of the nuclear pores, which would offer no barrier to Ca^{2+} diffusion, allowing rapid Ca^{2+} equilibration between both compartments (6,9).

In pancreatic β-cells, Ca^{2+} also signals several functions by means of the above-mentioned spatial and temporal features. Nutrients produce intracellular Ca^{2+} signals that activate insulin secretion but also induce the transcription of several Ca^{2+}-dependent genes such as insulin or immediate early genes such as c-*fos*, *nur*-77, or c-*myc* (10–13). Although Ca^{2+} dynamics and its spatial distribution in the β-cell have been further studied in relation to the exocytotic process (14,15), little has been examined about the pathways that link extracellular messages, Ca^{2+} signals, and nuclear function. In this regard, it would be particularly interesting to analyze nuclear and cytosolic Ca^{2+} dynamics, especially in light of recent findings that have demonstrated the presence of ATP-dependent K^+ (K_{ATP}) channels on the nuclear envelope of pancreatic β-cells, whose blockage triggers nuclear Ca^{2+} transients that affect nuclear function (8). In the current study, we used spot confocal microscopy to evaluate the dynamics of Ca^{2+} signals elicited by glucose, oleate, and K^+ in the nucleus and in the cytoplasm of INS-1 cells and pancreatic β-cells. We show that nutrients produce Ca^{2+} elevations of higher amplitude in the nucleus, probably by interaction with nuclear Ca^{2+} release mechanisms.

RESEARCH DESIGN AND METHODS

Cell isolation and culture. Islets from adult (8–10 weeks old) Swiss albino male mice (OF1) killed by cervical dislocation in accord with our institutional guidelines were isolated and then dispersed into single cells following a procedure described elsewhere (14). Isolated cells were cultured in RPMI-1640 medium (Invitrogen, Barcelona, Spain) containing 5.6 mmol/l glucose for

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K_{ATP} channel, ATP-dependent K^+ channel.

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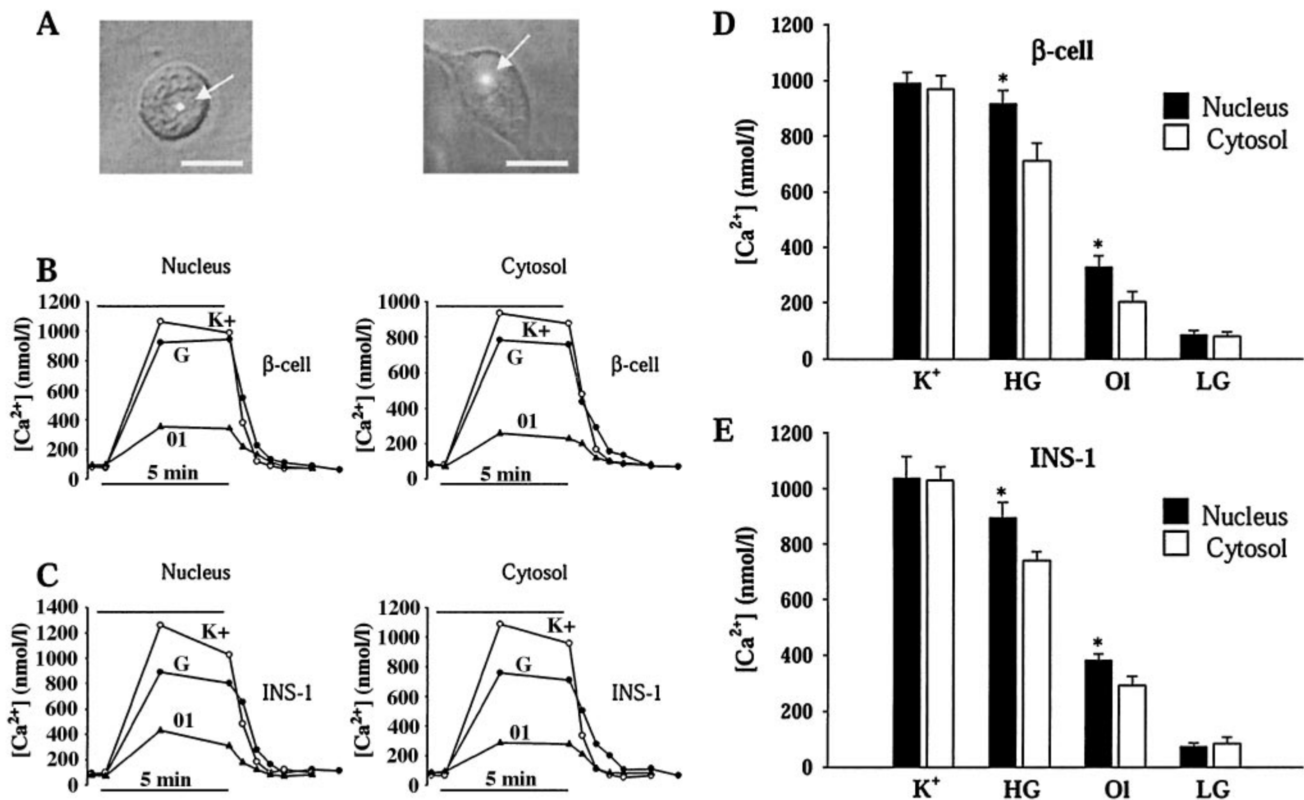

FIG. 1. Nuclear and cytosolic [Ca^{2+}] changes in cultured cells induced by different stimuli. *A*: Hybrid phase-contrast images of a pancreatic β-cell (left) and an INS-1 cell (right) showing the location of the fluorescence spot focused in the nucleus (bar = 10 μm). *B* and *C*: Isolated cells were stimulated with 40 mmol/l K^+ (○), 16.7 or 22 mmol/l glucose (G) (●) for β-cells or INS-1 cells, respectively, and 0.5 mmol/l oleate in 3 mmol/l glucose (Ol) (▲) for the time indicated by the line. Ca^{2+} changes in the nucleoplasm or in the cytoplasm of different pancreatic β-cells (*B*) and INS-1 cells (*C*) were monitored by spot confocal microscopy. These records are representative of more than seven cells for each condition from at least three different preparations. *D* and *E*: The amplitude of these [Ca^{2+}] changes, measured as the mean [Ca^{2+}] increase during the response, is shown for β-cells (*D*) and INS-1 cells (*E*). Data are means ± SE (P* < 0.05, unpaired *t* test). HG, high glucose (16.7 mmol/l for β-cells and 22 mmol/l for INS-1 cells); LG, low glucose (3 mmol/l). Means were obtained from 7–19 cells for each condition.**

12–16 h before experiments. Immunocytochemistry analysis of cultured cells revealed that around 80% of the preparation was insulin-producing cells (16). In addition, we selected β-cells according to their large size and low nuclear/cytoplasmic ratio compared with other islet cell types (16). Thus, we expected the majority of cells that we studied in the experiments to be pancreatic β-cells.

Cells from the pancreatic β-cell line INS-1 were grown in monolayer cultures as follows (12). When cells reached 80% confluence, they were washed twice with Krebs-Ringer bicarbonate buffer at pH 7.4, containing 5.6 mmol/l glucose and 0.07% BSA. Cells were subsequently incubated 2 more days in RPMI-1640 medium containing 5 mmol/l glucose and 10% FCS, but the second-day cells were trypsinized and transferred to poly-L-lysine–treated coverslips.

Fatty acids were prepared as previously described (12). Defatted BSA was used as a control condition (12).

Spot confocal microscopy in single islet cells. Cells were loaded with 2 μmol/l FLUO-3 acetoxymethyl ester (Molecular Probes, Leiden, the Netherlands) for 30 min at room temperature. Under these conditions, a homogenous distribution of the dye in the cytosol and in the nucleus was confirmed in the majority of cells (~83%) by imaging cultures as described earlier (14). Cells were perfused at a rate of 0.5 ml/min in a Krebs-Ringer buffer (in mmol/l): 119 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 5 HEPES, and 3 glucose gassed with a mixture of 95% O_2, 5% CO_2 (pH = 7.4). Ca^{2+} concentration ([Ca^{2+}]) changes were measured using spot confocal microscopy, which excels in measuring localized Ca^{2+}-induced fluorescence elevations because of its remarkable signal-to-noise ratio over conventional systems (8,14,17). The spot illumination-detection configuration has been previously described in detail (14,17). Briefly, a laser-illuminated pinhole (10 μm) was focused onto a spot through the objective either on the nucleus or the bulk cytosol of an isolated islet cell (Fig. 1*A*). The full-width half-maximal dimension of the illumination spot is 0.6 μm, whereas the depth of field is 1.1 μm. Thus, the predicted detection volume is about $0.6 \times 0.6 \times 1.1\ \mu m^3$, which is ~1.2 and 0.075% of the nuclear and cell volume (assuming a radius of 2 and 5 μm, respectively). Changes in fluorescence in this small volume were detected with a photodiode (HR008; UDT, Hawthorne, CA), which was connected to an Axopatch-200A amplifier (50 GΩ feedback; Axon Instruments, Foster City, CA). The laser illumination time was 30 ms. We minimized the number of measurements on the same spot to prevent probe photobleaching. Under our experimental conditions and sampling rate, bleaching was insignificant. Autofluorescence was determined to be <2%. Nuclear Ca^{2+} was measured by positioning the spot in the center of the nucleus, identified easily by transmitted light microscopy, whereas cytosolic Ca^{2+} was recorded by placing the spot in a cytosolic area adjacent to the nuclear periphery (separation ≈ 1 μm). Distances were measured from acquired images using a charge coupled device (CCD) camera (14).

FLUO-3 has higher absorbance and fluorescence emission in the nucleoplasm than in the cytoplasm (18). Consequently, a specific calibration curve of the fluorescence for each cellular compartment is required to compare Ca^{2+} signals. We estimated in situ the dissociation constant (K_d) for FLUO-3 in two different pools of cells where the illumination spot was focused either in the nucleoplasm (n = 8 for β-cells and n = 8 for INS-1 cells) or in the cytoplasm (n = 9 for β-cells and n = 8 for INS-1 cells). After a brief treatment with 0.5 μmol/l digitonin for 3 min (Sigma, Madrid, Spain), cells were then exposed to an intracellular solution containing different [Ca^{2+}] buffered with EGTA using a commercial calibration kit (Molecular Probes), but K^+ was adjusted to a concentration of 125 mmol/l. This procedure did not produce leakage of the fluorescent probe. Calibrations gave a K_d in the cytosol of 0.66 ± 0.05 and 0.61 ± 0.08 μmol/l and a K_d in the nucleoplasm of 0.51 ± 0.04 and 0.48 ± 0.05 μmol/l (means ± SE) for β-cells and INS-1 cells, respectively. [Ca^{2+}] was calculated according to the calibration equation: $[Ca^{2+}] = K_d (F - F_{min})/(F_{max} - F)$. Fluorescence was measured after each experiment either in the nucleoplasm or in the cytoplasm by exposure of cells to the extracellular solution with saturating [Ca^{2+}] (F_{max}) or absence of Ca^{2+} (F_{min}) (0 mmol/l Ca^{2+} plus 2 mmol/l EGTA), respectively, in the presence of 10 μmol/l ionomycin.

Nuclei preparation. Nuclei were isolated according to procedures published earlier (8). Briefly, isolated islet cells were suspended in an intracellular solution (in mmol/l: 125 KCl, 2 K_2PO_4, 40 HEPES, 0.1 $MgCl_2$, at pH 7.2, and 100 nmol/l Ca^{2+}) and disrupted by brief sonication. Nuclei were separated by centrifugation, resuspended in the intracellular solution, and then allowed to attach onto glass chambers. Isolated nuclei were loaded with the Ca^{2+} probe Calcium Green-1 dextran (30 μg/ml; 30 min at 4°C; Molecular Probes). For further details, see Quesada et al. (8).

RESULTS

Nutrients trigger Ca^{2+} signals of higher amplitude in the nucleoplasm. Spot confocal microscopy has been successfully applied in pancreatic β-cells, skeletal muscle cells, and neurons to monitor Ca^{2+} dynamics in local intracellular domains (8,14,17). We used this technique to measure [Ca^{2+}] changes either in the cytosol or in the nucleus of INS-1 cells and pancreatic β-cells exposed for 5 min to 40 mmol/l K^+, 16.7 or 22 mmol/l glucose, and 0.5 mmol/l oleate in 3 mmol/l glucose (Fig. 1*A*–*C*). All stimuli led to a [Ca^{2+}] rise in both intracellular compartments. When we examined the Ca^{2+} responses, we found no differences in duration between those generated in the nucleus and in the cytosol in any of the two types of insulin-secreting cells ($P > 0.05$; unpaired t test). However, significant differences in amplitude were obtained when comparing nuclear and cytosolic [Ca^{2+}] mean values in both kinds of cells (Fig. 1*D* and *E*). Whereas K^+ induced similar elevations of [Ca^{2+}] in both intracellular compartments, glucose and oleate produced Ca^{2+} signals of higher amplitude in the nucleus (Fig. 1*D* and *E*).

It has been shown that Ca^{2+} probes exhibit enhanced absorbance and fluorescence emission in the nucleoplasm (18). Accordingly, we performed two independent in situ calibrations in the nucleus and in the cytosol to get comparable values between both cell locations (9) (see RESEARCH DESIGN AND METHODS). The differences in amplitude observed between the two compartments cannot arise from calibration artifacts, otherwise such differences should be reflected concomitantly with all the stimuli and not just with glucose and oleate. Similar [Ca^{2+}] values in the nucleus and in the cytosol were obtained at rest with 3 mmol/l glucose as well as with stimulatory conditions with 40 mmol/l K^+. These data also support the reliability of the calibration, because Ca^{2+} is expected to be equilibrated between both compartments in such conditions in β-cells (19) (see DISCUSSION). Furthermore, the same observations have been confirmed in two different kinds of insulin-producing cells with similar phenotypes and physiological responses.

Potential involvement of nuclear Ca^{2+} release mechanisms. Plasma membrane depolarization, either by a rise in extracellular K^+ or by stimulatory glucose as a result of an ATP/ADP increase and K_{ATP} channel closure, leads to massive Ca^{2+} influx in the cytosol by activation of voltage-dependent Ca^{2+} channels (10). Likewise, oleate can produce a cytosolic increase of [Ca^{2+}], probably by direct action on plasma membrane K_{ATP} channels or voltage-dependent Ca^{2+} channels (20,21). However, this classic pathway is not sufficient to explain the higher values of amplitude of the Ca^{2+} signals induced by both nutrients in the nucleus. In Fig. 2, we show that tolbutamide, which closes K_{ATP} channels, can trigger Ca^{2+} transients in isolated β-cell nuclei. This result confirms previous findings that propose the existence of a new signaling pathway, linking extracellular messages with nuclear function by means of nuclear K_{ATP} channels. Their blockage results in depolarization of the nuclear membrane and the release of Ca^{2+} from the nuclear envelope to the nucleoplasm, probably through ryanodine channels or a voltage-sensitive mechanism (8).

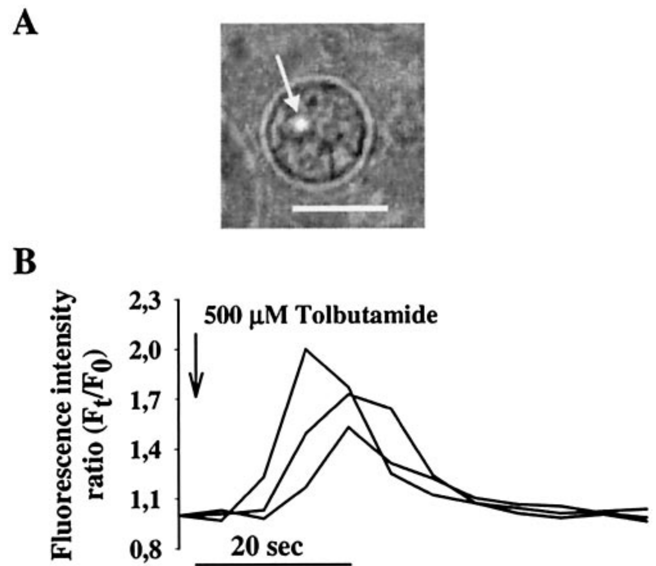

FIG. 2. Nuclear Ca^{2+} transients induced by blockage of nuclear K_{ATP} channels. *A*: Hybrid phase-contrast image showing the location of the fluorescence spot focused on an isolated nucleus from a β-cell, loaded with the fluorescent probe Calcium Green-1 dextran (bar = 4 μm). *B*: Closure of nuclear K_{ATP} channels with tolbutamide resulted in Ca^{2+} release from the nuclear envelope to the nucleoplasm. Traces represent three different cases.

DISCUSSION

Ca^{2+} dynamics in the nucleus and the cytosol were monitored in isolated INS-1 cells and pancreatic β-cells exposed to K^+, glucose, and oleate. Each stimulus produced a [Ca^{2+}] rise of similar duration in both intracellular compartments, suggesting that, in our experimental conditions, there is no apparent restriction to Ca^{2+} diffusion between nucleus and cytosol, in agreement with previous reports in pancreatic β-cells (19) and in other systems (6). This idea is also supported by the fact that the elevation in cytosolic [Ca^{2+}] produced by K^+-induced Ca^{2+} influx was equilibrated in the nucleoplasm. Conversely, significant differences in the amplitude of the Ca^{2+} signals induced by glucose and oleate were reported between the nucleoplasm and the cytoplasm. Unlike the nutrients, K^+ brought about similar values of amplitude in both cell locations, suggesting that the cytosolic [Ca^{2+}] increase resulting from extracellular Ca^{2+} entry by plasma membrane depolarization is rapidly transmitted throughout the cell. However, an extracellular Ca^{2+} influx or a source of Ca^{2+} located in the cytosol cannot provide an explanation for the higher nuclear levels of the ion induced by nutrients. Thus, glucose and oleate should involve additional pathways and Ca^{2+} sources that lead to higher [Ca^{2+}] in the nucleus. On account of nutrient metabolism, the basal ATP/ADP ratio, the concentration of diadenosine polyphosphates, and other messengers are augmented in the pancreatic β-cell, which in turn drive the closure of K_{ATP} channels, activating the well-known voltage-dependent

Ca^{2+} influx through the plasma membrane (10,20,22,23). In addition to this classic pathway, it has been found that ATP, AP_4A (diadenosine tetraphosphate), and tolbutamide block K_{ATP} channels located on the nuclear membrane, causing a Ca^{2+} discharge in the nucleoplasm by mechanisms sensitive to nuclear transmembrane potential (8). Thus, the metabolism of glucose and oleate may result in a concurrent action on plasma membrane and nuclear K_{ATP} channels, causing a Ca^{2+} rise from two different pools. Ca^{2+} influx from the extracellular pool would be equilibrated throughout the whole cell, producing equivalent $[Ca^{2+}]$ in the nucleus and the cytosol, whereas Ca^{2+} release from the nuclear pool would account for the higher amplitude observed within the nucleoplasm. Although our results are consistent with similar observations in other systems (6), some investigators have failed to report differences between nuclear and cytosolic $[Ca^{2+}]$ levels in pancreatic β-cells (19). These discrepancies may be explained by the use of different methods to monitor $[Ca^{2+}]$ changes, or different culture conditions, which have altered Ca^{2+} responses in β-cells (24,25).

Given that gene transcription is sensitive to amplitude-modulated $[Ca^{2+}]$ changes (1–3), we propose that nutrients could signal nuclear functions by ensuring an appropriate transitory level of $[Ca^{2+}]$ within the nucleoplasm higher than that possibly required for the activation of cytosolic functions. An exciting challenge in the future would be to find whether these amplitude-modulated Ca^{2+} signals in the nucleus are able to regulate gene expression in the pancreatic β-cell, which still remains elusive, and how these signals are integrated within the islets of Langerhans.

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