Published in final edited form as:

Pancreas. 2012 March ; 41(2): 238–244. doi:10.1097/MPA.0b013e3182277e56.

Serum Deprivation Induces Glucose Response and Intercellular Coupling in Human Pancreatic Adenocarcinoma PANC-1 Cells

Sahar Hiram-Bab, PhD^{*}, Yuval Shapira, MSc^{*}, Marvin C. Gershengorn, MD[†], and Yoram Oron, PhD^{*}

^{*}Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

[†]Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Yoram Oron, incumbent, Andy Lebach Chair of Clinical Pharmacology and Toxicology, Tel Aviv University.

Abstract

Objective—This study aimed to investigate whether the previously described differentiating islet-like aggregates of human pancreatic adenocarcinoma cells (PANC-1) develop glucose response and exhibit intercellular communication.

Methods—Fura 2–loaded PANC-1 cells in serum-free medium were assayed for changes in cytosolic free calcium ([Ca]_i) induced by depolarization, tolbutamide inhibition of K(ATP) channels, or glucose. Dye transfer, assayed by confocal microscopy or by FACS, was used to detect intercellular communication. Changes in messenger RNA (mRNA) expression of genes of interest were assessed by quantitative real-time polymerase chain reaction. Proliferation was assayed by the MTT method.

Results—Serum-deprived PANC-1 cell aggregates developed [Ca]_i response to KCl, tolbutamide, or glucose. These responses were accompanied by 5-fold increase in glucokinase mRNA level and, to a lesser extent, of mRNAs for K(ATP) and L-type calcium channels, as well as increase in mRNA levels of glucagon and somatostatin. Trypsin, a proteinase-activated receptor 2 agonist previously shown to enhance aggregation, modestly improved [Ca]_i response to glucose. Glucose-induced coordinated [Ca]_i oscillations and dye transfer demonstrated the emergence of intercellular communication.

Conclusions—These findings suggest that PANC-1 cells, a pancreatic adenocarcinoma cell line, can be induced to express a differentiated phenotype, in which cells exhibit response to glucose and form a functional syncytium similar to those observed in pancreatic islets.

Keywords

pancreatic adenocarcinoma; glucose response; cytosolic calcium; intercellular communication

PANC-1 cells are an established line of human pancreatic adenocarcinoma (PAC). Hui et al,¹ Hardikar et al,² and Wu et al³ reported that PANC-1 cells, when changed from serum-containing growth medium to serum-free medium (SFM), undergo differentiation and start

expressing glucagon, somatostatin, and insulin. The initial process involves aggregation to form spherical clusters that morphologically resemble pancreatic islets accompanied by mesenchymal-to-epithelial transition.^{3,4} We have previously reported that PANC-1 cells express 3 subtypes of proteinase-activated receptors (PARs 1, 2, 3).⁵ The stimulation of PAR-1 or PAR-2 evokes cytosolic free calcium ($[Ca]_i$) transients and accelerates PANC-1 cell aggregation in SFM.^{5,6}

β -Cells secrete insulin in response to glucose. This signaling mechanism depends on glucose metabolism and requires the functional presence of glucose sensing machinery. The first step is a rapid glucose uptake facilitated by GLUT2, followed by its phosphorylation and entrance into the glycolytic cycle leading to elevation of the ATP/ADP ratio. The increase in the ATP/ADP ratio causes closure of K(ATP) channels resulting in depolarization, activation of L-type voltage-sensitive calcium channels (VSCCs) and a rise in $[Ca]_i$ leading to insulin secretion.⁷ Fully differentiated β cells in islets form a characteristic syncytium that underlies coordinated calcium signaling and insulin secretion.^{8–10} Indeed, single β cells, which cannot establish intercellular communication via gap junctions, show alterations in secretion and insulin gene expression, which are corrected after restoration of β -cell contacts.^{11–13} Hence, the emergence of glucose signaling and the existence of intercellular communication are hallmarks of functional β -cell differentiation.

The present report addresses the question whether the early aggregation and differentiation of PANC-1 cells is accompanied by the emergence of functional glucose signaling machinery and of intercellular communication, as well as the effect of activation of PAR-2 receptors on these processes. We show that differentiating PANC-1 cell clusters exhibit glucose signaling and intercellular transport of a marker fluorescent probe. The activation of the glucose signaling pathway is modestly enhanced by a low concentration of trypsin.

MATERIALS AND METHODS

Materials

PANC-1 cells were from ATCC (Manassas, VA). Dulbecco modified Eagle medium (DMEM), F12, antibiotics, Hanks, and trypsin solutions, heat-inactivated fetal bovine serum and EZ-RNA-II RNA isolation kit were purchased from Biological Industries (Beit-Haemek, Israel). Fatty acid-free bovine serum albumin was from Gemini Bioproducts (West Sacramento, Calif.). Fura-2-AM and calcein were from Molecular Probes (Invitrogen, Carlsbad, Calif.). 1,1'-Diiododecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and 1,1'-diiododecyl-3,3,3',3'-tetramethylindocarbocyanine, 4-chlorobenzenesulfonate salt (DiD) were from Biotium (Hayward, Calif.). Tolbutamide (1-butyl-3-(4-methylphenylsulfonyl) urea), MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), and TCPK-treated trypsin were from Sigma-Aldrich (St Louis, Mo). Complementary strand DNA synthesis kit, Universal polymerase chain reaction (PCR) Master Mix, specific primers, and TaqMan PCR probes were from Applied Biosystems (Foster City, Calif.). All other chemicals were of analytical quality.

Cell Culture

PANC-1 cells were grown in serum-containing medium (SCM, DMEM with 10% fetal bovine serum, supplemented with 2 mM L-glutamine, penicillin [50 U/mL], and streptomycin [50 μ g/mL]) at 37°C and in 6%/94% CO₂ and air gas mixture. Twice a week, cells were harvested using trypsin (0.05%) and reseeded in SCM at 25% confluence. For aggregation, 25% to 50% confluent cells were washed 3 times with Hanks solution and cultured in SFM (DMEM/F12 1:1, 1% BSA, and antibiotics).

Determination of $[Ca]_i$

Cells were plated on glass no. 1.5 coverslip-based Lab-Tek chambers, Nalge-Nunc International (ThermoFisher Scientific, Wiesbaden, Germany) at subconfluent cell density. Cells were loaded with 5 μ M Fura 2-AM for 30 to 60 minutes in Hanks balanced salt solution (HBSS) and washed with HBSS, and the 340/380 nm excitation fluorescence ratio emission (510 nm) was determined using a Nikon TMD inverted microscope and large numerical aperture (1.30) $\times$ 40 oil immersion Nikon objective. Frames (340/380 nm) were acquired with intensified Photonic Science analog ISIS camera at 2 ratio images per second. Universal Imaging (Sunnyvale, Calif) Metafluor software (version 6.1) was used for acquisition and analysis. Agonists were added directly to the chamber using a perfusion system, which allowed agonists' washout. Where, because of aggregation of PANC-1 cells, visualization of individual cells was difficult, a 48-raster mask was used, where the size of a single-mask square unit approximated the diameter of a single cell. Data are presented as changes in fluorescence ratio of individual cells or raster units.

Proliferation Assay

PANC-1 cells were plated at 10% to 20% confluence in SCM, and the medium was changed the next day ($t = 0$) to SFM with indicated concentrations of trypsin for 7 days. MTT (0.5 mg/mL) was added for 2 hours, and proliferation was assayed as described in the manufacturer's protocol.

Microscopic Fluorescence Assay of Diffusible Dye Transfer

PANC-1 cells were cultured with calcein-AM (10 μ g/mL, 2 hours) or DiI (10 μ g/mL, 30 minutes) in HBSS before monodispersion with trypsin. Loaded single cells were seeded in 8-chamber Lab-Tek Permanox slides (Nalge-Nunc, ThermoFisher International, Rochester, NY) in SCM. After 6 to 12 hours, medium was replaced with SFM for additional 96 hours. Confocal images were captured with a Zeiss laser scanning inverted microscope (LSM510), equipped with Argon/2 (488 nm excitation line) and helium/neon lasers. Slides were viewed with 63 $\times$, 1.25 oil immersion Ph3 Neofluar objective at room temperature.

FACS Fluorescence Assay of Diffusible Dye Transfer

A subconfluent monolayer of cells growing in 25-mL flasks was incubated in 2 mL of HBSS with 15 to 20 μ g/mL calcein-AM for 2 hours, 5 μ g/mL DiD for 30 minutes, or both (for double-stained controls). Cells were washed 3 times with phosphate-buffered saline, harvested by trypsin, and reseeded at 3 (calcein-loaded)/1 (DiD-stained) cell ratio in 25-mL flasks in SCM for 12 hours. Cells were then washed 3 times SFM and incubated further in SFM for 48 hours. Cells were washed with phosphate-buffered saline, trypsinized, pelleted at 150g $\times$ 3 minutes, and resuspended in cold HBSS. Calcein-loaded and DiD-stained cells at 3:1 ratio mixture constituted the "freshly mixed" control. Calcein and DiD levels were quantified by flow cytometry on a fluorescence-activated cell sorter FACS Aria Flow Cytometer (Becton Dickinson; BD, Heidelberg, Germany). A total of 10,000 events were acquired. Data were analyzed using WinMdi software.

Quantitative Real-Time PCR

Quantitative real-time (qRT) PCR was performed as previously described.^{4,5} Results were normalized to GAPDH.

Statistics

All assays were performed at least in 2 independent experiments, each assayed in duplicates or triplicates. Student t test was used, and differences were considered significant when $P < 0.05$.

RESULTS

PANC-1 Cells Maintained in SFM Acquire $[Ca]_i$ Response to Depolarization, Tolbutamide, and Glucose

The emergence of glucose signaling, the obligatory process for β -cell function, can be interrogated either as a response to glucose or at 2 partial steps of the signaling pathway—the response of K(ATP) channels to inhibitory sulfonylurea compounds or depolarization-induced entry of Ca^{2+} via VSCCs. To assay the individual components of the pathway, we assayed $[Ca]_i$ response to depolarization by high K^+ , to tolbutamide (a first-generation clinically used sulfonylurea drug), or to glucose. We measured these responses in cells growing in SCM, and at 7 or 14 days after serum removal, before insulin expression.²

PANC-1 cells growing in SCM (DMEM supplemented with 10% fetal bovine serum) did not respond to any of the stimuli (not shown), whereas PANC-1 cells maintained in SFM for 7 or 14 days responded to stimulation with 50 mM KCl, 1 μ M tolbutamide, or 25 mM glucose. Figure 1A shows 3 representative $[Ca]_i$ responses to 25 mM glucose in single cells within a cluster. The $[Ca]_i$ responses were either a single or double transient (a) or a transient followed by prolonged oscillations (c). Although large responses were occasionally observed (a, c), most responses were smaller (b). Most of the responses to any of the 3 stimuli did not exceed a 50% increase over the baseline value (Fig. 1B). These results demonstrate that PANC-1 cells lack the necessary components of insulin secretion machinery while growing in SCM but acquire $[Ca]_i$ responses to stimuli that activate the glucose signaling pathway on serum removal.

Trypsin Enhances PANC-1 $[Ca]_i$ Response

Proteinase-activated receptor 2 stimulation in pancreatic ductal epithelium,¹⁴ acini,¹⁵ as well as PANC-1 cells⁵ activates the calcium mobilization pathway via activation of VSCCs and enhances secretion.^{16–18} We hypothesized that, because PAR-2 activation has a role in modulating Ca^{2+} stores in pancreatic acinar and ductal epithelial cells, it might also affect the glucose-sensing functions in PANC-1 cells. Moreover, the activation of PAR-1 or -2 by thrombin or trypsin caused accelerated aggregation and formation of smaller, presumably more viable clusters.⁵ We have, therefore, studied the effect of a single dose of trypsin, a physiological PAR-2 agonist, on PANC-1 cells' $[Ca]_i$ response to various stimuli. PANC-1 cells cultured for 7 days in SFM challenged on day 0 with a single dose of 1 U/mL trypsin were stimulated with glucose, tolbutamide, or KCl. Figure 2 shows a summary of 3 independent experiments, indicating that trypsin modestly but significantly ($P < 0.01$) increased $[Ca]_i$ response amplitude to glucose and tolbutamide, whereas the response to depolarization was not significantly altered.

Trypsin has been shown to act as a growth and survival factor.^{19,20} Indeed, 7 days of exposure to trypsin caused a dose-dependent biphasic increase in proliferation, with optimum between 0.03 and 1.0 U/mL (Fig. 3). PANC-1 cells challenged with 1 U/mL trypsin were more viable, exhibiting lower proportions of trypan blue-positive cells after 7 days in culture (Fig. 3, inset).

PANC-1 Cells Maintained in SFM Exhibit Intercellular Coupling

Intercellular communication is necessary for optimal secretory response, as confirmed in vitro in either mouse primary β cells²¹ or transformed mouse β cells¹¹ and directly demonstrated by electrophysiological measurements.²² Gap junctions mediate the functional cellular connections that are responsible for electrical and metabolic coupling. Indeed, the integrated secretory response of intact islets is greater than that of dispersed β cells,^{11–13} which show an increased basal and a decreased glucose-stimulated insulin secretion.

The existence of a functional syncytium is reflected in the response to glucose of intact islets, which exhibit synchronized oscillations of $[Ca]_i$ in adjacent β cells. To test whether serum deprivation promotes intercellular coupling, we assayed $[Ca]_i$ responses and compared their patterns in neighboring cells to those of cells in different locations in the preparation. Often the response pattern in neighboring cells was very similar, including coordinated fluctuations. The response pattern in distant cells, however, was usually different. The (a) and (b) tracings in Figure 1A show 2 cells exhibiting nonsynchronous $[Ca]_i$ fluctuations. Figure 4 shows synchronized initial $[Ca]_i$ transient and subsequent fluctuations in 3 adjacent cells and a different response pattern in a distant cell in the same preparation (gray tracing). These findings suggest that PANC-1 cells maintained in SFM develop intercellular communication. To verify that PANC-1 cells develop cell-to-cell communication, we performed diffusible dye transfer experiments. Cells were preloaded with either a diffusible anionic dye, calcein, or a nondiffusible lipophilic dye, DiI. The 2 populations of cells were mixed and seeded in SCM. After the cells had adhered to the plasticware, the medium was replaced with SFM for additional 4 days. Cells stained with both dyes demonstrated the existence of intercellular connections through which calcein was transferred to DiI-stained cells. Figure 5 shows a representative confocal micrograph of all 3 types of cell populations; calcein-stained, DiI-stained and double-stained. When control cells were treated with the same protocol, but cultured in SCM, a negligible proportion of double-stained cells was observed (<2%, not shown).

To estimate the extent of dye transfer, we repeated the experiment shown in Figure 5, using DiD (as a nondiffusible) and calcein (as a diffusible) markers. To increase the probability of dye transfer, the ratio of calcein-labeled to DiD-labeled cells was set to 3:1. Cells were incubated for 48 hours in SFM, trypsinized, and analyzed by FACS using calcein- and DiD-specific excitation wavelengths. Figure 6A shows dot plot graphs of a representative experiment, showing manually selected regions of double label. Analysis of 4 experiments (Fig. 6B) demonstrated that colabeling of PANC-1 cells with both calcein and DiD yielded $68.4\% \pm 9.5\%$ double-labeled cells, representing the maximal simultaneous loading of both markers. When a freshly mixed (3:1) suspension of calcein- and DiD-labeled cells was assayed, the proportion of doubly labeled cells was $5.7\% \pm 1.3\%$, representing the transfer-independent fluorescence signal spillover of the 2 dyes. After 48 hours of coincubation of the 2 populations in SFM, a $19.4\% \pm 5.9\%$ proportion of doubly labeled cells was yielded, that is, a net increase of $13.7\% \pm 4.6\%$ that could be attributed to dye transfer. Because at the 3:1 ratio of calcein to DiD-labeled cells, full transfer of calcein would result in one quarter of the population exhibiting double label, our results show 13.7% of the maximal 68.4%, that is, $19\% \pm 5.0\%$ dye transfer. This number is, therefore, close to the maximal value (Fig. 6B).

Glucose Signaling Response Is Accompanied by an Increase in the Expression of Proteins Involved in the Glucose Signaling Machinery

The data presented above strongly suggest that PANC-1 cells partially develop glucose signaling machinery and intercellular communications. We assayed by qRT-PCR a number of mRNAs of proteins that participate in the glucose signaling pathway (Table 1). The results (Fig. 7) indicate that the emergence of a functional glucose response is accompanied by an increase in mRNA levels for some of the participating proteins. The greatest increase (>5-fold) was in GCK mRNA, as early as 24 hours after changing the medium to SFM. Both L-type Ca channels (CACNA1C) and KATP channels mRNAs exhibited a trend toward increased expression, yielding approximately a 2-fold increase at 7 days. The β -cell-specific glucose transporter (GLUT2) mRNA exhibited no significant change. Although trypsin treatment improved $[Ca]_i$ signals (Fig. 2), it did not affect the mRNAs' levels of glucose signaling pathway proteins (not shown). Quantitative RT-PCR at 1 week in SFM also

confirmed the previously reported shift toward a more epithelial gene expression profile, including increases in epithelial marker genes (fold increase: CLDN, 3–9.5; CLDN, 4–2.9; CDH, 1–2.4) as well as increases mRNA levels of glucagon and somatostatin (Fig. 7 and Table 1). Unexpectedly, the mesenchymal marker gene *ACTA2* mRNA also increased by 2.4-fold.

The greater response in trypsin-treated cultures could be explained by the enhanced viability of the cells (Fig. 3) without the need for additional increase in the transcription of key signaling proteins.

DISCUSSION

PAC is one of the most intractable malignancies resistant to practically all therapeutic modalities. Although ranked in tenth place in cancer incidence, it is the fourth most common cause of cancer deaths in the Western world. It is generally accepted that aggressive cancer cells exhibit a more mesenchymal phenotype and that the trend toward invasion and metastasis is inversely related to their degree of differentiation. This concept provides the rationale for studying experimental manipulations that result in partial or full differentiation of cancer cells in general and of PAC cells in particular.

Hui et al,¹ Hardikar et al,² and Wu et al³ reported that PANC-1 cells, an established human PAC line, undergo partial differentiation on serum deprivation and start expressing epithelial marker genes, including endocrine markers, such as glucagon and insulin. Gershengorn et al⁴ and Wu et al³ subsequently reported that serum-deprived PANC-1 cells undergo mesenchymal-to-epithelial transition.

The objective of this study was to examine whether these changes in gene expression are accompanied by the emergence of endocrine functions. Specifically, we tested whether serum-deprived PANC-1 cells acquire responses mediated by the glucose-sensing signal transduction pathway and if they mimic the formation of a functional syncytium, which has been shown for pancreatic islet β cells.^{6,8,10,12}

Our results clearly demonstrate that serum-deprived PANC-1 cells respond to exocytotic stimuli in a manner characteristic of functional endocrine islet cells. Glucose elicits insulin secretion by changing the ATP/ADP ratio leading to closure of K(ATP) channels, which in turn causes membrane depolarization, opening of VSCCs, and stimulation of exocytosis due to increased $[Ca]_i$.⁷ PANC-1 cells proliferating in SCM did not respond with $[Ca]_i$ elevation to tolbutamide, depolarization or glucose. PANC-1 cells maintained in SFM for 7 or 14 days responded to all these stimuli. These results demonstrate that short-term serum deprivation of PANC-1 cells, before the onset of insulin expression, not only drives the expression of epithelial and endocrine markers^{2,3,23} but also induces $[Ca]_i$ response to the appropriate stimuli, characteristic of a functional β cell.

Although occasionally very large responses were observed, in most experiments, the responses to tolbutamide, depolarization, or glucose were modest (approximately 50% over the baseline $[Ca]_i$). We previously reported that stimulation of PARs enhanced PANC-1 cells aggregation in SFM.⁵ Because aggregation into islet-like spherical clusters may promote differentiation, we tested whether trypsin, a PAR-2 agonist, would increase PANC-1 cells responses. Indeed, trypsin significantly, although modestly, enhanced PANC-1 cells' responsiveness. This effect of trypsin could not be explained by an increase in the expression of glucose signaling proteins mRNAs. However, the increase in the responsiveness of trypsin-treated cells could be explained by the enhanced viability of PANC-1 cells.

The emergence of the glucose response was accompanied by an increase in the expression of GCK and, to a lesser degree, by an increase in K(ATP) channel mRNAs. L-type channels' mRNA exhibited a trend toward modest increase, whereas GLUT2 mRNA did not change. PANC-1 cells' aggregates exhibited early expression of mRNAs for both glucagon and somatostatin, ahead of the previously reported⁴ late expression of mRNA for insulin. Cells maintained in SCM and challenged with high K⁺ exhibited no measurable response, suggesting the absence of functional L-type Ca²⁺ channels. This precludes responses to either tolbutamide or glucose, even assuming that the densities of K(ATP) channels and GLUT2 transporters and GCK levels in these cells were sufficient for glucose entry and its phosphorylation. Our data suggest that the minor increase in L-type Ca²⁺ channels mRNA observed after 1 week was sufficient to produce a response to depolarization, which did not further increase at 2 weeks in SFM. On the other hand, responses to tolbutamide and glucose exhibited an increase between weeks 1 and 2, which could be attributed to the increased levels of GCK and/or K(ATP) transcripts. Although changes in mRNA level do not always correspond to changes in the level or activity of the cognate protein, the trend observed in PANC-1 cells was compatible with the dynamics of the glucose signaling pathway, as reflected by the 3 types of responses we assayed.

A single exposure to trypsin, a PAR-2 agonist previously shown by us to promote PANC-1 cells' aggregation in SFM, modestly improved responses to glucose and tolbutamide (Fig. 2); the apparent increase in the response to depolarization was not statistically significant. The increase in the responses was not accompanied by increases in the levels of mRNAs for any of the components of the glucose signaling pathway.

The onset of the glucose response was accompanied by the emergence of intercellular communication. This manifested as synchronized [Ca]_i responses and oscillations and could be directly demonstrated by dye transfer experiments, using both confocal microscopy and FACS analysis. The FACS data suggest that, during the first 48 hours in SFM, close to 80% of the target cells exhibit dye transfer. Hence, the generation of gap junctions apparently precedes the onset of the glucose-induced response and may be part of the process of accelerating and enhancing the function of the glucose signaling pathway.

REFERENCES

1. Hui H, Wright C, Perfetti R. Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes*. 2001; 50(4):785–796. [PubMed: 11289043]
2. Hardikar AA, Marcus-Samuels B, Geras-Raaka E, et al. Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates. *Proc Natl Acad Sci U S A*. 2003; 100(12):7117–7122. [PubMed: 12799459]
3. Wu Y, Li J, Saleem S, et al. c-Kit and stem cell factor regulate PANC-1 cell differentiation into insulin- and glucagon-producing cells. *Lab Invest*. 2010; 90(9):1373–1384. [PubMed: 20531294]
4. Gershengorn MC, Hardikar AA, Wei C, et al. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science*. 2004; 306(5705):2261–2264. [PubMed: 15564314]
5. Wei C, Geras-Raaka E, Marcus-Samuels B, et al. Trypsin and thrombin accelerate aggregation of human endocrine pancreas precursor cells. *J Cell Physiol*. 2006; 206(2):322–328. [PubMed: 16021635]
6. Charpentier E, Canela J, Meda P. beta cells preferentially exchange cationic molecules via connexin 36 gap junction channels. *Diabetologia*. 2007; 50(11):2332–2341. [PubMed: 17828386]
7. Jensen MV, Joseph JW, Ronnebaum SM, et al. Metabolic cycling in control of glucose-stimulated insulin secretion. *Am J Physiol Endocrinol Metab*. 2008; 295(6):E1287–E1297. [PubMed: 18728221]

8. Bavamian S, Klee P, Britan A, et al. Islet-cell-to-cell communication as basis for normal insulin secretion. *Diabetes Obes Metab.* 2007; 9:118–132. [PubMed: 17919186]
9. Gilon P, Ravier MA, Jonas J-C, et al. Control mechanisms of the oscillations of insulin secretion in vitro and in vivo. *Diabetes.* 2002; 51(Suppl 1):S144–S151. [PubMed: 11815474]
10. Pedersen MG, Bertram R, Sherman A. Intra- and inter-islet synchronization of metabolically driven insulin secretion. *Biophys J.* 2005; 89(1):107–119. [PubMed: 15834002]
11. Ravier MA, Guldenagel M, Charollais A, et al. Loss of connexin36 channels alters β -cell coupling, islet synchronization of glucose-induced Ca^{2+} and insulin oscillations, and basal insulin release. *Diabetes.* 2005; 54(6):1798–1807. [PubMed: 15919802]
12. Benninger RKP, Zhang M, Head WS, et al. Gap junction coupling and calcium waves in the pancreatic islet. *Biophys J.* 2008; 95(11):5048–5061. [PubMed: 18805925]
13. Serre-Beinier, Vr; Bosco, D.; Zulianello, L., et al. Cx36 makes channels coupling human pancreatic β -cells, and correlates with insulin expression. *Hum Mol Genet.* 2009; 18(3):428–439. [PubMed: 19000992]
14. Kim MH, Choi B-H, Jung SR, et al. Protease-activated receptor-2 increases exocytosis via multiple signal transduction pathways in pancreatic duct epithelial cells. *J Biol Chem.* 2008; 283(27):18711–18720. [PubMed: 18448425]
15. Singh VP, Bhagat L, Navina S, et al. Protease-activated receptor-2 protects against pancreatitis by stimulating exocrine secretion. *Gut.* 2007; 56(7):958–964. [PubMed: 17114298]
16. Ammala C, Larsson O, Berggren PO, et al. Inositol trisphosphate—dependent periodic activation of a Ca^{2+} -activated K^{+} conductance in glucose-stimulated pancreatic [β]-cells. *Nature.* 1991; 353(6347):849–852. [PubMed: 1719424]
17. Parekh AB. Store-operated Ca^{2+} entry: dynamic interplay between endoplasmic reticulum, mitochondria and plasma membrane. *J Physiol.* 2003; 547(2):333–348. [PubMed: 12576497]
18. Tamarina NA, Kuznetsov A, Rhodes CJ, et al. Inositol (1,4,5)-trisphosphate dynamics and intracellular calcium oscillations in pancreatic β -cells. *Diabetes.* 2005; 54(11):3073–3081. [PubMed: 16249428]
19. Yada K, Shibata K, Matsumoto T, et al. Protease-activated receptor-2 regulates cell proliferation and enhances cyclooxygenase-2 mRNA expression in human pancreatic cancer cells. *J Surg Oncol.* 2005; 89(2):79–85. [PubMed: 15660373]
20. Iwaki K, Shibata K, Ohta M, et al. A small interfering RNA targeting proteinase-activated receptor-2 is effective in suppression of tumor growth in a Panc1 xenograft model. *Int J Cancer.* 2008; 122(3):658–663. [PubMed: 17935125]
21. Zhang M, Goforth P, Bertram R, et al. The Ca^{2+} dynamics of isolated mouse [β]-cells and islets: implications for mathematical models. *Biophys J.* 2003; 84(5):2852–2870. [PubMed: 12719219]
22. Rolland, JFo; Henquin, JC.; Gilon, P. Feedback control of the ATP-sensitive K^{+} current by cytosolic Ca^{2+} contributes to oscillations of the membrane potential in pancreatic β -cells. *Diabetes.* 2002; 51(2):376–384. [PubMed: 11812744]
23. Zhang T, Wang H, Saunee NA, et al. Insulinoma-associated antigen-1 zinc-finger transcription factor promotes pancreatic duct cell trans-differentiation. *Endocrinology.* 2010; 151(5):2030–2039. [PubMed: 20215568]

FIGURE 1.

PANC-1 cells increase $[Ca]_i$ responses to glucose, depolarization and tolbutamide stimulation. PANC-1 cells were maintained in SFM for 7 or 14 days, washed with Hanks solution (1 mM glucose), loaded with Fura 2-AM, and assayed for stimulated $[Ca]_i$ responses as described in Materials and Methods.

A, PANC-1 cells were stimulated with 25 mM glucose.

Representative tracings of 340/380 nm ratios in individual cells were either single transient (a); 2 cells exhibiting double transient (b); or a transient followed by prolonged oscillations (c).

B, PANC-1 cells maintained in SCM or for 7 and 14 days in SFM were challenged with 50 mM KCl (K^+), 1 μ M tolbutamide (Tol), or 25 mM glucose (Glu). Mean $\pm$ SE fold increase of 340/380 nm ratios of 4 experiments are shown.

FIGURE 2.

Trypsin enhances PANC-1 $[Ca]_i$ responses. PANC-1 cells exposed to a single dose of trypsin (1 U/mL, +) and maintained for 7 days in SFM were loaded with Fura-2-AM and challenged with glucose (25 mM), tolbutamide (1 μ M), or KCl (50 mM). Data show fold increase of 340/380 nm ratios of 3 experiments. * $P < 0.01$.

FIGURE 3.

Trypsin enhances PANC-1 viability. PANC-1 cells were challenged with the indicated concentrations of 1 U/mL trypsin and maintained for 7 days in SFM. Proliferation was assayed with MTT. Inset, Effect of 1 U/mL trypsin on cell viability. Trypan blue-positive cells were counted with a hemocytometer. Data are presented as percentage of trypan blue-positive cells. * $P < 0.01$.

FIGURE 4.

PANC-1 cells maintained in SFM exhibit synchronized oscillations. $[Ca]_i$ responses to glucose were assayed as described in Materials and Methods and legend to Figure 1. Traces in 3 adjacent cells and a different response pattern in a distant cell in the same preparation (gray tracing). Addition of 25 mM glucose marked by arrow).

FIGURE 5.

PANC-1 cells maintained in SFM exhibit dye transfer. Confocal micrographs of calcein-positive (green), DiI-positive (red), and double-positive (merged) cells after incubation in SFM for 48 hours of 1:1 mix of calcein- and DiI-stained cells (Materials and Methods).

FIGURE 6. PANC-1 cells maintained in SFM exhibit dye transfer. Cells were loaded with calcein-AM, DiD, or both dyes, incubated for 48 hours in SFM and assayed by flow cytometry. A, Dot profiles of calcein- (FL1) and/or DiD- (FL4) positive cells. Double-positive cells were gated (R1). B, Mean $\pm$ SE percentage of double-positive cells (R1) in 4 independent experiments.

FIGURE 7.

The onset of glucose signaling is accompanied by changes in the expression of proteins involved in the glucose signaling. Changes in mRNA levels of genes associated with glucose signaling and endocrine phenotype were assessed 1, 4, and 7 days after serum removal. Threshold mRNA values of each transcript were normalized to threshold levels of GAPDH mRNA. Bars represent changes relative to proliferating cells (d0). Experiments were performed in duplicate for each data point.

TABLE 1
 Normalized Threshold Levels of qRT-PCR of Designated mRNAs at the Indicated Times After Serum Removal

Day	GCK	Glut2	K ATP	L type	GCG	SST
0	36.1 ± 0.2	37.0 ± 0.2	27.7 ± 0.5	36.0 ± 0.4	38.3 ± 0.5	37.9 ± 0.6
1	33.8 ± 0.4	37.0 ± 0.1	27.1 ± 0.2	36.4 ± 0.5	37.7 ± 0.2	36.7 ± 0.3
4	33.7 ± 0.2	37.4 ± 1.2	27.0 ± 0.4	35.6 ± 0.5	36.5 ± 0.6	34.3 ± 0.3
7	33.6 ± 0.1	39.0 ± 0.5	26.5 ± 0.6	34.8 ± 0.3	37.7 ± 0.7	35.1 ± 0.8