Glucose-Induced $[Ca^{2+}]_i$ Abnormalities in Human Pancreatic Islets

Important Role of Overstimulation

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Chronic hyperglycemia desensitizes β-cells to glucose. To further define the mechanisms behind desensitization and the role of overstimulation, we tested human pancreatic islets for the effects of long-term elevated glucose levels on cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) and its relationship to overstimulation. Islets were cultured for 48 h with 5.5 or 27 mmol/l glucose. Culture with 27 mmol/l glucose obliterated postculture insulin responses to 27 mmol/l glucose. This desensitization was specific for glucose versus arginine. Desensitization was accompanied by three major $[Ca^{2+}]_i$ abnormalities: *1*) elevated basal $[Ca^{2+}]_i$, *2*) loss of a glucose-induced rise in $[Ca^{2+}]_i$, and *3*) perturbations of oscillatory activity with a decrease in glucose-induced slow oscillations (0.2–0.5 min^{-1}). Coculture with 0.3 mmol/l diazoxide was performed to probe the role of overstimulation. Neither glucose nor diazoxide affected islet glucose utilization or oxidation. Coculture with diazoxide and 27 mmol/l glucose significantly ($P < 0.05$) restored postculture insulin responses to glucose and lowered basal $[Ca^{2+}]_i$ and normalized glucose-induced oscillatory activity. However, diazoxide completely failed to revive an increase in $[Ca^{2+}]_i$ during postculture glucose stimulation. In conclusion, desensitization of glucose-induced insulin secretion in human pancreatic islets is induced in parallel with major glucose-specific $[Ca^{2+}]_i$ abnormalities. Overstimulation is an important but not exclusive factor behind $[Ca^{2+}]_i$ abnormalities. *Diabetes* 49:1840–1848, 2000

Hyperglycemia impairs β-cell function in type 2 diabetic patients as evidenced by improved function after blood glucose normalization (1–3). The mechanisms behind this effect are largely unclarified regarding the signal-secretion mechanisms that are altered. Calcium signaling is a potential target for desensitization because key elements in glucose recognition as a secretagogue are an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (4) and oscillations of $[Ca^{2+}]_i$ (5–7). Decreased glucose-induced insulin secretion associates with defects in glucose-stimulated $[Ca^{2+}]_i$ (8–10), and toxic influences can profoundly affect oscillatory activity (11). Also, studies in rodents indicate influences of hyperglycemia on $[Ca^{2+}]_i$ (10,12–14). However, it is not known whether prolonged glucose elevation exerts effects on $[Ca^{2+}]_i$ fluxes in human pancreatic islets and, if so, whether the effects are similar to those in rodent islets.

Both direct and indirect actions of hyperglycemia can be construed to cause altered β-cell function (15–18). Using animal models of diabetes, we (15,19) and others (20) have demonstrated that overstimulation exerts a profound desensitizing influence on insulin responses to glucose. These studies used diazoxide as a probe for avoiding overstimulation. Diazoxide is known to block insulin secretion by opening ATP-dependent K^+ channels in cell membranes of β-cells, thereby hyperpolarizing the cells and offsetting opposite effects by glucose (21). In previous studies, we demonstrated that desensitization by overstimulation involves Ca^{2+} inflow (15). However, the effects of overstimulation on Ca^{2+} fluxes as tested by diazoxide have been insufficiently clarified in animal studies and have not been investigated at all in human β-cells.

Against this background, we aimed to test the influence of 48 h of elevated glucose levels in human pancreatic islets in vitro on subsequent insulin and $[Ca^{2+}]_i$ responses to glucose. The specificity of such effects was tested by comparison with responses to arginine, which is a nonnutrient secretagogue. On finding that long-term elevated glucose profoundly affected $[Ca^{2+}]_i$ parameters, we tested the extent to which relief of overstimulation by diazoxide could normalize glucose-induced insulin release and $[Ca^{2+}]_i$ parameters.

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AM, acetoxymethyl ester; ANOVA, analysis of variance; BSA, bovine serum albumin; $[Ca^{2+}]_i$, cytoplasmic free Ca^{2+} concentration; CV, coefficient of variation; KRBB, Krebs-Ringer bicarbonate buffer.

RESEARCH DESIGN AND METHODS

Materials. Bovine serum albumin (BSA) (fraction V) and forskolin were purchased from Sigma (St. Louis, MO), diazoxide (Hyperstat) was purchased from Schering-Plough Labo (Heist-op-den-Berg, Belgium), and fura-2/acetoxymethyl ester (AM) was purchased from Molecular Probes Europe (Leiden, Holland). D-[U-^{14}C]glucose and D-[5-^{3}H]glucose were obtained from Du Pont-NEN (Boston, MA).

Preparation and preprotocol maintenance of human islets. Human islets were isolated from pancreases retrieved from 24 heart-beating organ donors sent to the Central Unit of Beta-Cell Transplant (Brussels) as previously described (22). The age of the donors was 40.8 ± 3.2 years (means ± SE, range 19–59 years). Aliquots of the preparations (n = 24) were examined by electron microscopy and indicated <0.5% exocrine cells. The prevalence of insulin$^+$ and glucagon$^+$ cells was determined by light microscopic examination of immunocytochemically stained islets (23), which showed 58 ± 2% insulin$^+$

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cells and 13 ± 2% glucagon+ cells. After isolation, islets were cultured in Ham's F10 medium containing 6.1 mmol/l glucose and were supplemented with 0.5% BSA, 0.08 mg/ml penicillin, and 0.1 mg/ml streptomycin. The culture period in Brussels varied between 2 and 32 days (11 ± 2 days) before the islets were sent by air to Uppsala, Sweden, in the same medium as described above supplemented with 2% newborn calf serum and 10 mmol/l HEPES (24). There the islets were cultured in RPMI-1640 medium containing 5.5 mmol/l glucose, 2 mmol/l glutamine, 10% fetal calf serum, 100 U/ml benzylpenicillin, and 0.1 mg/ml streptomycin for 2–5 days before being transferred to our laboratory in Stockholm (transfer time ~1 h).

After arrival at our laboratory, the islets were transferred to new RPMI-1640 medium (with the same additions as above) and were cultured for 2 days before experiments. The time that elapsed from islet isolation in Brussels to the start of the actual experiment was 20 ± 2 days (10–35 days).

Islet culture. Islets were cultured in RPMI medium with 27 or 5.5 mmol/l glucose with or without diazoxide for 48 h while free-floating at 37° C with an atmosphere of 5% CO_2 95% air. The concentration of diazoxide when present was 325 µmol/l. Samples of the culture media were secured for insulin assay.

Measurements of insulin release. After culture, islets were washed and transferred to 5 ml Krebs-Ringer bicarbonate buffer (KRBB) (25) containing 10 mmol/l HEPES, 0.2% BSA, and 3.3 mmol/l glucose. Islets were preincubated in this medium for 30 min at 37°C. Islets were then transferred to tubes containing 300 µl KRBB with 3.3 and 27 mmol/l glucose or, in some experiments, 3.3 mmol/l glucose and 10 mmol/l arginine. A total of 3 to 4 tubes with 3–5 islets each were run for each experimental condition. The final postculture incubations were carried out for 60 min at 37°C in a shaking water bath. At the end of the incubations, aliquots of the incubation medium were secured for insulin assay. At the same time, islets were retrieved and sonicated for 10–15 s. Sonication was followed by extraction of insulin overnight at 4°C in 200 µl acid ethanol (70% vol/vol).

Islet glucose oxidation and glucose utilization. Glucose metabolism was measured after 30 min of preincubation in KRBB containing 3.3 mmol/l glucose. The parameters of glucose oxidation and utilization were determined by simultaneously measuring the formation of $^{14}CO_2$ from D-[U-^{14}C]glucose (26) and of $^{3}H_2O$ from D-[5-^{3}H]glucose (27) from the same batch of islets as described previously in detail (28).

Measurements of $[Ca^{2+}]_i$. After culture, the islets were loaded in 2 ml KRBB with 3.3 mmol/l glucose and 2 µmol/l fura-2/AM for 40 min at 37°C under gentle agitation. Single islets were transferred to a small open perifusion chamber (volume 150 µl) with a coverslip bottom. Islets were gently fixed under a grid made of titan. The medium flow rate was 150 µl/min and was controlled by a peristaltic pump. The dead space of the perifusion system corresponded with a lag period of ~40 s. Fura-2 fluorescence was measured at 37°C with a SPEX Fluorolog-2 system coupled with an inverted epifluorescence microscope (Zeiss Axiovert 35M) equipped with a 40 × 0.75 NA Plan-Neofluar objective. The measuring diaphragm was closed enough to exclude the brighter peripheral zone of the islet, thus minimizing the contribution of non–β-cell fluorescence. Fura-2 was alternatingly excited at 340 and 380 nm by a beam chopper, and emitted light was collected at 515 nm by a photon-counting photometer. A 340/380-nm ratio was obtained at 1 Hz. Data were stored in the system computer. The apparent concentration of cytoplasmic Ca^{2+} was calculated using the measured 340/380-nm ratios and calibrations by the equation described by Grynkiewicz et al. (29) with a dissociation constant (K_d) of 224 nmol/l. Linear correction was performed for each experiment to correct for trends at baseline before and after the stimulation period.

Insulin assay. Insulin was measured by radioimmunoassay using charcoal separation (30). Human insulin was used as a standard. The insulin antibodies used had been raised in our laboratory against porcine insulin.

Analysis of oscillations in $[Ca^{2+}]_i$. Fourier analysis is a mathematical technique for assessing the contribution of oscillations of any periodicity to the total oscillatory activity in a data set. With this analysis, any oscillatory phenomenon can be described in terms of its component sinusoidal forms. Fourier analyses were pooled for islets cultured with 5.5 or 27 mmol/l glucose in the absence or presence of diazoxide, and the means ± SE of the spectral densities were obtained at each frequency. The period analyzed was between 300 and 1,300 s (i.e., the stable stimulated period of $[Ca^{2+}]_i$ measurements). The time series results for individual data sets were smoothed using a three-point moving average to reduce rapid fluctuations in the data because of assay or experimental noise. Time series analysis was performed using spectral analysis with STATISTICA software (StatSoft). Any linear time trends and low-frequency oscillations in $[Ca^{2+}]_i$ were eliminated using regression analysis (detrending) to filter out peaks with periods >20 min. Spectral analysis results were presented as a histogram of spectral density showing the total amplitude of oscillations and as relative spectral density graphs (expressed as the percentage of total density) showing the frequency distribution of oscillations.

Expression of results. Data are means ± SE. In the case of islet insulin contents, results were adjusted for insulin released into postculture media. For significance testing with multiple comparisons, the one-way repeated-measures analysis of variance (ANOVA) was carried out. When the normality test failed, ANOVA on ranks was performed. Differences between two groups were evaluated using Student's *t* test. A P value < 0.05 was considered significant.

The variability of basal and stimulated $[Ca^{2+}]_i$ levels was greater within versus between preparations. As an example, for basal $[Ca^{2+}]_i$ from 27-mmol/l glucose cultured islets, the coefficient of variation (CV) was 17.4% for islets from a single preparation, whereas the interpreparational CV was 14.5%. Therefore, each islet studied was entered as one observation.

RESULTS

Prolonged β-cell stimulation by 27 mmol/l glucose specifically desensitizes glucose-induced insulin release. Culture for 48 h with 27 vs. 5.5 mmol/l glucose increased the amount of insulin accumulated in culture media by fivefold. Reciprocally, islet insulin contents were decreased by 75%. After culture, islets cultured with 27 mmol/l glucose were almost totally unresponsive to postculture stimulation with glucose, whereas islets cultured with 5.5 mmol/l glucose responded with a 6.8-fold increase in secretion (Table 1).

Fractional release (in percentage of insulin content) during final incubations with 3.3 mmol/l glucose was enhanced 8.6-fold in islets cultured in 27 mmol/l glucose vs. those cultured in 5.5 mmol/l glucose. However, during final incubations with 27 mmol/l glucose, fractional release failed to increase in islets, whereas a 5.5-fold increase was elicited in islets cultured with 5.5 mmol/l glucose.

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TABLE 1
Influence of glucose concentrations ± 325 µmol/l diazoxide during culture on glucose-induced insulin release to culture media, on release in 60-min final incubations, and on islet insulin content

Culture conditions (48 h)	Culture media (µU · islet^{-1} · 48 h^{-1})	Final incubations (µU · islet^{-1} · 60 h^{-1}): 3.3 mmol/l glucose	Final incubations (µU · islet^{-1} · 60 h^{-1}): 27 mmol/l glucose	Incremental release	Islet insulin content (µU/islet)
27 mmol/l glucose	208 ± 70	10.7 ± 2.3	11.7 ± 2.6	0.94 ± 0.7	101 ± 32
5.5 mmol/l glucose	41.7 ± 16.4*	3.5 ± 0.8*	23.9 ± 5.8*†	20.4 ± 5.2*	400 ± 121*
27 mmol/l glucose + diazoxide	75.6 ± 24.7‡	22.9 ± 5.9‡	49.8 ± 15.6†‡	26.9 ± 10.4‡	365 ± 101‡
5.5 mmol/l glucose + diazoxide	17.6 ± 7.0‡	3.6 ± 0.7	13.3 ± 4.0†‡	9.7 ± 3.5‡	432 ± 148

Data are means ± SE for 14 (27 mmol/l glucose culture) or 12 (5.5 mmol/l glucose culture) experiments. *P < 0.05 vs. 27 mmol/l glucose culture; †P < 0.05 vs. final incubation in 3.3 mmol/l glucose; ‡P < 0.05 vs. no diazoxide.

TABLE 2
Influence of glucose concentrations ± 325 μmol/l diazoxide during culture on arginine-induced insulin release

Culture conditions (48 h)	Final incubations ($\mu U \cdot islet^{-1} \cdot 60\ min^{-1}$) 3.3 mmol/l glucose	3.3 mmol/l glucose + 10 mmol/l arginine	Incremental release
27 mmol/l glucose	5.3 ± 1.5	8.5 ± 1.9*	3.2 ± 1.0
5.5 mmol/l glucose	1.4 ± 0.3†	1.8 ± 0.4†	0.4 ± 0.3†
27 mmol/l glucose + diazoxide	9.5 ± 3.7	14.4 ± 4.3*	4.9 ± 3.1
5.5 mmol/l glucose + diazoxide	2.1 ± 0.6	2.6 ± 0.8	0.4 ± 0.2

Data are means ± SE for 6 experiments. *$P < 0.05$ vs. final incubation in 3.3 mmol/l glucose; †$P < 0.05$ vs. 27 mmol/l glucose.

Diazoxide protects against desensitization of glucose-induced insulin secretion after high-glucose culture. Diazoxide was added to culture media to probe for the effects of overstimulation behind the desensitization to glucose. Coculture with diazoxide significantly reduced insulin accumulation in culture media after culture with both 5.5 and 27 mmol/l glucose (Table 1). However, in absolute terms, the accumulation was much more inhibited with 27 mmol/l glucose. Coculture with diazoxide and 5.5 mmol/l glucose did not affect islet insulin content, whereas coculture with diazoxide and 27 mmol/l glucose increased islet insulin content 3.6-fold (Table 1).

Coculture with diazoxide and 27 mmol/l glucose restored a marked insulin response to renewed stimulation with glucose during postculture incubations (Table 1). In addition, previous diazoxide significantly increased the insulin release during basal conditions (i.e., in the presence of 3.3 mmol/l glucose). These effects could not be explained by an effect of diazoxide per se. Hence, after coculture with diazoxide and 5.5 mmol/l glucose, a significant decrease was evident in glucose-induced insulin secretion (Table 1).

Fractional release after coculture with diazoxide and 27 mmol/l glucose was decreased in final incubations with 3.3 mmol/l glucose compared with islets cultured in 27 mmol/l glucose only (from 11 ± 1.3 to 5.8 ± 0.6%), although fractional release was increased relative to islets cultured with 5.5 mmol/l glucose (1.7 ± 0.6%). Fractional release in final incubations with 27 mmol/l glucose increased to 10 ± 0.8% after culture with diazoxide and 27 mmol/l glucose (i.e., to approximately the same levels seen after culture with 27 mmol/l glucose alone [12 ± 1.3%]).

High-glucose culture or diazoxide does not desensitize glucose-induced arginine release. The desensitization to glucose strikingly contrasted with arginine-induced insulin responses. Islets cultured with high glucose thus displayed a definite response to this amino acid, whereas a response was lacking in low-glucose cultured islets (Table 2). Furthermore, arginine-induced insulin secretion was not significantly affected by previous diazoxide (Table 2).

High-glucose culture enhances basal $[Ca^{2+}]_i$. After culture, islets were first perifused with 3.3 mmol/l glucose for 20–30 min preceding stimulation. The levels of basal $[Ca^{2+}]_i$ recorded immediately before stimulation were markedly increased (by 61%) by culture with 27 vs. 5.5 mmol/l glucose (Table 3).

Previous diazoxide counteracts high-glucose culture effects on basal $[Ca^{2+}]_i$. Coculture with diazoxide in the presence of 27 mmol/l glucose significantly lowered the elevated

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TABLE 3
Influence of culture conditions on $[Ca^{2+}]_i$ changes in human islets stimulated by an acute increase in glucose concentration from 3.3 to 27 mmol/l

	Culture conditions (48 h) 5.5 mmol/l glucose	27 mmol/l glucose	5.5 mmol/l glucose + 325 μmol/l diazoxide	27 mmol/l glucose + 325 μmol/l diazoxide
Basal $[Ca^{2+}]_i$ (nmol/l)	95.9 ± 7.3	154 ± 12*	96.0 ± 7.4	124 ± 8.6†
Initial decrease	6 of 7	—	6 of 7	—
Initial decrease (nmol/l)	5.2 ± 0.7	—	4.9 ± 1.1	—
Increase of 5 nmol/l Ca^{2+} (s)	103 ± 3.4	—	108 ± 8.5	—
Incremental Ca^{2+} at first peak (nmol/l)	82.4 ± 10.0	—	71.0 ± 10.3†	—
Delay to first peak of Ca^{2+} (s)	199 ± 24.3	—	201 ± 21.4	—
Incremental mean Ca^{2+} (nmol/l)	68.6 ± 5.7	8.4 ± 4.0*	57.0 ± 7.2†	9.9 ± 4.8*
Off response	3 of 7	9 of 10	1 of 7	9 of 10

Data are means ± SE or *n* for 10 (27 mmol/l glucose) or 7 (5.5 mmol/l glucose) experiments. After loading with 2 μmol/l fura-2/AM for 40 min, islets were perifused for 20–30 min with a medium containing 3.3 mmol/l glucose. Thereafter, the concentration of glucose was increased to 27 mmol/l. Basal $[Ca^{2+}]_i$ was calculated at 3.3 mmol/l glucose before switching to 27 mmol/l glucose. Initial decrease was defined as a decrease >2 nmol/l. Increase of 5 nmol/l Ca^{2+}, incremental Ca^{2+} at first peak, and delay to first peak of Ca^{2+} were calculated after switching to 27 mmol/l. Incremental mean Ca^{2+} was calculated by integrating all $[Ca^{2+}]_i$ values between 300 and 1,300 s of stimulation with 27 mmol/l glucose. Off-response was defined as an increase >10 nmol/l after changing the medium back to 3.3 mmol/l glucose from 27 mmol/l glucose. *$P < 0.01$ vs. 5.5-mmol/l glucose cultured islets; †$P < 0.03$ vs. no diazoxide.

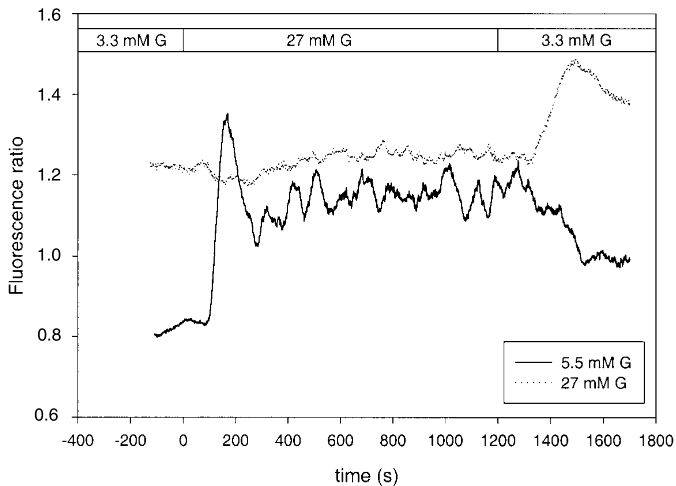

FIG. 1. Influence of 5.5 or 27 mmol/l glucose (G) during 48 h culture on $[Ca^{2+}]_i$ changes in human islets stimulated postculture by an acute increase in glucose concentration from 3.3 to 27 mmol/l. Shown are traces from single islets. The initial equilibration period was 20–30 min. Only the last 3 to 4 min are shown.

$[Ca^{2+}]_i$ caused by high-glucose culture (Table 3). The restorative effect of diazoxide amounted to 53% of the glucose effect. This restorative effect contrasted with results after coculture of diazoxide and 5.5 mmol/l glucose, in which case previous diazoxide did not affect basal $[Ca^{2+}]_i$.

High-glucose culture abolishes $[Ca^{2+}]_i$ responses to glucose. In islets cultured with 5.5 mmol/l glucose, six of seven islets first responded with a minor and transient drop in $[Ca^{2+}]_i$ on stimulation with 27 mmol/l glucose (Table 3). This was followed by a peak in $[Ca^{2+}]_i$, which was followed by irregular oscillations from an elevated plateau. A trace from a registration of a single islet is shown in Fig. 1, and the means ± SE of seven experiments are shown in Fig. 2*A* and are quantified in detail in Table 3. The $[Ca^{2+}]_i$ findings were vastly different in 27-mmol/l glucose cultured islets. Neither an initial drop of $[Ca^{2+}]_i$ nor a subsequent increase in $[Ca^{2+}]_i$ was then observed on stimulation with 27 mmol/l glucose. In fact, the increase in $[Ca^{2+}]_i$ was replaced by a small decrease in $[Ca^{2+}]_i$ after stimulation with high glucose in 7 of 10 islets from six different donors. A trace from a registration from a single islet is shown in Fig. 1, and the means ± SE of 10 experiments are shown in Fig. 2*A* and Table 3. Furthermore, after a change from

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FIG. 2. Influence of glucose (G) concentrations during 48 h culture in the absence (*A* and *C*) or the presence (*B* and *D*) of 325 µmol/l diazoxide (D) on $[Ca^{2+}]_i$ changes in human islets. In *A* and *B*, islets were stimulated postculture by an acute increase in glucose concentration from 3.3 to 27 mmol/l. In *C* and *D*, islets were stimulated postculture by 10 mmol/l arginine on a background of 3.3 mmol/l glucose. For *A* and *B*, data are means ± SE of 10 (27-mmol/l glucose culture) or 7 (5.5-mmol/l glucose culture) experiments. For *C* and *D*, data are means ± SE of six experiments. The initial equilibration period was 20–30 min. Only the last 3 to 4 min are shown.

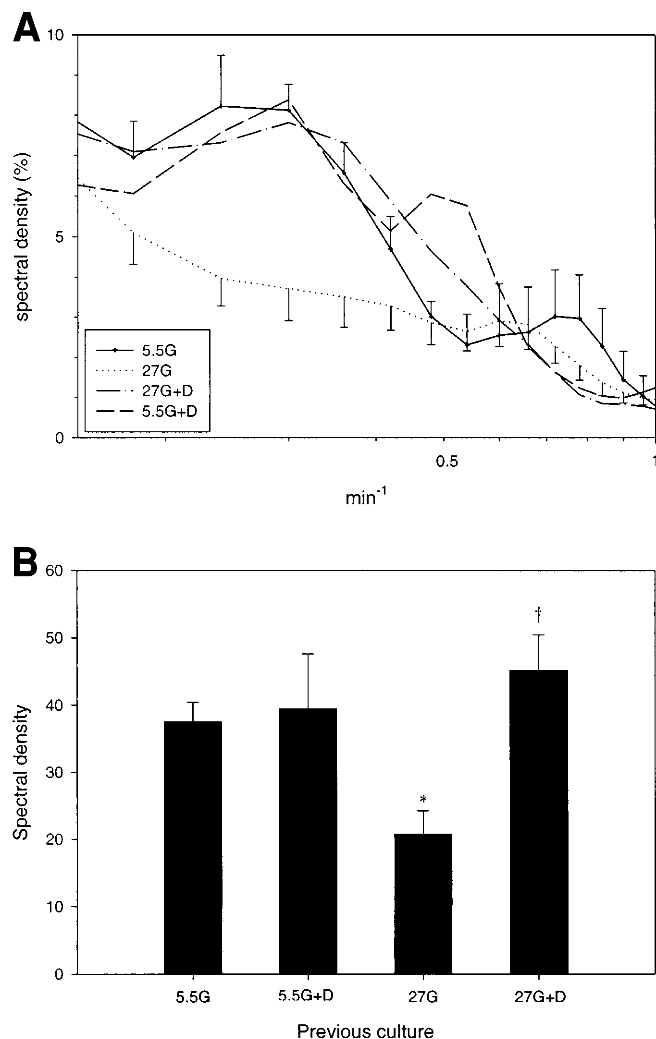

FIG. 3. Results of spectral analysis showing the relative spectral density of glucose-induced $[Ca^{2+}]_i$ oscillations in human islets previously cultured with 5.5 or 27 mmol/l glucose (G) in the absence or presence of 325 µmol/l diazoxide (D) for 48 h (*A*). For clarity, some error bars have been deleted. Significance for differences for 27- vs. 5.5-mmol/l glucose cultured islets was $P < 0.005$ and for 27-mmol/l glucose cultured islets vs. 27-mmol/l glucose and diazoxide cultured islets was $P < 0.005$. *B*: The amplitude of $[Ca^{2+}]_i$ oscillations in terms of total spectral density. *$P < 0.04$ for 27- vs. 5.5-mmol/l glucose cultured islets; †$P < 0.02$ for 27-mmol/l glucose cultured islets vs. 27-mmol/l glucose and diazoxide cultured islets.

27 to 3.3 mmol/l glucose, a marked positive deflection (off-response) was seen in 9 of 10 islets after culture with 27 mmol/l glucose (Table 3). After culture with 5.5 mmol/l glucose, an off-response was present in three of seven islets (Table 3).

Previous diazoxide fails to restore glucose-induced stimulation of $[Ca^{2+}]_i$. Previous diazoxide did not restore a stimulated $[Ca^{2+}]_i$ response to 27 mmol/l glucose in 27-mmol/l glucose cultured islets to any extent (Fig. 2*B*, Table 3). Culture with diazoxide and 5.5 mmol/l glucose decreased somewhat (by <20%) the glucose-induced measurement in $[Ca^{2+}]_i$ (Table 3).

Arginine-induced $[Ca^{2+}]_i$ responses are not desensitized by high-glucose culture. Responses to arginine were examined to assess the specificity of the desensitized and abnormal $[Ca^{2+}]_i$ responses to glucose. Definite stimulation by 10 mmol/l arginine in the presence of 3.3 mmol/l glucose was recorded in islets cultured with both 5.5 and 27 mmol/l glucose. In contrast to stimulation with glucose, a clear albeit relatively small positive deflection was recorded both after high- and low-glucose culture (Fig. 2*C*). No oscillations clearly correlated with the stimulation with arginine could be detected.

Diazoxide fails to affect arginine-induced $[Ca^{2+}]_i$ responses. Previous diazoxide did not affect the arginine-induced pattern of stimulation except that the stimulated level of $[Ca^{2+}]_i$ was somewhat decreased after high-glucose culture (Fig. 2*D*).

High-glucose culture diminishes slow $[Ca^{2+}]_i$ oscillations. The pattern of oscillations during stimulation with 27 mmol/l glucose was markedly different in islets cultured with 27 vs. 5.5 mmol/l glucose. Slow oscillations (0.2–0.5 min^{-1}) occurred less frequently in 27- vs. 5.5-mmol/l glucose cultured islets ($P < 0.005$, Fig. 3*A*). Not only the frequency but also the amplitude of slow oscillations (0.2–0.5 min^{-1}) was decreased in 27- vs. 5.5-mmol/l glucose cultured islets in terms of total spectral density ($P < 0.04$, Fig. 3*B*).

Previous diazoxide normalizes glucose-induced oscillatory activity. Coculture with diazoxide normalized the pattern of oscillations in terms of relative spectral density seen in islets cultured with 27 mmol/l glucose alone. Hence, after coculture with diazoxide and 27 mmol/l glucose, slow oscillations were totally restored ($P < 0.005$ vs. no previous diazoxide, Fig. 3*A*). These effects of diazoxide could not be explained by a drug effect per se because previous diazoxide after culture with low glucose tended to exert the opposite effect (Fig. 3*A*).

Previous diazoxide also restored the amplitude of slow oscillations (0.2–0.5 min^{-1}) in terms of total spectral density after 27-mmol/l glucose culture ($P < 0.02$, Fig. 3*B*). The 5.5-mmol/l glucose culture with diazoxide did not affect the overall amplitude of slow oscillations (Fig. 3*B*).

Previous glucose or diazoxide do not affect islet glucose utilization and oxidation. We tested whether the glucose concentration and/or presence of diazoxide during culture would affect glucose metabolism when assessed after culture. However, neither glucose utilization nor oxidation was affected by the culture conditions (Table 4).

DISCUSSION

The starting point of this study was the demonstration that long-term elevated glucose levels completely and specifically desensitize glucose-induced insulin secretion from human pancreatic islets. The desensitizing effect of long-term elevated glucose levels that we observe is much more pronounced than in rodent islets (13,15). Desensitization mimics the severe attenuation of glucose-induced insulin release in type 2 diabetic patients. Although we recognize that all causes of decreased insulin secretion in type 2 diabetes cannot be encompassed by in vitro experiments, our results should still provide insight into mechanisms behind abnormal insulin release in this disease.

The present study demonstrates (for the first time, to our knowledge) that long-term exposure to elevated glucose levels induces profound abnormalities in the regulation of $[Ca^{2+}]_i$ in human pancreatic islets. One abnormality was an increase in basal $[Ca^{2+}]_i$, and another abnormality was a marked attenuation of a $[Ca^{2+}]_i$ response to an acute elevation of glucose. High-glucose culture conditions have been shown to elevate basal $[Ca^{2+}]_i$ in islets from *ob/ob* mice (13); however, attenuation of a $[Ca^{2+}]_i$ response to glucose to the degree of

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TABLE 4
Influence of glucose concentrations ± 325 μmol/l diazoxide during culture on postculture glucose utilization and oxidation

Culture conditions (48 h)	Glucose utilization ($pmol \cdot islet^{-1} \cdot 2h^{-1}$)		Glucose oxidation ($pmol \cdot islet^{-1} \cdot 2h^{-1}$)	
	3.3 mmol/l glucose	27 mmol/l glucose	3.3 mmol/l glucose	27 mmol/l glucose
27 mmol/l glucose	74.4 ± 9.3	237.5 ± 32.7	12.9 ± 3.2	38.5 ± 7.0
6 mmol/l glucose	81.3 ± 12.4	233.5 ± 57.3	11.2 ± 1.8	28.4 ± 5.9
27 mmol/l glucose + diazoxide	71.2 ± 8.8	197.3 ± 29.3	13.5 ± 3.8	36.0 ± 6.5
6 mmol/l glucose + diazoxide	69.6 ± 15.8	200.9 ± 35.0	9.8 ± 1.7	30.1 ± 3.9

Data are means ± SE for six experiments.

that in the present study has not been observed in cultured rodent islets (12–14). We note that the attenuated $[Ca^{2+}]_i$ response that we observe was also found in islets from a single patient with type 2 diabetes (31).

Our study also demonstrated that long-term elevated glucose profoundly perturbs glucose-induced oscillatory activity. These effects were obvious despite the nonoptimal glucose concentration used for examining this particular parameter. (Oscillations are usually best recorded during stimulation with 10–15 mmol/l glucose, at least in rodent islets. The reason for using 27 mmol/l rather than a lower concentration of glucose was to ensure that the effect of glucose on other $[Ca^{2+}]_i$ parameters as well as on insulin secretion was maximal.) Importantly, glucose-induced slow $[Ca^{2+}]_i$ oscillations (0.2–0.5 min^{-1}) were reduced after high-glucose culture. To view our own findings in perspective, we note that slow $[Ca^{2+}]_i$ oscillations are a well-established essential feature of the physiological $[Ca^{2+}]_i$ response to glucose both in rodent (5) and in human (31,32) islets and in isolated β-cells (33). Normal $[Ca^{2+}]_i$ oscillatory activity could minimize the risk of cell injury by $[Ca^{2+}]_i$-dependent proteases (34); furthermore, oscillation of $[Ca^{2+}]_i$ may be less desensitizing on intracellular processes than a sustained stimulation. From another viewpoint, loss of slow oscillations may signify a stressed β-cell because toxic influences lead to loss of slow oscillations (11). Of further potential importance is the synchronization between slow $[Ca^{2+}]_i$ oscillations and insulin release that has been demonstrated in rodent islets, at least under certain experimental conditions (35,36).

Our data with arginine indicate that the abnormalities in glucose-induced regulation of $[Ca^{2+}]_i$ are specific for glucose, and the $[Ca^{2+}]_i$ increase in response to arginine is unaffected or possibly enhanced by high-glucose culture. These observations are congruent with previous ones in neonatally streptozotocin-induced diabetic rats (10). Both glucose- and arginine-induced increases in $[Ca^{2+}]_i$ result from depolarization of the cell membrane followed by inflow of Ca^{2+} through voltage-dependent channels. However, depolarization by glucose is coupled with glucose closure of the ATP-dependent K^+ channel, an effect that is secondary to an increase in the cytosolic ATP/ADP ratio as a result of glucose metabolism. Depolarization by arginine, on the other hand, is induced by the electrogenic properties of the arginine molecule (37). These data make the possibility unlikely that a deficiency in $[Ca^{2+}]_i$ channel functioning is causing the abnormalities presently seen with glucose as a stimulus.

The effects of arginine were tested with a low concentration of glucose, which in some previous studies had only marginal effects on insulin secretion and on $[Ca^{2+}]_i$. The results obtained after culture with 5.5 mmol/l glucose are consistent with these observations and show no effects on insulin secretion and modest effects on $[Ca^{2+}]_i$. Hyperglycemia is known to left shift the dose response for glucose-induced insulin secretion as well as to potentiate insulin secretion of other secretagogues (38). Consistent with this, we observed a stronger insulin response to arginine after culture with 27 mmol/l glucose as well as a tendency for a more marked $[Ca^{2+}]_i$ response.

Our experiments with diazoxide served to probe the importance of overstimulation for the desensitized insulin response to glucose and the $[Ca^{2+}]_i$ abnormalities seen after high-glucose culture. The choice of diazoxide was motivated by its inhibitory effect on glucose-induced insulin secretion, the swift reversibility thereof after 48 h of exposure time in vivo in rats (19) and after 20–22 h of culture of rat pancreatic islets (15), and the low toxicity in clinical settings (39).

We found that diazoxide during high-glucose culture preserved an insulin response to glucose during postculture conditions (i.e., when the drug was no longer present). The incremental increase in secretion during a glucose challenge was of the same magnitude as the response of islets cultured with 5.5 mmol/l glucose alone. The beneficial effects of previous diazoxide on glucose-induced insulin secretion could not be explained by an effect of the drug per se. Hence, the presence of diazoxide during culture with normal glucose levels (5.5 mmol/l) had (for unknown reasons) an inhibitory (not a stimulatory) effect on the postculture insulin response to glucose. Altogether, these results strongly indicate an important role for overstimulation behind the desensitized insulin response to glucose.

Our study shows that previous diazoxide had normalizing effects on two important $[Ca^{2+}]_i$ abnormalities. First, elevated basal levels of $[Ca^{2+}]_i$ after high-glucose culture were decreased by the copresence of diazoxide during culture. Importantly, this effect was glucose dependent because it was not seen after culture with diazoxide with normal glucose levels. Also, a previous study indicated that any effect of diazoxide per se would increase rather than decrease $[Ca^{2+}]_i$ (40).

Previous diazoxide reversed a second Ca^{2+} abnormality, i.e., oscillatory activity. Thus, the copresence of diazoxide during high-glucose culture normalized the slow (0.2–0.5 min^{-1}) $[Ca^{2+}]_i$ oscillations during glucose stimulation. Again, this effect appeared not to be an effect of the drug per se because slow oscillations after coculture of diazoxide together with low glucose were not increased, as was the case after coculture with high glucose.

However, previous diazoxide failed to restore to any degree a net $[Ca^{2+}]_i$ response to glucose. Notably, this failure was dissociated from a restoring effect on glucose-induced

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insulin secretion. The discrepancies are unlikely to be explained by a difference in time scale for insulin (60 min) and $[Ca^{2+}]_i$ measurements (1,600 s) because the latter measurements were without any tendency for $[Ca^{2+}]_i$ to increase with the duration of glucose stimulation. Our results with $[Ca^{2+}]_i$ responses contrast with findings in isolated β-cells of the rat, in which a partial deficiency in glucose-induced $[Ca^{2+}]_i$ response could be corrected (along with glucose-induced insulin secretion) by including diazoxide during high-glucose culture (8). Species differences as well as experimental differences (isolated cells in the previous study vs. islets in the present study) could possibly explain these discrepancies.

A further abnormality not corrected by diazoxide was the exaggerated $[Ca^{2+}]_i$ off-response seen at the cessation of postculture stimulation with glucose. The mechanisms behind such off-responses are presently obscure and require further study.

The question arises regarding to which extent any of the observed abnormalities are influenced by the participation of non–β-cells. In human islets, α-cells surround a core of β-cells. The content and regulation of fura-2–associated $[Ca^{2+}]_i$ in α-cells could thus be included in the present measurements. However, α-cells normally respond to glucose reciprocally with β-cells (41). Therefore, that α-cells could be responsible for the elevation of basal $[Ca^{2+}]_i$ after high-glucose culture or the loss of a $[Ca^{2+}]_i$ increase to postculture stimulation with glucose seems unlikely. Regarding synchronous oscillations in response to glucose, studies using confocal microscopy in mouse islets indicate that only β-cells oscillate (42,43). Arginine-induced $[Ca^{2+}]_i$ effects could, on the other hand, be influenced by α-cell participation because this amino acid is a potent stimulator of glucagon secretion (41) and induces an increase in $[Ca^{2+}]_i$ in mouse islets (37,42). Note, however, that $[Ca^{2+}]_i$ responses to arginine tended to be higher rather than lower after high-glucose culture. Because previous hyperglycemia depresses a glucagon response to arginine (44), one would have imagined the opposite finding in our experiments if participation from the α-cells were to dominate the arginine-induced $[Ca^{2+}]_i$ response.

What roles do the $[Ca^{2+}]_i$ abnormalities observed play in desensitization of glucose-induced insulin release after high-glucose culture? That glucose-induced insulin secretion normally requires inflow of extracellular Ca^{2+} (with the increase in cytosolic $[Ca^{2+}]_i$ acting as a stimulus for insulin secretion) is well established. Consensus exists that some elevation of $[Ca^{2+}]_i$ is a necessary condition for insulin secretion in response to other physiological stimuli (6). Thus, the loss of stimulation of $[Ca^{2+}]_i$ by glucose is a potential cause of the desensitized insulin response. However, in our study, basal $[Ca^{2+}]_i$ was markedly elevated by the high-glucose culture before postculture stimulation with glucose, thus questioning the need for further $[Ca^{2+}]_i$ elevation to induce insulin secretion. In this context, note that the fractional release of insulin at low glucose was markedly elevated during basal glucose conditions; this implies a near-maximal effect by $[Ca^{2+}]_i$ before the introduction of 27 mmol/l glucose.

The chronic elevation of basal $[Ca^{2+}]_i$ may possibly inhibit insulin secretion. Consistent with such a notion, supraphysiological concentrations of extracellular Ca^{2+} inhibit secretion (45). Such inhibition may be linked to or precede $[Ca^{2+}]_i$ toxicity, a concept that has been advanced for different cell types (34), including pancreatic β-cells (46,47).

Along this line of reasoning, the lowering effect of previous diazoxide on basal $[Ca^{2+}]_i$ could be important in preserving sensitivity to glucose, especially because this effect was not seen after the presence of diazoxide during normal glucose cultures. However, we note that diazoxide did not reduce elevated basal $[Ca^{2+}]_i$ levels to levels seen after culture with 5.5 mmol/l glucose. Furthermore, the reduction in basal $[Ca^{2+}]_i$ levels by diazoxide was accompanied by increased rather than decreased release of insulin under basal conditions (although fractional release was decreased vs. culture without diazoxide). One may speculate that a combination of moderately elevated $[Ca^{2+}]_i$ along with a glucose-sensitizing effect to $[Ca^{2+}]_i$ as part of a K^+-ATP channel–independent action of glucose (48,49) could be responsible for the increased basal secretion.

A role for a decrease in slow $[Ca^{2+}]_i$ oscillations for desensitization of glucose-induced insulin secretion is compatible with the importance of such oscillations in the signal-secretion chain of events. The mechanisms responsible for this type of oscillatory activity have yet to be determined. Some oscillatory activity is coupled with glucose metabolism; $[Ca^{2+}]_i$ oscillations, for example, have been found to be coupled to oscillations in NADH and ATP/ADP concentrations (50). In our study, we did not find any gross abnormalities in the glycolytic or oxidative metabolism of glucose either as a result of the glucose concentration during culture or as a result of the presence of diazoxide. (Incidentally, these findings attest to the viability of the islets after high-glucose culture). However, our results do not rule out subtle abnormalities of glucose metabolism that could affect, for example, cytosolic ATP/ADP ratios and that could be important in the present context.

Our findings indicating that overstimulation is responsible for the loss of glucose-induced slow $[Ca^{2+}]_i$ oscillations could be clinically important. As mentioned, such oscillations may at least in part underlie pulsatility of insulin secretion. Pulsatile insulin secretion may have better biological effects than continuous insulin release (51), and disturbances in pulsatility of insulin secretion are a characteristic of type 2 diabetic patients (52) and their relatives (53). In obese humans, observations have indicated that increased demands for secretion, such as those that occur during obesity, will diminish orderly pulsatility of insulin, whereas weight loss will restore such pulsatility (54). On these grounds, our results with diazoxide are compatible with the notion that loss of pulsatile insulin secretion in type 2 diabetic patients is related to overstimulation of the patients' β-cells.

In conclusion, the present study has demonstrated in human pancreatic islets that a prolonged high-glucose environment in parallel abolishes glucose-induced insulin secretion and glucose-induced $[Ca^{2+}]_i$ elevation, increases basal $[Ca^{2+}]_i$, and abolishes slow (0.2–0.5 min^{-1}) $[Ca^{2+}]_i$ oscillations. Relieving overstimulation by diazoxide restores secretion, partially reverses elevated basal $[Ca^{2+}]_{i,}$, and restores slow (0.2–0.5min^{-1}) $[Ca^{2+}]_i$ oscillations but fails to restore a $[Ca^{2+}]_i$ elevation in response to glucose. These results document an important but not exclusive role of overstimulation for desensitization-associated Ca^{2+} abnormalities.

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REFERENCES

1. Kosaka K, Kuzuya Y, Akanuma Y, Hagura R: Increase in insulin response after treatment of overt maturity-onset diabetes is independent of the mode of treatment. *Diabetologia* 18:23–28, 1980
2. Garvey W, Olefsky J, Griffin J, Hamman R, Kolterman O: The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. *Diabetes* 34:222–234, 1985
3. Yki-Järvinen H: Glucose toxicity. *Endocr Rev* 13:415–431, 1992
4. Wollheim CB, Pozzan T: Correlation between cytosolic free Ca^{2+} and insulin release in an insulin-secreting cell line. *J Biol Chem* 259:2262–2267, 1984
5. Grapengiesser E, Gylfe E, Hellman B: Glucose-induced oscillations of cytoplasmic Ca^{2+} in the pancreatic beta-cell. *Biochem Biophys Res Commun* 151:1299–1304, 1988
6. Henquin JC, Jonas JC, Gilon P: Functional significance of Ca^{2+} oscillations in pancreatic β-cells. *Diabete Metab* 24:30–36, 1998
7. Soria B, Martin F: Cytosolic calcium oscillations and insulin release in pancreatic islets of Langerhans. *Diabete Metab* 24:37–40, 1998
8. Okamoto Y, Ishida H, Taminato T, Tsuji K, Kurose T, Tsuura Y, Kato S, Imura H, Seino Y: Role of cytosolic Ca^{2+} in impaired sensitivity to glucose of rat pancreatic islets exposed to high glucose in vitro. *Diabetes* 41:1555–1561, 1992
9. Zaitsev SV, Efanova I, Östenson C-G: Delayed Ca^{2+} response to glucose in diabetic GK-rat. *Biochem Biophys Res Commun* 239:129–133, 1997
10. Tsuji K, Taminato T, Ishida H, Okamoto Y, Tsuura Y, Kato S, Kurose T, Okada Y, Imura H, Seino S: Selective impairment of the cytoplasmic Ca^{2+} response to glucose in pancreatic β-cells of streptozotocin-induced non-insulin-dependent diabetic rats. *Metabolism* 42:1424–1428, 1993
11. Grapengiesser E, Gylfe E, Hellman B: Disappearance of glucose-induced oscillations of cytoplasmic Ca^{2+} in pancreatic β-cells exposed to streptozotocin or alloxan. *Toxicology* 63:263–271, 1990
12. Gilon P, Jonas JC, Henquin JC: Culture duration and conditions affect the oscillations of cytoplasmic calcium concentration induced by glucose in mouse pancreatic islets. *Diabetologia* 37:1007–1014, 1994
13. Bergsten P: Slow and fast oscillations of cytoplasmic Ca^{2+} in pancreatic islets correspond to pulsatile insulin release. *Am J Physiol* 268:E282–E287, 1995
14. Antoine M-H, Lebrun P, Herchuelz A: Cell culture conditions influence glucose-induced $[Ca^{2+}]_i$ responses in isolated rat pancreatic β cells. In *Physiology and Pathophysiology of the Islets of Langerhans.* Soria B, Ed. New York, Plenum, 1997, p. 227–230
15. Björklund A, Grill V: B-cell insensitivity in vitro: reversal by diazoxide entails more than one event in stimulus-secretion coupling. *Endocrinology* 132: 1319–1328, 1993
16. Donath MY, Gross DJ, Cerasi E, Kaiser N: Hyperglycemia-induced β-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes* 48:738–744, 1999
17. Kilpatrick ED, Robertson RP: Differentiation between glucose-induced desensitization of insulin secretion and β-cell exhaustion in the HIT-T15 cell line. *Diabetes* 47:606–611, 1998
18. Tajiri Y, Möller C, Grill V: Long term effects of aminoguanidine on insulin release and biosynthesis: evidence that the formation of advanced glycosylation end products inhibits β cell function. *Endocrinology* 138:273–280, 1997
19. Sako Y, Grill V: Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* 39: 1580–1583, 1990
20. Leahy JL: Impaired β-cell function with chronic hyperglycemia: "overworked β-cell" hypothesis. *Diabetes Rev* 4:298–319, 1996
21. Trube G, Rorsman P, Shosaku O: Opposite effects of tolbutamide and diazoxide on the ATP dependent K^+ channel in the mouse pancreatic β-cells. *Pflugers Arch* 407:493–499, 1986
22. Eizirik DL, Korbutt GS, Hellerström C: Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the β-cell function. *J Clin Invest* 90:1263–1268, 1992
23. Pipeleers DG, In't Veld P, Maes E, Shuit F, Gepts W: A new in vitro model for the study of pancreatic α and β cells. *Endocrinology* 117:806–816, 1985
24. Ling Z, Pipeleers DG: Prolonged exposure of human β cells to elevated glucose levels results in sustained cellular activation leading to loss of glucose regulation. *J Clin Invest* 98:2805–2812, 1996
25. Cohen PP: Methods for preparation and study of tissues: suspending media for animal tissues. In *Manometric Techniques*. Umbreit WW, Burris RH, Stauffer JF, Eds. Minneapolis, Burgess, 1985, p. 149–150
26. Keen H, Field JB, Pastan IH: A simple method for in vitro metabolic studies using small volumes of tissue and medium. *Metab Clin Exp* 12:143–147, 1963
27. Ashcroft SJH, Weerasinghe LCC, Bassett JM, Randle PJ: The pentose cycle and insulin release in mouse pancreatic islets. *Biochem J* 126:525–532, 1972
28. Zhou Y-P, Berggren P-O, Grill V: A fatty acid-induced decrease in pyruvate dehydrogenase activity is an important determinant of β-cell dysfunction in the obese diabetic *db/db* mouse. *Diabetes* 45:580–586, 1996
29. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
30. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
31. Kindmark H, Köhler M, Arkhammar P, Efendic S, Larsson O, Linder S, Nilsson T, Berggren PO: Oscillations in cytoplasmic free calcium concentration in human pancreatic islets from subjects with normal and impaired glucose tolerance. *Diabetologia* 37:1121–1131, 1994
32. Martin F, Soria B: Glucose-induced $[Ca^{2+}]_i$ oscillations in single human pancreatic islets. *Cell Calcium* 20:409–414, 1996
33. Hellman B: Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic beta cells. *Diabetologia* 37 (Suppl. 2):11–20, 1994
34. McConkey DJ, Orrenius S: The role of calcium in the regulation of apoptosis. *Biochem Biophys Res Commun* 239:357–366, 1997
35. Bergsten P, Grapengiesser E, Gylfe E, Tengholm A, Hellman B: Synchronous oscillations of cytoplasmic Ca^{2+} and insulin release in glucose-stimulated pancreatic islets. *J Biol Chem* 269:8749–8753, 1994
36. Gilon P, Shepherd RM, Henquin JC: Oscillations of secretion driven by oscillations of cytoplasmic Ca^{2+} as evidenced in single pancreatic islets. *J Biol Chem* 268:22265–22268, 1993
37. Smith PA, Sakura H, Coles B, Gummerson N, Proks P, Ashcroft FM: Electrogenic arginine transport mediates stimulus-secretion coupling in mouse pancreatic β-cells. *J Physiol* 499:625–635, 1997
38. Leahy JL, Weir GC: Unresponsiveness to glucose in a streptozotocin model of diabetes: inappropriate insulin and glucagon responses to a reduction of glucose concentration. *Diabetes* 34:653–659, 1985
39. Björk E, Berne C, Kämpe O, Wibell L, Oskarsson P, Karlsson FA: Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. *Diabetes* 45:1427–1430, 1996
40. Grimmsmann T, Rustenbeck I: Direct effects of diazoxide on mitochondria in pancreatic β-cells and on isolated liver mitochondria. *Br J Pharmacol* 123: 781–788, 1998
41. Gerich JE, Charles MA, Grodsky GM: Regulation of pancreatic insulin and glucagon secretion. *Annu Rev Physiol* 38:353–388, 1976
42. Asada N, Shibuya I, Iwanaga T, Niwa K, Kanno T: Identification of α- and β-cells in intact isolated islets of Langerhans by their characteristic cytoplasmic Ca^{2+} concentration dynamics and immunocytochemical staining. *Diabetes* 47:751–757, 1998
43. Nadal A, Quesada I, Soria B: Homologous and heterologous asynchronicity between identified α-, β- and δ-cells within intact islets of Langerhans in the mouse. *J Physiol* 157:85–93, 1999
44. Grill V, Adamson U, Rundfeldt M, Andersson S, Cerasi E: Glucose memory of pancreatic β and α2 cells: evidence for common time-dependent actions of glucose on insulin and glucagon secretion in the perfused rat pancreas. *J Clin Invest* 64:700–707, 1979
45. Hellman B: Calcium transport in pancreatic β-cells: implications for glucose regulation of insulin release. *Diabetes Metab Rev* 2:215–241, 1986
46. Efanova I, Zaitsev SV, Zhivotovsky B, Köhler M, Efendic S, Orrenius S, Berggren P-O: Glucose and tolbutamide induce apoptosis in pancreatic β-cells. *J Biol Chem* 273:33501–33507, 1998
47. Wang L, Bhattacharjee A, Zuo Z, Hu F, Honkanen RE, Berggren PO, Li M: A low voltage-activated Ca^{2+} current mediates cytokine-induced pancreatic β-cell death. *Endocrinology* 140:1200–1204, 1999
48. Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T: Dual, functional role of membrane depolarization/Ca^{2+} influx in rat pancreatic β-cell. *Diabetes* 41:438–443, 1992
49. Gembal M, Gilon P, Henquin JC: Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse β cells. *J Clin Invest* 89:1288–1295, 1992
50. Prentki M, Tornheim K, Corkey BE: Signal transduction mechanisms in nutrient-induced insulin secretion. *Diabetologia* 40 (Suppl. 2):S32–S41, 1997

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51. Matthews DR: Physiological implications of pulsatile hormone secretion. *Ann N Y Acad Sci* 618:28–37, 1991
52. Lang DA, Matthews DR, Burnett M, Turner RC: Brief, irregular oscillations of basal plasma insulin and glucose concentration in diabetic man. *Diabetes* 30:435–439, 1981
53. Schmitz O, Porksen N, Nyholm B, Skjaerbaek C, Butler PC, Veldhuis JD, Pincus SM: Disorderly and nonstationary insulin secretion in relatives of patients with NIDDM. *Am J Physiol* 272:E218–E226, 1997
54. Matthews DR: Oscillatory insulin secretion: a variable phenotypic marker. *Diabet Med* 13 (Suppl. 6):53–58, 1996

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