Effects of High Glucose on Insulin Secretion by Isolated Rat Islets and Purified β-Cells and Possible Role of Glycosylation

FRANCESCO PURRELLO, MARIO VETRI, CONCETTA GATTA, DAMIANO GULLO, AND RICCARDO VIGNERI

We investigated the effect of 24 h of exposure to various glucose concentrations on insulin secretion by isolated rat pancreatic islets and purified rat β-cells. Compared with islets cultured with standard medium (5.5 mM glucose), islets cultured with 16.7 mM glucose showed a higher basal insulin release (means ± SE, 3.0 ± 0.5 vs. 0.7 ± 0.2%, $n = 8$, $P < .005$) and reduced glucose-stimulated insulin secretion (2.4 ± 0.3 vs. 5.8 ± 0.4%, $n = 8$, $P < .005$). Similar results were also obtained with purified β-cells. The effect of high glucose was time dependent (present after 12 h, maximal after 24 h) and reversible: when islets cultured with high glucose were transferred to standard medium, normal responsiveness to glucose was restored within 8 h and normal basal release within 24 h. Mannitol, 3-*O*-methylglucose, and 2-deoxyglucose were not able to mimic the effects of glucose. Islets or purified β-cells cultured in the presence of high glucose had a normal response when stimulated with glyburide, dibutyryl cyclic AMP, and isobutylmethylxanthine. Tunicamycin, an inhibitor of N-terminal glycosylation, prevented glucose-induced desensitization when added during 24 h of islet culture with 16.7 mM glucose. Swainsonine, another agent that influences glycosylation, had a similar effect. Our study indicates *1*) that 24 h of exposure to high glucose induces a specific and reversible impairment of insulin secretion in response to glucose, *2*) that this is a direct effect of glucose on β-cells, and *3*) that islet glucose metabolism and glycosylation processes may play a critical role in determining glucose desensitization. *Diabetes* 38:1417–22, 1989

Glucose is the major nutrient regulator for β-cell insulin secretion. When β-cells are chronically exposed to high glucose levels, subsequent insulin secretion in response to glucose may be impaired (1–3). This has been documented both in vitro and in vivo. In isolated rat islets, long-term perifusion with high glucose concentrations led to a progressive loss of response to the continuous glucose stimulation (4,5). Similarly, in animal models with genetic (6) or experimentally induced (7,8) hyperglycemia, insulin release in response to glucose was blunted. Furthermore, β-cell ability to suppress insulin secretion when plasma glucose concentration is low was impaired in normal subjects who had maintained hyperglycemia with the clamp technique (9), and the altered insulin secretion of type II (non-insulin-dependent) diabetic patients was improved by correcting hyperglycemia with diet, hypoglycemic agents, or insulin (10–12). All these observations led to the hypothesis that chronic hyperglycemia may adversely affect insulin secretion in diabetic patients and therefore worsen the progression of the disease (1).

In this study, we characterized the effect of 24 h of exposure to high glucose levels in pancreatic islets and purified β-cells isolated from Wistar rat pancreases and cultured under controlled conditions. In both of these systems, we show that cultures with high glucose concentrations altered both basal and glucose-stimulated insulin secretion, an effect that has been referred to as glucose-induced "desensitization" (4). The molecular mechanisms of this desensitization were also investigated.

RESEARCH DESIGN AND METHODS

Crude collagenase, trypsin, and bovine deoxyribonuclease were obtained from Boehringer Mannheim (Mannheim, FRG). Tunicamycin, swainsonine, cycloheximide, 3-*O*-methylglucose, and 2-deoxyglucose were obtained from Sigma (London, UK). Percoll was purchased from Pharmacia (Uppsala, Sweden). Culture medium CMRL-1066, Ham's F10 medium, heat-inactivated fetal calf serum (FCS), glutamine, and antibiotics were obtained from Gibco (Glasgow, UK). Guinea pig anti-insulin and rabbit anti-glucagon antibodies

From the Endocrinology Department, University of Catania, Garibaldi Hospital, Catania, Italy.

Address correspondence and reprint requests to Francesco Purrello, Cattedra di Endocrinologia, Ospedale Garibaldi, Catania, Italy.

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were a gift from D. Pipeleers (Brussels). Anti-guinea pig and anti-rabbit IgG fluorescein-labeled antibodies (SwAGp/FITC, GAR/FITC) were obtained from Nordic Immunological (The Netherlands).

Islet preparation. Pancreatic islets were isolated by the collagenase method (13) from 200- to 250-g fed male Wistar rats injected with 0.2 ml i.p. of a 0.2% pilocarpine solution 2 h before death by decapitation. Pancreases were distended by injecting 10 ml Krebs-Ringer-HEPES buffer (KRHB; 14) containing 1.5 mg/ml collagenase and were then removed, cleaned of lymph nodes and fat, and minced. After 18–22 min of incubation at 37°C under continuous shaking in KRHB containing 1.5 mg/ml collagenase, the pancreatic digest was filtered through a 500-μm nylon filter and washed three times with KRHB. The filter was washed in the same buffer and further incubated at 37°C for 10 min with continuous shaking. Islets were then handpicked under stereomicroscope observation. With this technique, 300–400 islets were isolated from each pancreas. This procedure was completed within 120 min.

β-Cell preparation. β-Cells were isolated from 1500 to 2000 pancreatic islets according to the method described by Pipeleers and Pipeleers-Marical (15). Islets were transferred to Ca^{2+}-free KRHB at 30°C with 1 mM EGTA and were gently resuspended with a Pasteur pipette. After 8 min, 16.5 μg/ml trypsin and 2 μg/ml DNAse were added, and a further 8- to 10-min incubation at 30°C was allowed. Islet cell dissociation was monitored by observing a drop of the suspension with a microscope, and the procedure was stopped by adding KRHB containing 1.67 mM calcium and 1% newborn calf serum. The suspension was then filtered through a 100-μm nylon filter, and the filtered aliquot containing isolated cells was centrifuged through a Percoll layer (density 1.040 g/ml). The pellet was gently resuspended in Ham's F10 medium containing 10% FCS and incubated for 20–30 min at 37°C.

At the end of this incubation the different types of islet cells were separated by counterflow centrifugation (centrifugal elutriation) at 15°C (15) with a Beckman J-21B centrifuge and a Beckman JE-6 rotor with speed (1960 rpm) controlled by a stroboscope (Palo Alto, CA). The separation chamber was perfused with Ca^{2+}-free KRHB with 1 g/dl bovine serum albumin. The flow rate was controlled by a peristaltic pump (Watson-Marlow 502S). Isolated pancreatic cells were resuspended in the mixing chamber (30-ml volume), and the pump was started at a flow rate of 6 ml/min, causing pancreatic cells to accumulate in the separation chamber. The fraction (90 ml) collected during this period contained no cells (15). The flow rate was subsequently increased to 18 ml/min (fraction 1), 50 ml/min (fraction 2), and 70 ml/min (fraction 3). For each of these flow rates, a 120-ml fraction was collected in sterile polycarbonate tubes. Tubes were then centrifuged at 1500 rpm, the pellets from each fraction were pooled, and an aliquot was taken for cell counting or staining with immunofluorescent antibodies.

Pancreatic cells were recovered as ~20% in fraction 1, 30% in fraction 2, and 50% in fraction 3. Immunostaining indicated that fraction 1 contained mainly (>70%) glucagon cells, whereas fractions 2 and 3 contained mainly (>90 and >80%, respectively) insulin-secreting cells. Fractions 2 and 3 were therefore pooled in experiments with purified β-cells.

Immunostaining was carried out in cells washed with phosphate-buffered saline (PBS) and resuspended in an equal volume of PBS and 4% formaldehyde in PBS. After 30 min at room temperature, cells were centrifuged and washed two times with PBS and once with PBS containing 0.5% bovine serum albumin. A drop of cell suspension (~50,000 cells) was then layered on a glass slide and dried overnight. The slides were then exposed to 50% ethanol at −20°C for 20 min, washed three times with PBS, and incubated for 1 h at room temperature with guinea pig anti-insulin or rabbit anti-glucagon antibodies. The slides were then washed four times with PBS and incubated 45 min at room temperature with fluorescein-conjugated anti-guinea pig or anti-rabbit IgG antiserums. After four more washes with PBS, cells were incubated with 2% formaldehyde for 30 min at room temperature and dried.

Experimental protocol. Purified islets or pancreatic β-cells were incubated in medium (CMRL-1066 or Ham's F10, respectively) containing 10% heat-inactivated FCS, L-glutamine, antibiotics, and different concentrations of glucose from 5.5 to 16.7 mM. In experiments with the glucose analogues 3-*O*-methylglucose and 2-deoxyglucose or with mannitol, 11.2 mM of the corresponding glucose analogue was added to the glucose concentration of the medium (5.5 mM) to reach a final glucose concentration of 16.7 mM in the medium. Incubations were carried out at 37°C in a 95% O_2/5% CO_2 atmosphere. At the end of culture, islets or β-cells were centrifuged, washed, resuspended in KRHB containing 1.4 mM glucose, and allowed to recover for 60 min at 37°C. Glucose or other secretagogues at various concentrations were then added, and insulin in the medium or in acid-alcohol cell extracts was measured after 30–60 min by radioimmunoassay with porcine insulin as a standard. Cell viability was studied with trypan blue or neutral red and was >90% in both islets and cells cultured at 5.5 and 16.7 mM glucose.

Results were expressed as the percentage of total islet insulin content that was released into the medium. Glucose-stimulated insulin secretion was calculated as the difference

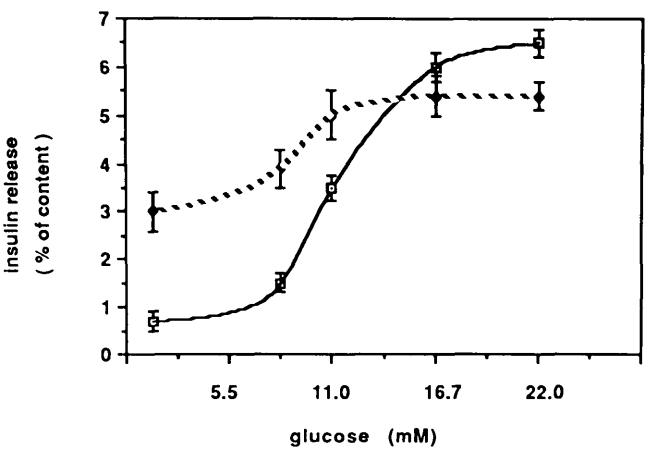

FIG. 1. Pancreatic islets were cultured for 24 h in CMRL-1066 medium containing 5.5 (*solid line*) or 16.7 (*broken line*) mM glucose. Islets were then washed, incubated in Krebs-Ringer-HEPES buffer with 1.4 mM glucose for 60 min, and then transferred to buffer with various glucose concentrations. Insulin released in medium was measured after 60 min and incubation at 37°C and was expressed as percentage of total islet insulin content. Results are means ± SE of 8 experiments.

between insulin secretion in response to maximal glucose stimulation and insulin release measured under basal conditions.

RESULTS

Glucose-stimulated insulin secretion. Increasing glucose concentrations elicited different secretory responses in islets cultured for 24 h in standard medium (5.5 mM glucose, control islets) or medium containing high glucose (16.7 mM) (Fig. 1). In islets preexposed to high glucose, insulin release under basal conditions (i.e., with a nonstimulatory [1.4 mM] glucose) was higher than in control islets, and insulin secretion in response to maximal glucose stimulation (16.7 or 22 mM) was reduced (Fig. 1). In eight experiments, basal insulin release (mean ± SE) was 0.7 ± 0.2% in control islets and 3.0 ± 0.5% in islets cultured in parallel with 16.7 mM glucose ($P < .005$). The increased basal insulin release of islets cultured with 16.7 mM glucose was energy dependent (release decreased to 0.5 ± 0.2% in the presence of 0.3 mM 2,4-dinitrophenol) and temperature dependent (release decreased to 1.2 ± 0.4% when incubation was at 4°C) but not Ca^{2+} dependent (2.5 ± 0.4% in Ca^{2+}-free buffer). When exposed to 22 mM glucose for 1 h, glucose-stimulated insulin secretion was 5.8 ± 0.4% in control islets and 2.4 ± 0.3% in islets cultured with high glucose ($P < .005$). The effect of the glucose concentration during 24 h of culture on basal and glucose-stimulated insulin secretion is shown in Fig. 2. A significant difference with respect to control islets was observed in islets cultured with 13.8 mM glucose ($P < .01$). The increased insulin release due to 24 h of exposure to high glucose did not significantly reduce islet insulin content (52 ± 3 vs. 45 ± 4 ng/islet, $n = 8$, NS). Therefore, even when expressed in absolute values, basal insulin release is increased and glucose-stimulated insulin release is decreased in islets cultured for 24 h with high glucose concentration.

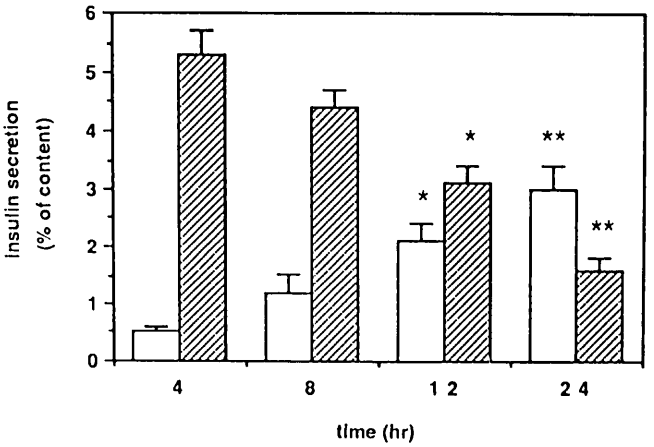

FIG. 3. Time course of glucose effect on insulin secretion. Islets were incubated for indicated times in CMRL-1066 medium containing 16.7 mM glucose. Basal (*open bars*) and glucose-stimulated (*hatched bars*) insulin secretion were measured as described in RESEARCH DESIGN AND METHODS. Results are means ± SE of 3 experiments. *$P < .05$, **$P < .005$, vs. islets cultured with 5.5 mM glucose.

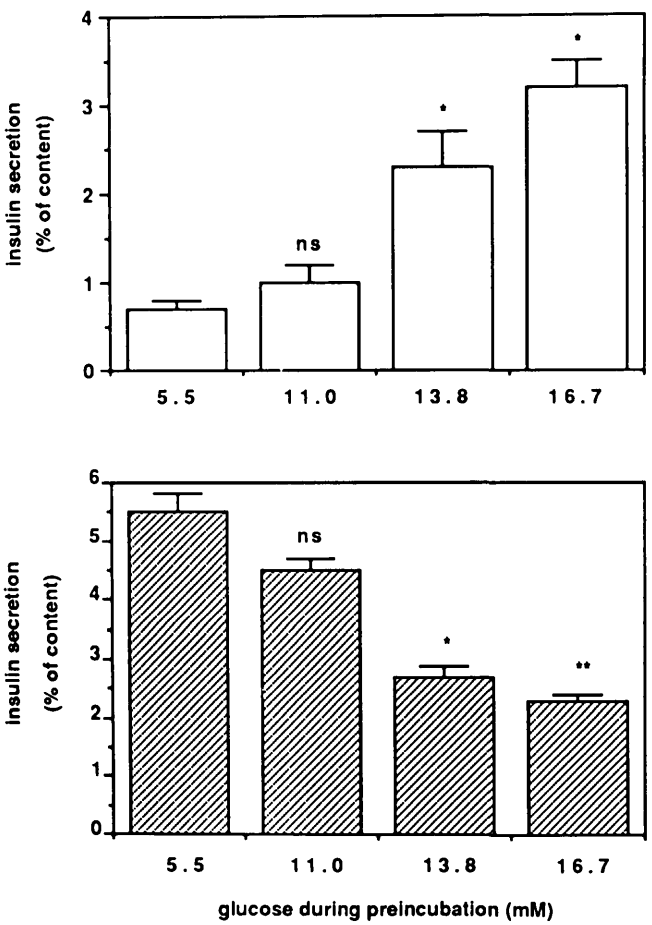

FIG. 2. Pancreatic islets were cultured for 24 h in CMRL-1066 medium containing 5.5, 11, 13.8, or 16.7 mM glucose. Basal (*top*) and glucose-stimulated (*bottom*) insulin secretion was measured as indicated in RESEARCH DESIGN AND METHODS. Basal insulin release was calculated with 1.4 mM glucose. Glucose-stimulated insulin secretion is expressed as difference between insulin released in response to maximal stimulating glucose concentration (22 mM) and basal insulin release. Results are means ± SE of 3 experiments. *$P < .01$, **$P < .005$, vs. islets cultured with 5.5 mM glucose.

The effect of high glucose concentration during culture was time dependent (Fig. 3). A significant effect was present after 12 h, and maximal effect was reached after 24 h, because no further increase was observed at 48 h (data not shown). Under these experimental conditions, the effect of glucose was reversible. When islets cultured with 16.7 mM glucose were transferred to 5.5 mM glucose, glucose-stimulated insulin secretion returned to control islet values within 8 h, and basal insulin release returned to control values within 24 h (Table 1). In parallel experiments, islets cultured with 5.5 mM glucose for two consecutive 24-h periods showed no difference in basal or glucose-stimulated insulin secretion at the end of 48 h compared with islets cultured with 5.5 mM glucose for 24 h (data not shown). Mannitol (11.2 mM) added to 5.5 mM glucose (raising the glucose concentration to 16.7 mM) was without effect on either basal or glucose-stimulated insulin secretion (Table 2). Also, two nonmetabolizable glucose analogues were without effect: when either 11.2 mM 3-*O*-methylglucose (transported within the cells but not further metabolized) or 11.2 mM 2-deoxyglucose (transported and phosphorylated but not further metabolized) was added to the standard culture medium, no significant change compared with control islets was observed in either basal or glucose-stimulated insulin secretion (Table 2).

TABLE 1
Reversibility of effect of high glucose on insulin secretion (%)

Culture condition	1.4 mM glucose	22 mM glucose	Glucose-stimulated change
5.5 mM glucose for 24 h	0.5 ± 0.1	4.8 ± 0.9	4.3 ± 0.9
16.7 mM glucose for 24 h	2.8 ± 0.5*	3.9 ± 0.8	1.1 ± 0.7*
16.7 mM glucose for 24 h + 5.5 mM glucose for 8 h	1.3 ± 0.2†	5.7 ± 0.8	4.4 ± 0.8
16.7 mM glucose for 24 h + 5.5 mM glucose for 24 h	0.8 ± 0.2	6.0 ± 0.6	5.2 ± 0.6

Insulin secretion was measured after incubating the islets in Krebs-Ringer-HEPES buffer for 60 min at 37°C with 1.4 or 22 mM glucose. Glucose-stimulated insulin secretion was calculated by subtracting basal release from values obtained in response to maximal glucose stimulation (22 mM). Results are means ± SE of 4 experiments.
*$P < .01$, †$P < .05$, vs. 5.5 mM glucose for 24 h.

Culturing purified β-cells for 24 h with 5.5 or 16.7 mM glucose caused effects similar to those obtained with intact islets. Basal insulin release was increased (1.8 ± 0.1 vs. 0.8 ± 0.1% in experimental and control cells, respectively, $P < .05$), and glucose-stimulated insulin secretion was decreased (0.52 ± 0.13 vs. 2.69 ± 0.07%, $P < .005$; Table 3). These data indicate that the presence of other islet cells is not necessarily required to observe glucose-induced impairment of the β-cell's ability to secrete insulin in response to glucose.

Sulphonylurea- or cyclic AMP–stimulated insulin secretion. Exposure to 16.7 mM glucose did not alter the secretory capacity of either pancreatic islets or purified β-cells in response to glyburide, dibutyryl cyclic AMP, or isobutylmethylxanthine (Fig. 4; Table 3). The insulin secretion stimulated by these secretagogues was in fact not significantly different whether islets or β-cells were cultured with 5.5 or 16.7 mM glucose.

Other studies. Because a high environmental glucose concentration may affect glycosylation reactions and these reactions may in turn play a role in cell surface transport of secretory proteins (16,17), we investigated the effect of agents that influence glycosylation on glucose-induced desensitization. Tunicamycin, an agent that reportedly influences glycosylation by interfering with the dolichol-phosphate reaction and inhibiting N-terminal glycosylation in rough endoplasmic reticulum (18), was added at 0.5 and 2 μg/ml during 24 h of islet culture. Tunicamycin did not affect glucose-stimulated insulin secretion in control islets but prevented the effect of high glucose in blunting glucose-stimulated insulin secretion (Table 4). Tunicamycin had no effect on basal insulin release either in control islets or islets cultured with 16.7 mM glucose (data not shown).

Similar results, although less evident, were obtained with 0.5 and 2 μg/ml swainsonine, an inhibitor of the Golgi mannosidase II that removes the α-1,3– and α-1,6–linked mannose residues from the $GlcNAc_3Man_5$ structure and induces the formation of hybrid oligosaccharide chains (19,20) (Table 4). In contrast to the effect observed with glycosylation inhibitors, the addition of 2 μg/ml cycloheximide, an inhibitor

TABLE 2
Effect of glucose analogues on insulin secretion (%)

Culture condition	1.4 mM glucose	22 mM glucose	Glucose-stimulated change
5.5 mM glucose	0.3 ± 0.1	4.7 ± 0.3	4.4 ± 0.3
16.7 mM glucose	2.4 ± 0.3	3.7 ± 0.4	1.3 ± 0.2*
5.5 mM glucose + 11.2 mM mannitol	0.2 ± 0.1	4.6 ± 0.4	4.4 ± 0.3
5.5 mM glucose + 11.2 mM 2-DG	0.1 ± 0.1	4.7 ± 0.4	4.6 ± 0.4
5.5 mM glucose + 11.2 mM 3-OMG	0.1 ± 0.1	4.4 ± 0.3	4.3 ± 0.3

Islets were cultured for 24 h in CMRL-1066 medium containing glucose, mannitol, 2-deoxyglucose (2-DG), or 3-*O*-methylglucose (3-OMG) at indicated concentrations. Insulin secretion was measured after incubating the islets in Krebs-Ringer-HEPES buffer for 60 min at 37°C with 1.4 or 22 mM glucose. Glucose-stimulated insulin secretion was calculated by subtracting basal release from the values obtained in response to maximal glucose stimulation (22 mM). Results are means ± SE of 4 experiments.
*$P < .01$ vs. 5.5 mM glucose.

TABLE 3
Effect of high glucose on insulin secretion (%) by purified β-cells

	Culture condition	
	5.5 mM glucose	16.7 mM glucose
Basal insulin release	0.8 ± 0.1	1.8 ± 0.1*
Stimulation		
22 mM glucose	2.7 ± 0.1	0.5 ± 0.1†
22 mM glucose + 1 mM IBMX	9.8 ± 1.1	11.6 ± 1.1
10 μM glyburide	1.0 ± 0.2	1.7 ± 0.4

Purified β-cells were cultured for 24 h in Ham's F10 medium containing 5.5 or 16.7 mM glucose. Cells were then washed, transferred in Krebs-Ringer-HEPES buffer with 1.4 mM glucose for 30 min and then incubated for 60 min at 37°C in buffer with glucose or other secretagogues. Basal insulin release was calculated with 1.4 mM glucose. Glucose-, isobutylmethylxanthine (IBMX)-, and glyburide-stimulated insulin secretion were calculated by subtracting the respective basal insulin release in each experiment. Results are means ± SE of 4 experiments.
*$P < .05$, †$P < .005$, vs. 5.5 mM glucose.

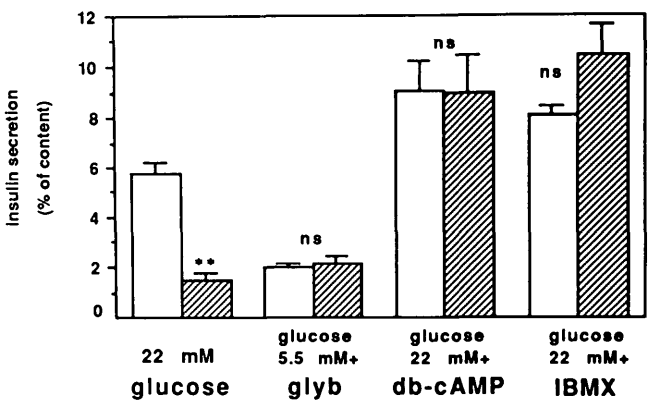

FIG. 4. Pancreatic islets were cultured for 24 h in CMRL-1066 medium containing 5.5 (*open bars*) or 16.7 (*hatched bars*) mM glucose. Islets were then transferred to Krebs-Ringer-HEPES buffer and incubated for 60 min at 37°C in presence of 22 mM glucose, 10 μM glyburide (glyb), 22 mM glucose plus 1 mM dibutyryl cyclic AMP (db-cAMP), or 22 mM glucose plus 1 mM isobutylmethylxanthine (IBMX). Insulin secretion induced by these secretagogues was calculated by subtracting basal insulin release. Results are means ± SE of 5 experiments. **$P < .005$.

TABLE 4
Effect of glycosylation inhibitors on glucose-stimulated insulin secretion (%)

Inhibitors	Culture condition	
	5.5 mM glucose	16.7 mM glucose
None	4.77 ± 0.10	1.02 ± 0.20*
0.5 μg/ml tunicamycin	3.96 ± 0.56	2.79 ± 0.28
2 μg/ml tunicamycin	4.12 ± 0.09	3.94 ± 0.19
0.5 μg/ml swainsonine	4.24 ± 0.43	1.49 ± 0.34†
2 μg/ml swainsonine	4.08 ± 0.58	2.37 ± 0.29

Islets were cultured for 24 h in CMRL-1066 medium containing 5.5 or 16.7 mM glucose. Glucose-stimulated insulin secretion was measured after incubating the islets in Krebs-Ringer-HEPES buffer for 60 min at 37°C with 22 mM glucose and was calculated by subtracting basal release. Results are means ± SE of 7 experiments. *$P < .005$, †$P < .05$, vs. 5.5 mM glucose.

of protein synthesis, did not influence basal insulin release but significantly decreased glucose-stimulated insulin secretion in control islets and in islets cultured with high glucose (Table 5).

DISCUSSION

Our study demonstrates that 24 h of exposure of rat pancreatic islets and purified β-cells to high glucose levels causes increased insulin release in the presence of nonstimulatory glucose concentrations and, at the same time, impairs insulin secretion in response to a maximal glucose stimulation. The increased basal insulin release caused by 24 h of exposure to high glucose is not due to membrane damage or leaking from dead cells because *1*) cell viability is >90% in these cells indicated by trypan blue and neutral red, *2*) normal basal insulin release may be restored when islets are transferred to a standard culture medium with 5.5 mM glucose, and *3*) the effect of high glucose is decreased at low temperatures, or in the presence of 2,4-dinitrophenol. In contrast, increased basal insulin release is unaffected by Ca^{2+} deprivation in the buffer; a similar Ca^{2+} independency of non-glucose-stimulated insulin release has been reported by others (21).

TABLE 5
Effect of cycloheximide on insulin secretion (%)

Culture condition	1.4 mM glucose	22 mM glucose	Glucose-stimulated change
5.5 mM glucose	0.6 ± 0.2	4.9 ± 0.8	4.3 ± 0.7
+2 μg/ml cycloheximide	0.6 ± 0.3	3.5 ± 0.4	2.8 ± 0.3*
16.7 mM glucose	3.3 ± 0.8†	4.3 ± 0.9	1.0 ± 0.2
+2 μg/ml cycloheximide	3.0 ± 0.7†	3.5 ± 0.7	0.5 ± 0.1*

Islets were cultured for 24 h in CMRL-1066 medium containing 5.5 or 16.7 mM glucose with or without 2 μg/ml cycloheximide. Insulin secretion was measured after incubating the islets in Krebs-Ringer-HEPES buffer for 60 min at 37°C with 1.4 or 22 mM glucose. Glucose-stimulated insulin secretion was calculated by subtracting basal release from values obtained in response to maximal glucose stimulation (22 mM). Results are means ± SE of 3 experiments.
*$P < .05$ vs. no cycloheximide.
†$P < .01$ vs. 5.5 mM glucose.

The impaired insulin secretion in response to a maximal glucose stimulation by islets chronically exposed to high glucose concentrations has been observed in perifused rat islets and has been called glucose-induced desensitization (4). The findings with perifused islets suggest that the high local insulin concentrations are not responsible for the decreased secretory response of islets cultured in the presence of high glucose. In the perifusion system, in fact, secreted insulin is continuously removed from the islet environment. The high basal insulin secretion and the maintained response to other secretagogues that we observed in islets exposed to high glucose are also in contrast to the negative-feedback effect of insulin on insulin secretion by pancreatic islets.

Under our experimental conditions, pancreatic islet glucose-induced desensitization is a time-dependent phenomenon. In addition, this effect is fully reversible; when transferred to a lower-glucose medium, islets recover their ability to respond to a maximal glucose stimulation. This recovery is more prompt than the recovery of normal basal insulin release, suggesting that the mechanism(s) responsible for the alteration of basal and glucose-stimulated insulin secretion may not be completely identical. Interestingly, the inhibitors of glycosylation also have different effects on basal and glucose-stimulated insulin secretion.

Exposure to high glucose concentrations specifically altered the ability of pancreatic islets to respond to glucose stimulation without affecting their ability to release insulin in response to other stimuli, e.g., glyburide, dibutyryl cyclic AMP, and isobutylmethylxanthine. These observations indicate that the glucose-induced desensitization was not caused by β-cell exhaustion due to intracellular insulin depletion and also suggest that the high basal secretion per se is not responsible for the subsequent impaired response to glucose. Further circumstantial evidence supports this conclusion: tunicamycin restores insulin secretion in response to glucose despite a persistent increased basal release, and, in the reversibility experiments, insulin secretion in response to glucose was restored 8 h after high glucose was removed despite still-elevated basal insulin release.

The mechanism of glucose-induced desensitization in β-cells is not known. Evidence indicates that this phenomenon is not a consequence of impaired insulin synthesis. Instead, glucose is known to increase steady-state insulin mRNA (22–24) and insulin synthesis at the translational level (25). Moreover, in experiments similar to ours, insulin synthesis has been reported unchanged and the conversion rate of proinsulin to insulin increased in the presence of high glucose concentrations (26). Therefore, it is likely that glucose-induced desensitization involves releasing mechanisms. Our study contributes to improve our understanding of this phenomenon. First, the observation that glucose also has a similar effect in highly purified β-cells suggests a direct action of glucose on β-cells without the necessary intervention of other islet cells via a paracrine action. Second, the observation that the glucose analogues that are transported (3-O-methylglucose) or transported and phosphorylated (2-deoxyglucose) are unable to mimic glucose effects suggests that β-cell glucose metabolism may be a necessary prerequisite for the induction of glucose-induced desensitization. Third, studies with agents that influence the glyco-

sylation process support the possibility that the increased availability of environmental glucose may cause an increased intracellular concentration of glycosylated compounds and that these compounds, in turn, may be responsible for desensitization, either directly or through increased glycosylation of protein(s).

Glucose-induced desensitization in rat pancreatic islets chronically exposed to high glucose concentrations is a phenomenon characterized by a higher basal insulin release and a lower responsiveness to further glucose stimulation. This pattern resembles what is commonly observed in patients with type II diabetes mellitus. Our in vitro model may therefore be of interest in studying the molecular mechanisms of glucose toxicity in diabetes.

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