Metabolism
Clinical and Experimental

VOL 51, NO 6 JUNE 2002

Glucose Desensitization in INS-1 Cells: Evidence of Impaired Function Caused by Glucose Metabolite(s) Rather Than by the Glucose Molecule per se

Birgitte Brock, Jane H. Mogensen, Søren Gregersen, and Kjeld Hermansen

Type 2 diabetes characteristically involves disturbances of the β-cell function including reduced insulin secretion in response to elevated glucose. In experimental diabetes, β cells are often "blind" to glucose, and clonal β-cell lines chronically exposed to glucose show impaired glucose sensing. The present study focuses on the effect of long-term exposure to high-glucose concentrations on insulin secretion, insulin store, and insulin mRNA content in the β-cell line INS-1. The cellular insulin mRNA content has been shown to be reduced by approximately 90% on such exposure for 4 days. This decrement could be partly counteracted by subsequent culture for 4 days at low glucose, while daily alternate culture in high and low glucose did not prevent the insulin mRNA content from being reduced. The insulin release from cells cultured at high glucose was simultaneously reduced by 50%. This change was, however, not reversed by subsequent culture at low glucose, a pattern also found for the intracellular insulin stores. The suppression of insulin mRNA, insulin release, and intracellular insulin stores induced by high glucose was completely neutralized by the metabolic glucokinase blocker, mannoheptulose, while 2-deoxyglucose, a phosphoglucose isomerase blocker, had no impact. This suggests that glucokinase activity may have a negative regulatory effect. Addition of D-glyceraldehyde (DG) induced an increase in insulin release, while insulin mRNA remained unaltered. It would therefore seem that at least one glucose metabolite is involved in the glucose desensitization in INS-1 cells, which opens the prospect of regulatory factor(s), which possess(es) negative, as well as positive, actions.

TYPE 2 DIABETES IS characterized by an impaired ability of the β cell to maintain appropriate insulin secretion in response to elevated plasma glucose.[1-3] The impaired insulin secretion is partially reversed by normalizing elevated glucose levels in vivo[4,5] and in vitro,[6] and it has therefore been suggested that hyperglycemia per se may alter normal β-cell function.[4,7,8]

Attention has increasingly focused on methods for characterizing pathophysiologic changes contributing to or caused by hyperglycemia. The glucose toxicity hypothesis proposes that in chronic hyperglycemia the glucose molecule and/or its metabolites suppress the secretory potential of the β cell.[9-12] The use of the term glucose toxicity is, however, controversial. It has been suggested that this expression should be reserved for irreversible effects on the process of insulin gene transcription and/or expression, while reversible glucose-induced effects should be designated glucose desensitization.[13] Leahy[14] has proposed that glucose toxicity will eventually be recognized as a global impairment of the glucose-regulated β-cell function involving more than one molecular cause. According to the latter concept, high glucose initially augments β-cell glucose sensitivity and thus causes depletion of a substance, a cofactor, or a substrate, which is required to maintain an insulin secretion that is partly reversible by normalization of the circulating glucose level.[15] Glucose per se is known to regulate the transcription of insulin mRNA.[16-20] Chronic exposure of human pancreatic islets to high glucose in vitro, furthermore, impairs the β-cell function by reducing the insulin mRNA content,[8,21,22] the insulin stores, and the basal insulin release.[21] A similar effect on mRNA levels has been found in rat islets long-term cultured in high glucose, and desensitization to glucose was found in transformed β-cell lines.[8,23,24] This invites

From the Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark.

Submitted December 27, 2000; accepted December 18, 2001.

Supported by the Aarhus University Research Foundation, the Institute for Experimental Clinical Research, Aarhus University, the Faculty of Medicine, Aarhus University, Aarhus Amtssygehus' forskningsfond, Aarhus University Hospital, the Danish Diabetes Association, the Novo-Nordisk Foundation, Poul and Erna Sehested Hansens Foundation, and Margrethe Møller Fonden.

Address reprint requests to Birgitte Brock, MD, PhD, Department of Clinical Pharmacology, The Bartholin Building, University of Aarhus, DK-8000 Aarhus, Denmark.

0026-0495/02/5106-0004$35.00/0

doi:10.1053/meta.2002.32722

the question whether long-term exposure to glucose per se affects insulin release, intracellular insulin stores, and insulin mRNA content and, if so, this can be ascribed to glucose per se and/or glucose metabolites, cofactors or enzymes within the glycolytic pathway. The present report addresses this issue by subjecting the glucose-sensitive β-cell line INS-1 to a long-term exposure to high glucose, reexposed cells to lower glucose, and exposed cells alternately to high and low concentrations of glucose. We also explored the effect of blocking glucokinase and phosphoglucose isomerase on the alterations obtained from glucose exposure and, lastly, supplied cells with additional trioses, all in an attempt to narrow down the area from which to find key players contributing to the glucose desensitization phenomenon.

MATERIALS AND METHODS

Culturing of Cells

The glucose-sensitive insulin-secreting cell line INS-1 (kindly provided by Professor C.B. Wollheim, Geneva, Switzerland) was cultured in RPMI 1640 medium (Gibco, BRL, Paisley, UK) containing 11 mmol/L glucose and supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (Life Technologies, Rockville, MD),[25] 10 mmol/L N-(2hydroxyethyl)piperazine-N′-(2-ethanesulphonic acid) (HEPES), 100 IU/mL penicillin (Life Technologies), 100 mg/mL streptomycin (Life Technologies), 1 mmol/L sodium pyruvate, and 50 mmol/L β-mercaptoethanol in a humidified (95% air/5% CO_2) atmosphere. The experiments were performed in the same medium, but supplemented with specific sugars and trioses according to the protocol. For RNA extraction, cells were seeded in 50-mL culture flasks (NUNC, Roskilde, Denmark) at a density of 0.75×10^6 cells/mL. Cells were cultured for a total of 7 days and exposed to 11 mmol/L glucose for 3 days and the ensuing time to 3.3 mmol/L glucose (Sigma Chemical, St Louis, MO), 6.6 mmol/L glucose, 16.7 mmol/L glucose, 6.6 mmol/L glucose plus 5 mmol/L mannoheptulose (M)[26,27] (Sigma), 16.7 mmol/L glucose plus 5 mmol/L mannoheptulose, 6.6 mmol/L glucose plus 2.5 mmol/L 2-deoxyglucose (D) (Sigma), 16.7 mmol/L glucose plus 2.5 mmol/L 2-deoxyglucose, 6.6 mmol/L glucose plus 0.1 mmol/L D-glyceraldehyde (DG) (Sigma), and 6.6 mmol/L glucose plus 0.5 mmol/L dihydroxyacetone (DHA) (Sigma). Cells cultured for a total of 11 days were exposed to 11 mmol/L glucose for 3 days and were cultured the ensuing time (8 days) at 6.6 mmol/L glucose, 8 days at 26.6 mmol/L glucose, 4 days at 6.6 mmol/L glucose plus 4 days at 26.6 mmol/L glucose, 4 days at 26.6 mmol/L glucose plus 4 days at 6.6 mmol/L glucose, and for 8 days at alternately 6.6 mmol/L glucose and 26.6 mmol/L glucose with daily changes, respectively.

Insulin Secretion

INS-1 cells were plated (3.0×10^5 cells/mL into 24-well plates) (NUNC) at 11 mmol/L glucose and cultured for 3 days. Four days before measurement of the insulin secretory capacity, cells were cultured at the carbohydrate concentrations mentioned above. To determine the insulin release, the INS-1 cells were incubated for 60 minutes in a HEPES-buffered medium containing 125 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 1.28 mmol/L $CaCl_2$, 25 mmol/L HEPES, and 0.1% human serum albumin (Hoechst, Behring, Germany) plus specific sugars/trioses according to the protocol. Insulin release was measured as insulin output (insulin secretion in response to 1 hour exposure to the HEPES buffer containing the same type and concentration of sugar as the culture medium without prior preincubation) and as the glucose-stimulated insulin secretion (GSIS) (insulin secretion in response to 1 hour exposure to HEPES buffer containing 16.7 mmol/L glucose). The incubation medium was collected for subsequent insulin analysis. The cells were lysed in 0.1 mol/L NaOH, and total protein was determined by Bradford's method using Bio-rad protein assay dye reagent (Bio-Rad Laboratories, Hercules, CA). The insulin secretion/total protein ratio was calculated.

Insulin Content

INS-1 cells were plated (3.0×10^5 cells/well into 24-well plates) (NUNC) at 11 mmol/L glucose and cultured for 3 days. Four days before measurement of insulin content, cells were cultured at 6.6 mmol/L glucose, 16.7 mmol/L glucose, 6.6 mmol/L glucose plus 5 mmol/L mannoheptulose, and 16.7 mmol/L glucose plus 5 mmol/L mannoheptulose. Cells were washed in Earle's basal medium (Gibco, BRL) at room temperature before adding 1 mL of ice cold medium in which the cells were scraped off with a rubber policeman. After centrifugation (5,000 rpm, 5 minutes, 4°C), the medium was discharged and the intact cells were divided in 2 parts: one part was resuspended in a glycine- bovine serum albumin (BSA) buffer containing 0.75% glycine and 0.25% BSA adjusted to pH 8.8 for insulin determination after sonication (Bronson sonicator 2 · 15 seconds on ice; Bronson Ultrasonic, Danburg, CT) and centrifugation at $30,000 \times g$ for 30 minutes at 4°C. The supernatant was collected for subsequent insulin analysis. The other part was resuspended in 0.1 mol/L NaOH for protein determination.

mRNA Analysis

The cells were lysed in the culture flask using 1 mL Trizol (Gibco). Total RNA was extracted according to the protocol provided by the manufacturer with the modification of an extra phenol/chloroform (Sigma) extraction. Integrity and concentration measurements were controlled on a 1% agarose gel (SeaKem GTG, FMC Bioproducts, Rockland, ME). RNA samples (10 μg) were denatured by incubation in formamide, subjected to electrophoresis in a 1% agarose gel containing formaldehyde and formamide, and transferred to a nylon membrane (HYBOND-N, Amersham, Buckinghamshire, England). The insulin mRNA sequence was detected by Northern blot hybridization with a nick-translated (Promega, Madison, WI) ^{32}P-labeled cDNA probe containing rat insulin II cDNA.[26] Autoradiography was performed at -80°C using Fuji medical x-ray film (Fuji, Fuji, Japan). The autoradiograms were analyzed by densitometric scanning. The 18s and 28s ribosomal RNA bands were used to ensure concentration and integrity.

Insulin Assay

Samples of the incubation medium were immediately frozen for later insulin analysis. The concentration of insulin in the medium was determined by radioimmunoassay with a guinea pig antiporcine antibody (Novo Nordisk, Bagsvaerd A/S, Denmark) and mono-^{125}I-(Tyr A14)-labeled human insulin (Novo Nordisk) as tracer and rat insulin (Novo Nordisk) as standard. Free and bound radioactivity was separated using ethanol.[28]

Statistical Analysis

Students unpaired *t* test was used for statistical comparisons of the results regarding insulin release, while Mann-Whitney's nonparametric test was used for statistical comparison of the mRNA results.

RESULTS

Studies of Insulin mRNA Content

Reduction of the glucose level from 16.7 mmol/L to 6.6 mmol/L reduced insulin content by 71% ($P < .05$) in INS-1 cells cultured for 4 days (Fig 1A). Culturing at 3.3 mmol/L reduced insulin content by 64% ($P < .05$). Elevation of glucose from 6.6 mmol/L to 26.6 mmol/L for 4 days decreased the

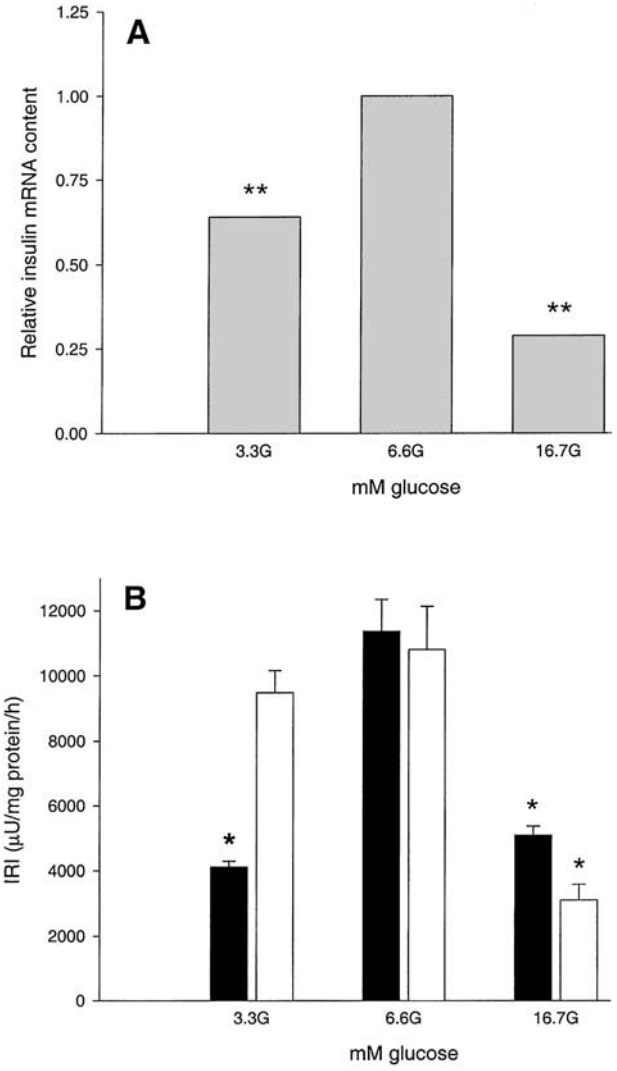

Fig 1. Effect of glucose exposure on insulin secretion and insulin mRNA contents. INS-1 cells were cultured for 4 days in 3.3 mmol/L, 6.6 mmol/L, and 16.7 mmol/L glucose. (A) Relative insulin mRNA content: 0.64 (0.50 to 0.74), 1.0 (0.95 to 1.08), and 0.29 (0.26 to 0.33). Results are given as means (range) from 4 separate experiments and is given relative to the content at 6.6 mmol/L glucose. (*$P < .0002$; **$P < .05$ compared with 6.6 mmol/L glucose). (B) Insulin secretion determined as insulin output (■) and GSIS (□) (see Materials and Methods). The values are mean ± SEM from 9 to 10 individual incubations performed in 2 separate experiments and expressed as immunoreactive insulin (IRI)/milligram total cell protein/hour (μU/mg/h).

insulin mRNA content considerably ($P < .05$) (Fig 2A), whereas exposure to 26.6 mmol/L for 8 days almost abolished the expression of insulin mRNA ($P < .05$) (Fig 2A). Cells first cultured for 4 days at 26.6 mmol/L glucose and then reexposed to 6.6 mmol/L glucose for another 4 days only partly restored the insulin mRNA content, ie, by 76% ($P < .05$) (Fig 2A). To further characterize the relationship between glucose exposure and downregulation of insulin mRNA, INS-1 cells were cultured with daily changes between 6.6 and 26.6 mmol/L glucose for 8 days (Fig 2A). Cells cultured like this prior to RNA extraction contained about one tenth of the amount of insulin mRNA found in cells continuously cultured at 6.6 mmol/L glucose ($P < .05$). The response of these cells was comparable to cells cultured for 4 days at high-glucose concentrations (Fig 2A). Addition of 5 mmol/L mannoheptulose to inhibit glucokinase to 6.6 mmol/L glucose did not alter the insulin mRNA content (Fig 3A [6.6 G *v* 6.6 G+M]). However, 5 mmol/L mannoheptulose prevented the glucose-induced downregulation of insulin RNA found in cells cultured for 4 days at 16.7 mmol/L glucose and even seemed to upregulate the expression as compared with 6.6 mmol/L glucose (by 51%, $P = .07$) (Fig 3A [6.6 G *v* 16.7 G+M]). Addition of 2.5 mmol/L 2-deoxy-

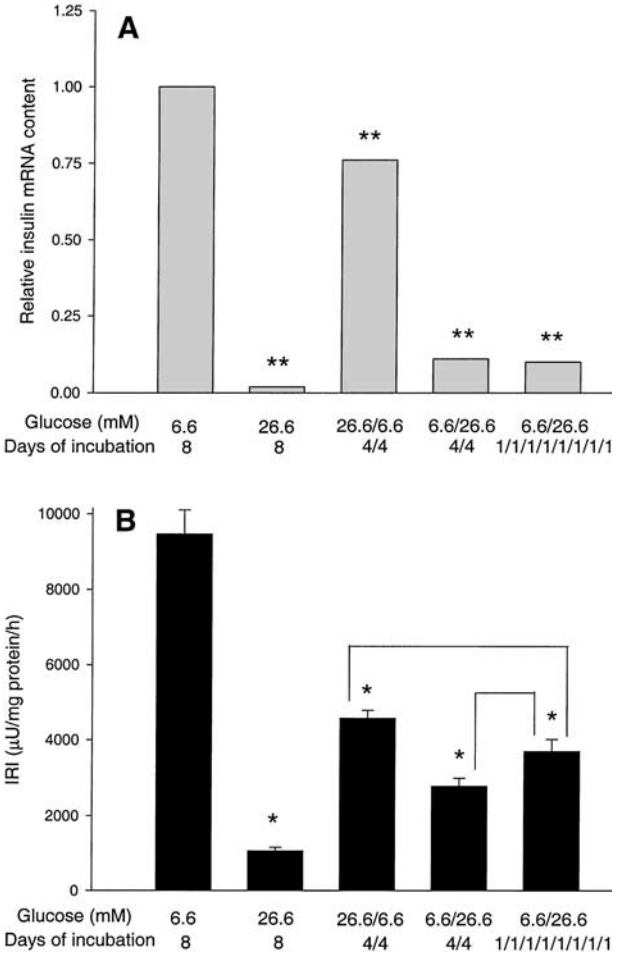

Fig 2. Effect of alternate glucose exposure on insulin secretion and mRNA content. Cells were incubated in 6.6 mmol/L glucose for 8 days, 26.6 mmol/L glucose for 8 days, 26.6 mmol/L glucose for 4 days followed by 6.6 mmol/L for 4 days, 6.6 mmol/L glucose for 4 days followed by 26.6 mmol/L for 4 days or alternate daily between 26.6 mmol/L and 6.6 glucose for 8 days ending on 26.6 mmol/L, respectively. (A) Relative insulin mRNA content: 1 (0.93 to 1.05), 0.02 (0.0 to 0.06), 0.76 (0.60 to 0.93), 0.11 (0.02 to 0.20), 0.10 (0.02 to 0.18). Results are given as means (range) of 4 separate experiments. (*$P < .0001$; **$P < .05$ compared with 6.6 mmol/L glucose). (B) Insulin output. The values are mean ± SEM from 8 individual incubations performed in 2 separate experiments.

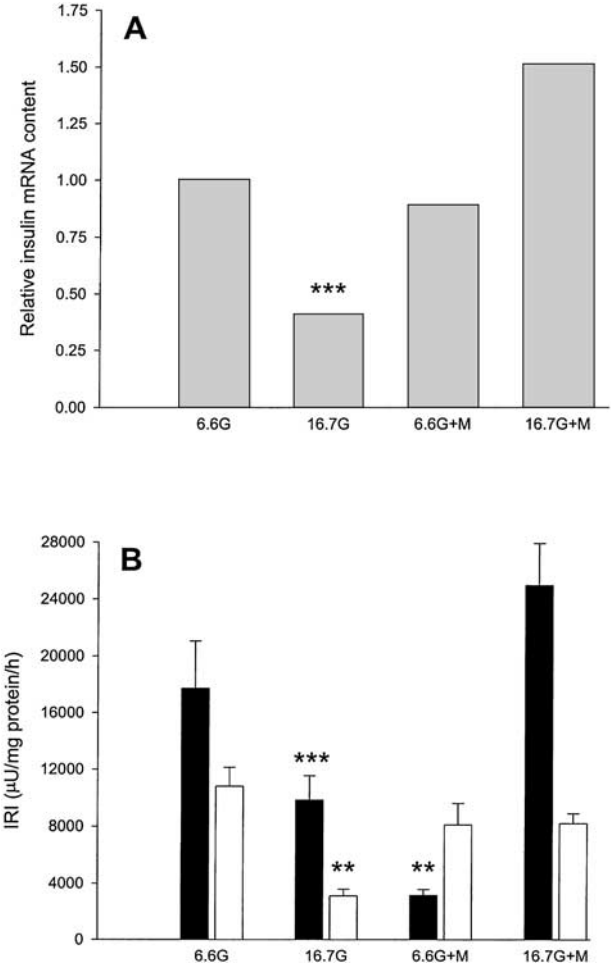

Fig 3. Effects of long-term exposure to the metabolic blocker mannoheptulose. INS-1 cells were cultured for 4 days at 6.6 mmol/L glucose (6.6 G), 16.7 mmol/L glucose (16.7 G), 6.6 mmol/L glucose and 5 mmol/L mannoheptulose (6.6 G+M), and 16.7 mmol/L glucose and 5 mmol/L mannoheptulose (16.7 G+M). (A) Relative insulin RNA content: 1 (0.62 to 1.38), 0.41 (0.01 to 0.77), 0.89 (0.51 to 1.14), 1.51 (1.09 to 2.39). The values are means (range) from 6 separate experiments. (*$P < .01$; **$P < .0002$; ***$P < .05$ compared with 6.6 mmol/L glucose). (B) Insulin secretion determinded as insulin output (■) and GSIS (□). The values are mean ± SEM from 9 individual incubations performed in 2 separate experiments.

glucose did not counteract the glucose-induced (16.7 mmol/L) downregulation of insulin mRNA content (Fig 4A [16.7 G *v* 16.7 G+D]), but the concentration was low, which has to be taken into account. In contrast, deoxyglucose blocking of the isomerase reduced the amount of insulin mRNA in cells exposed to 6.6 mmol/L glucose by 53% (Fig 4A [6.6 G *v* 6.6 G+D] [$P < .05$], an effect resembling the decrease observed at 3.3 mmol/L glucose [Fig 1A]). We examined the effect of the trioses per se by supplementing 6.6 mmol/L glucose with a triose (0.1 mmol/L DG or 0.5 mmol/L DHA) without blocking the upstream glucose metabolism. This did not influence the insulin mRNA content as compared with the effect of 6.6 mmol/L glucose (Fig 5A).

Studies on Insulin Release

The relationship between long-term glucose exposure and insulin release was determined by comparing the insulin output and GSIS from cells cultured for 4 days (Fig 1B) at 3.3 mmol/L, 6.6 mmol/L, and 16.7 mmol/L glucose. The insulin output was reduced by 55% at 16.7 mmol/L ($P < .0002$) and by 64% at 3.3 mmol/L ($P < .0002$) as compared with 6.6 mmol/L glucose. GSIS was 3.5 times higher at 6.6 than at 16.7 mmol/L ($P < .0002$), but identical at 3.3 and 6.6 mmol/L (Fig 1B). The most prominent decrease (89%) in insulin output compared with the 6.6-mmol/L glucose reference level was observed in cells cultured for 8 days at 26.6 mmol/L glucose ($P < .0001$) (Fig 2B). Insulin release dropped by 71% ($P < .0001$) (Fig 2B)

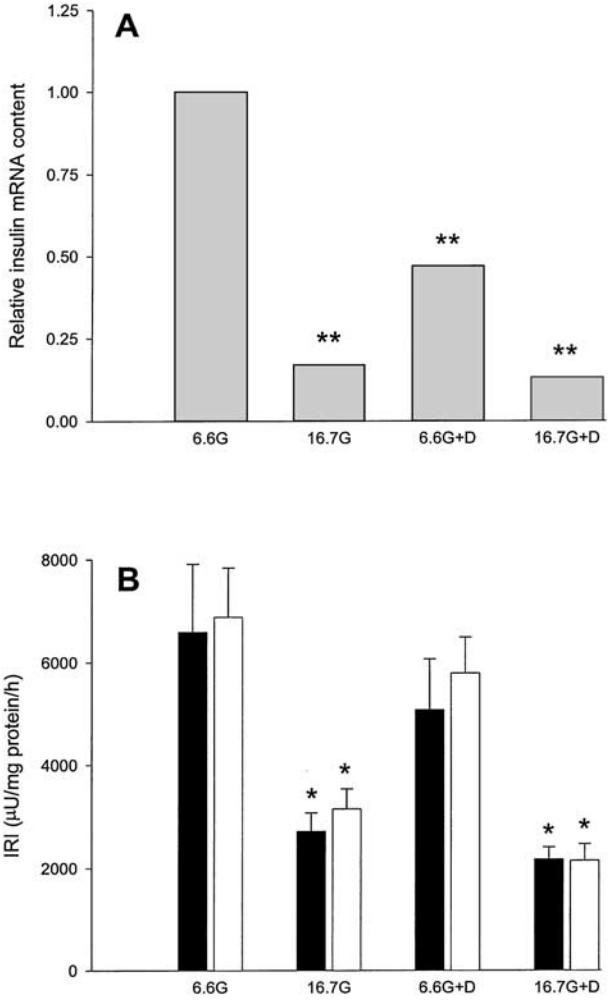

Fig 4. Effect of long-term exposure to the metabolic blocker 2-deoxyglucose. Cells were cultured for 4 days at 6.6 mmol/L glucose (6.6 G), 16.7 mmol/L glucose (16.7 G), 6.6 mmol/L glucose and 2.5 mmol/L 2-deoxyglucose (6.6 G+D), and 16.7 mmol/L glucose and 2.5 mmol/L 2-deoxyglucose (16.7 G+D). (A) Relative insulin mRNA content: 1 (0.86 to 1.08), 0.17 (0.05 to 0.35), 0.47 (0.34 to 0.60), 0.12 (0.03 to 0.20). The values are means (range) from 3 separate experiments. (*$P < .005$; **$P < .05$ compared with 6.6 mmol/L glucose). (B) Insulin output (■) and GSIS (□). The values are mean ± SEM from 21 to 24 individual incubations performed in 6 separate experiments.

when cells were cultured for 4 days at 6.6 mmol/L glucose and then for another 4 days at 26.6 mmol/L and by 52% ($P < .0001$) when cells were cultured for 4 days at 26.6 mmol/L glucose and then for 4 days at 6.6 mmol/L glucose (Fig 2B). Daily alternations between 6.6 and 26.6 mmol/L glucose interestingly caused the cellular insulin output to drop by 61%. This contrasted cells cultured 4 days at 6.6 mmol/L glucose and a subsequent 4 days at 26.6 mmol/L ($P < .02$), as well as from cells cultured 4 days at 26.6 mmol/L glucose and a subsequent 4 days at 6.6 mmol/L glucose ($P < .03$) (Fig 2B). Cells cultured 4 days at 16.7 mmol/L glucose plus 5 mmol/L mannoheptulose had the same insulin output as cells cultured in 6.6 mmol/L glucose (Fig 3B). Exposure to 6.6 mmol/L glucose plus 5 mmol/L mannoheptulose caused the insulin output to decline by 82% compared with the effect of a 6.6-mmol/L glucose culture ($P < .0002$) (Fig 3B [6.6 G *v* 6.6 G+M]) thus resembling the output from cells cultured at 3.3 mmol/L glucose (Fig 1B). We observed similar GSIS outcome in cells cultured at 6.6 mmol/L glucose supplemented with 5 mmol/L mannoheptulose, at 16.7 mmol/L glucose supplemented with 5 mmol/L mannoheptulose, and at low glucose concentration (Fig 3B). In contrast, the addition of 2.5 mmol/L 2-deoxyglucose to 16.7 mmol/L glucose left the insulin output and GSIS unchanged as compared with 16.7 mmol/L glucose (Fig 4B). Compared with 6.6 mmol/L glucose alone, supplemention with 0.1 mmol/L DG caused the insulin output to increase by 250% ($P < .02$) (Fig 5B [6.6 G *v* 6.6 G+DG]), but DHA supplementation had no effect (Fig 5B (6.6 G *v* 6.6 G+DHA]). At the 6.6-mmol/L glucose level, GSIS was similar whether cells were cultured in glucose alone or supplemented with 0.1 mmol/L DG or 0.5 mmol/L DHA (Fig 5B).

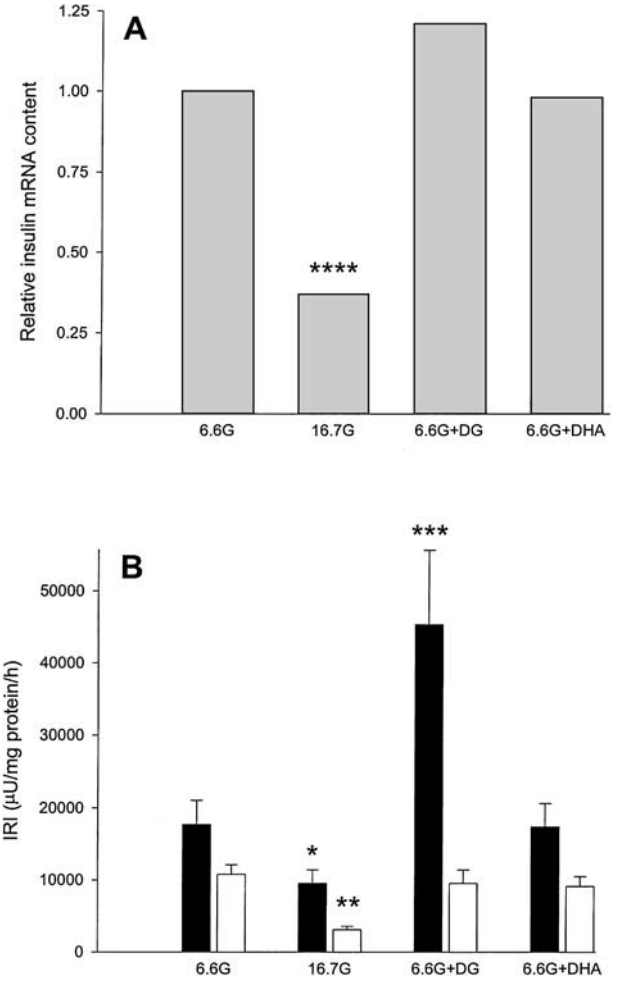

Fig 5. Effect of long-term exposure to the trioses DG and DHA. Cells were cultured for 4 days at 6.6 mmol/L glucose (6.6 G), 16.7 mmol/L glucose (16.7 G), 6.6 mmol/L glucose and 0.1 mmol/L DG (6.6 G+DG), and 6.6 mmol/L glucose and 0.5 mmol/L DHA (16.7 G+DHA). (A) Relative insulin mRNA content: 1 (0.53 to 1.47), 0.37 (0.01 to 0.79), 1.21 (0.95 to 1.55), 0.98 (0.74 to 1.20). The values are means (range) from 6 separate experiments. (*$P < .01$, **$P < .001$, ***$P < .02$, ****$P < .05$ compared with 6.6 mmol/L glucose). (B) Insulin output (■) and GSIS (□). The values are mean ± SEM from 9 individual incubations performed in 2 separate experiments.

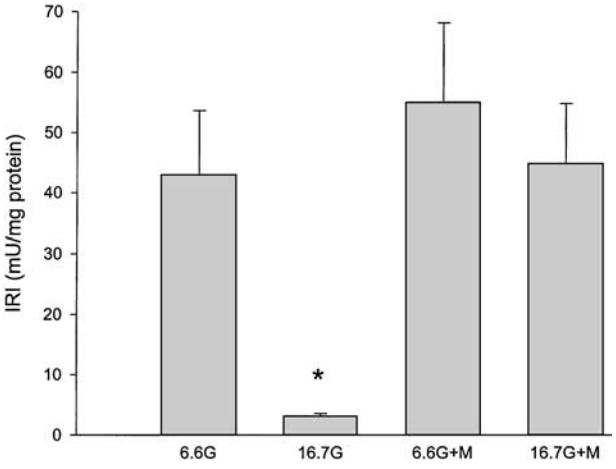

Fig 6. Insulin content. Cells were cultured for 4 days at 6.6 mmol/L glucose (6.6 G), 16.7 mmol/L glucose (16.7 G), 26.6 mmol/L glucose (26.6 G), 6.6 mmol/L glucose and 5 mmol/L mannoheptulose (6.6 G+M), and 16.7 mmol/L glucose and 5 mmol/L mannoheptulose (16.7 G+M). Results are mean ± SEM from 10 to 12 individual incubations performed in 2 separate experiments (*$P < .001$ compared with 6.6 mmol/L glucose).

Studies on Insulin Content

To elucidate the influence of glucose on insulin storage, INS-1 cells were cultured for 4 days at 6.6 mmol/L glucose, 16.7 mmol/L glucose, 26.6 mmol/L glucose, 6.6 mmol/L glucose supplemented with 5 mmol/L mannoheptulose, and 16.7 mmol/L glucose supplemented with 5 mmol/L mannoheptulose (Fig 6). Exposure to 16.7 mmol/L glucose reduced the intracellular insulin content by 93% ($P < .001$) and exposure to 26.6 mmol/L glucose reduced it by 97% ($P < .001$) (Fig 6). Addition of 5 mmol/L mannoheptulose to 16.7 mmol/L glucose totally counteracted the depletion of intracellular insulin storage.

DISCUSSION

Chronic hyperglycemia progressively impairs insulin secretion in vivo[5,29] and elevated glucose exhausts β cells in vitro.[4,30-32] In this report, we focus on the impact of glucose per

se on the insulin mRNA content, insulin release, and insulin storage in INS-1 cells, and we examine the impact of inhibition of glucose phosphorylation, partly inhibition of phosphoglucose isomerase, and supplementation with the trioses DG and DHA. In the INS-1 cells, it was shown that the glucose-induced downregulation of insulin release, insulin content, and level of insulin RNA could be counteracted by the addition of the specific inhibitor of glucose phosphorylation, mannoheptulose[33,34] at a concentration known to inhibit glucose metabolism half-maximally.[35] Addition of mannoheptulose to low glucose (6.6 mmol/L) reduced the insulin output, but not the GSIS. This secretion pattern resembles the insulin secretion pattern found in cells exposed to lower glucose (3.3 mmol/L); 5.0 mmol/L mannoheptulose also counteracted the glucose-induced depletion of intracellular insulin. These effects could not be abolished by 2.5 mmol/L 2-deoxyglucose, which interferes with the phosphoglucose isomerase. Zawalich et al[36] found that the addition of 10 mmol/L 2-deoxyglucose reduced glucose metabolism by only 22% and insulin release by approximately 42%. Addition of 2.5 mmol/L 2-deoxyglucose was therefore not expected to have a larger influence on insulin release and insulin mRNA content. In addition, 2-dexyglucose is known to lower intracellular phosphate[37] and thereby decrease insulin secretion in response to a glucose challenge.[38] Despite this, we found a decrease of insulin mRNA in cells cultured at 6.6 mmol/L glucose supplemented with 2.5 mmol/L, pointing to some influence of the hexose in the applied concentration. Since higher concentrations of 2-deoxyglucose were toxic to the cells, it was not possible to conduct long-term experiments using the higher concentrations used elsewhere.[36] None of the changes can be related to an osmotic effect since neither addition of 10.1 mmol/L fructose nor 10.1 mmol/L galactose caused a downregulation of insulin mRNA.[27] The 2 trioses, DG and DHA, are known to stimulate insulin release,[26,36,39,40] with the former being trice as potent as the latter and equivalent in potency to glucose in initiating insulin release.[36,39-41] Due to the triosephosphate isomerase, a higher concentration of DHA was used as compared with DG. Four days of exposure to DG had a potentiating effect on insulin output, but had no effects on GSIS. However, it tended to increase the amount of insulin mRNA as compared with 6.6 mmol/L glucose, pointing to an additional effect of DG phosphate per se or metabolite(s) distal of its metabolism. Exposure to 0.5 mmol/L DHA did not affect any of the variables investigated. Taken together, these data support the hypothesis that alterations in glucose sensing and insulin gene turnover are critically dependent on glucose metabolism or glucose-dependent alterations as previously described,[40,42] rather than changes in actual number of glucose molecules. Besides an increased expression of insulin mRNA at 16.7 mmol/L glucose supplemented with 5 mmol/L mannoheptulose, we found consistency between the level of the insulin mRNA and GSIS (Fig 3). In contrast, the insulin output differed substantially between the different sugars. These results emphasize that the persistent insulin release, at least from INS-1 cells, is neither well correlated with nor reflects the insulin mRNA content. Interestingly, the decrease in intracellular insulin content paralleled the decrease in insulin mRNA.

We also explored the impact of alternate glucose exposure on insulin secretion and insulin mRNA content in INS-1 cells. While 4 days culture in the low-glucose concentration could almost counteract the decline in insulin mRNA caused by high glucose (75% of maximum content), the insulin output was recovered less (48% of maximal secretion) (Fig 2), which corroborates results from Korsgren et al[4] and Ogawa et al.[5] In contrast, daily alternations in glucose exposure (between 6.6 mmol/L and 26.6 mmol/L) to some degree preserve the insulin secretory capacity. To our knowledge, this intriguing observation has not been described before.

In this report, we have presented data on reduced intracellular insulin content, GSIS, and downregulation of insulin mRNA, all caused by long-term exposure to high glucose. It is tempting to speculate that at least 2 regulatory factors may modulate the insulin release with one factor promoting insulin secretion and one factor reducing insulin secretion in response to glucose. The data suggest that the putative glucose metabolite(s) inducing glucose desensitization has to be found in the glycolytic pathway between glucose-6-phosphate and the triosephosphates, pointing to a pivotal role for glucokinase in the regulation of insulin response to long-term glucose exposure. Desensitization was prevented by mannoheptulose, not by 2-deoxyglucose, and it was not induced by any of the trioses. We therefore wish to suggest that glucose-6-phosphate may be a candidate prompting development of glucose desensitization. This is supported by the results of Gasa et al,[43] who suggest the glucose molecule to be involved in the regulation of glucokinase expression and by Molina et al,[44] who propose a rate-limiting step in insulin secretion to be found prior to the metabolism of the triosephosphates. Noma et al[31] and Efrat et al[45] corroborate the importance of the glucokinase activity. When it comes to a tentative promoting factor, glucose per se is an obvious candidate.

The results discussed above favor the "overworked β-cell" concept[14] since we found that exposing the INS-1 cells to a low-glucose concentration partly reversed the glucose-induced downregulation of insulin mRNA content with a lesser increase in the contemporary insulin output. Moreover, our results support the use of the INS-1 cell line as a tool in studies of the glucose toxicity phenomenon. In conclusion, it appears that a glucose metabolite plays a key role in the glucose desensitization of the pancreatic β cell.

ACKNOWLEDGMENT

Lisbet Blak, Kirsten Eriksen, and Jytte Søholt are all thanked cordially for their technical assistance. Professor Michael Væth, PhD, is gratefully acknowledged for his advice on biostatistics.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM. A balanced overview. Diabetes Care 15:318-368, 1992

2. Yki-Jarvinen H: Glucose toxicity. Endocr Rev 13:415-431, 1992

3. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. Diabetes Care 13:610-630, 1990

4. Korsgren O, Jansson L, Sandler S, et al: Hyperglycemia-induced B cell toxicity. The fate of pancreatic islets transplanted into diabetic mice is dependent on their genetic background. J Clin Invest 86:2161-2168, 1990

5. Ogawa Y, Noma Y, Davalli AM, et al: Loss of glucose-induced insulin secretion and GLUT2 expression in transplanted beta-cells. Diabetes 44:75-79, 1995

6. Robertson RP, Zhang HJ, Pyzdrowski KL, et al: Preservation of insulin

mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. J Clin Invest 90:320-325, 1992

7. Dohan FC, Lukens FDW: Experimental diabetes produced by the administration of glucose. Endocrinology 42:244-262, 1948

8. Olson LK, Redmon JB, Towle HC, et al: Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. J Clin Invest 92:514-519, 1993

9. Meglasson MD, Matscinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab Rev 2:163-214, 1986

10. Robertson RP: Type II diabetes, glucose "non-sense," and islet desensitization. Diabetes 38:1501-1505, 1989

11. Leahy JL: Natural history of beta-cell dysfunction in NIDDM. Diabetes Care 13:992-1010, 1990

12. Malaisse WJ: The beta cell in NIDDM: Giving light to the blind. Diabetologia 37:S36-S42, 1994 (suppl 2)

13. Robertson RP, Olson LK, Zhang H-J: Differentiating glucose toxicity from glucose desensitization: A new message from the insulin gene. Diabetes 43:1085-1089, 1994

14. Leahy JL: Impaired beta-cell function with chronic hyperglycemia: "Overworked beta-cell hypothesis." Diabetes Rev 4:298-319, 1996

15. Hosokawa YA, Leahy JL: Parallel reduction of pancreas insulin content and insulin secretion in 48-h tolbutamide-infused normoglycemic rats. Diabetes 46:808-813, 1997

16. Hammonds P, Schofield PN, Ashcroft SJ, et al: Regulation and specificity of glucose-stimulated insulin gene expression in human islets of Langerhans. FEBS Lett 223:131-137, 1987

17. Hammonds P, Schofield PN, Ashcroft SJ: Glucose regulates preproinsulin messenger RNA levels in a clonal cell line of simian virus 40-transformed B cells. FEBS Lett 213:149-154, 1987

18. Nielsen DA, Welsh M, Casadaban MJ, et al: Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. J Biol Chem 260:13585-13589, 1985

19. Welsh M, Nielsen DA, MacKrell AJ, et al: Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. J Biol Chem 260:13590-13594, 1985

20. Brunstedt J, Chan SJ: Direct effect of glucose on the preproinsulin mRNA level in isolated pancreatic islets. Biochem Biophys Res Commun 106:1383-1389, 1982

21. Marshak S, Leibowitz G, Bertuzzi F, et al: Impaired beta-cell functions induced by chronic exposure of cultured human pancreatic islets to high glucose. Diabetes 48:1237-1244, 1999

22. Eizirik DL, Korbutt GS, Hellerstrom C: Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the beta-cell function. J Clin Invest 90:1263-1268, 1992

23. Robertson RP, Zhang HJ, Pyzdrowski KL, et al: Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. J Clin Invest 90:320-325, 1992

24. Purrello F, Buscema M, Rabuazzo AM, et al: Glucose modulates glucose transporter affinity, glucokinase activity, and secretory response in rat pancreatic beta-cells. Diabetes 42:199-205, 1993

25. Asfari M, Janjic D, Meda P, et al: Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 130:167-178, 1992

26. Hermansen K: Pancreatic D-cell recognition of D-glucose: Studies with D-glucose, D-glyceraldehyde, dihydroxyacetone, D-mannoheptulose, D-fructose, D-galactose, and D-ribose. Diabetes 30:203-210, 1981

27. Brock B, Hermansen K: The glucose-induced down regulation of GLUT2 mRNA, insulin mRNA and insulin secretion in INS-1 cells is not caused by the glucose molecule per se. Diabetes 46:213, 1997 (suppl 1, abstr)

28. Gregersen S, Hermansen K, Yanaihara N, et al: Galanin fragments and analogues: Effects on glucose-stimulated insulin secretion from isolated rat islets. Pancreas 6:216-220, 1991

29. Heimberg H, DeVos A, Vandercammen A, et al: Heterogeneity in glucose sensitivity among pancreatic B-cells is correlated to differences in glucose phosphorylation rather than glucose transport. EMBO J 12:2873-2879, 1993

30. Tiedge M, Hohne M, Lenzen S: Insulin secretion, insulin content and glucose phosphorylation in RINm5F insulinoma cells after transfection with human GLUT2 glucose- transporter cDNA. Biochem J 296:113-118, 1993

31. Noma Y, Bonner-Weir S, Latimer JB, et al: Translocation of glucokinase in pancreatic beta-cells during acute and chronic hyperglycemia. Endocrinology 137:1485-1491, 1996

32. Kaiser N, Corcos AP, Sarel I, et al: Monolayer culture of adult rat pancreatic islets on extracellular matrix: Modulation of B-cell function by chronic exposure to high glucose. Endocrinology 129: 2067-2076, 1991

33. Coore HG, Randle PJ, Simon E, et al: Block of insulin secretion from the pancreas by D-mannoheptulose. Nature 197:1264-1266, 1963

34. Coore HG, Randle PJ: Inhibition of glucose phosphorylation by mannoheptulose. Biochem J 91:56-59, 1964

35. Zawalich WS, Pagliara AS, Matschinsky FM: Effects of iodoacetate, mannoheptulose and 3-O-methyl glucose on the secretory function and metabolism of isolated pancreatic islets. Endocrinology 100: 1276-1283, 1977

36. Zawalich WS, Dye ES, Rognstad R, et al: On the biochemical nature of triose- and hexose-stimulated insulin secretion. Endocrinology 103:2027-2034, 1978

37. Ohta M, Nelson D, Wilson JM, et al: Relationships between energy level and insulin secretion in isolated rat islets of Langerhans. Manipulation of [ATP]/[ADP][Pi] by 2-deoxy-D-glucose. Biochem Pharmacol 43:1859-1864, 1992

38. Campillo JE, Castillo M, Valdivia MM, et al: Effect of phosphate omission on glucose-induced insulin release in vitro. Experientia 35:1260-1261, 1979

39. Hellman B, Idahl LA, Lernmark A, et al: The pancreatic beta-cell recognition of insulin secretagogues. Comparisons of glucose with glyceraldehyde isomers and dihydroxyacetone. Arch Biochem Biophys 162:448-457, 1974

40. Jain K, Logothetopoulos J, Zucker P: The effects of D- and L-glyceraldehyde on glucose oxidation, insulin secretion and insulin biosynthesis by pancreatic islets of the rat. Biochim Biophys Acta 399:384-394, 1975

41. MacDonald MJ, Fahien LA: Insulin release in pancreatic islets by a glycolytic and a krebs cycle intermediate: Contrasting patterns of glyceraldehyde phosphate and succinate. Arch Biochem Biophys 279: 104-108, 1990

42. Malaisse WJ, Herchuelz A, Levy J, et al: The stimulus-secretion coupling of glucose-induced insulin release XIX. The insulinotropic effect of glyceraldehyde. Mol Cell Endocrinol 4:1-12, 1976

43. Gasa R, Fabregat ME, Gomis R: The role of glucose and its metabolism in the regulation of glucokinase expression in isolated human pancreatic islets. Biochem Biophys Res Commun 268:491-495, 2000

44. Molina JM, Premdas FH, Lipson LG: Insulin release in aging: Dynamic response of isolated islets of Langerhans of the rat to D-glucose and D-glyceraldehyde. Endocrinology 116:821-826, 1985

45. Efrat S, Leiser M, Surana M, et al: Murine insulinoma cell line with normal glucose-regulated insulin secretion. Diabetes 42:901-907, 1993