Long-Term Exposure of Isolated Rat Islets of Langerhans to Supraphysiologic Glucose Concentrations Decreases Insulin mRNA Levels

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Chronic hyperglycemia has been postulated to contribute to β-cell dysfunction in type 2 diabetic patients. A deleterious effect of prolonged exposure to high glucose concentrations on insulin gene expression has been demonstrated in insulin-secreting cell lines. This study was designed to investigate in isolated rat islets the effects of long-term exposure to supraphysiologic glucose concentrations on insulin, GLUT2, and glucokinase gene expression. The acute effects of glucose on gene expression were investigated by culturing rat islets in 2.8 or 16.7 mmol/L glucose for 24 hours. Insulin, GLUT2, and glucokinase mRNA levels were assessed by semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR). As expected, glucose acutely increased relative insulin and GLUT2 mRNA levels by 2.8- ± 0.5-fold (n = 5, $P < .005$) and 1.8- ± 0.3-fold (n = 5, $P < .05$), respectively, but had no effect on glucokinase gene expression (1.1- ± 0.1-fold increase, n = 4, NS). These results validate the use of semiquantitative RT-PCR to detect changes in gene expression in rat islets. Islets were then cultured in 5.6 or 16.7 mmol/L glucose for 2, 4, or 6 weeks. Relative insulin mRNA levels were higher in islets cultured in high glucose after 2 weeks (1.8 ± 0.1 *v* 1.0 ± 0.1, n = 4, $P < .05$), identical after 4 weeks (0.9 ± 0.1 *v* 1.00 ± 0.2, n = 4, NS), and significantly lower after 6 weeks (0.6 ± 0.1 *v* 1.0 ± 0.2, n = 6, $P < .05$). Relative GLUT2 mRNA levels were higher in islets cultured in high glucose after 2 weeks (1.7 ± 0.2 *v* 1.0 ± 0.2, n = 3, $P < .05$) and then identical in both groups after 4 weeks (1.0 ± 0.1 *v* 1.0 ± 0.1, n = 3, NS) and 6 weeks (1.0 ± 0.2 *v* 1.0 ± 0.1, n = 6, NS). Relative glucokinase mRNA levels were identical under both culture conditions at 2 (1.4 ± 0.4 *v* 1.0 ± 0.2, n = 3, NS), 4 (0.8 ± 0.5 *v* 1.0 ± 0.3, n = 3, NS), and 6 (0.9 ± 0.2 *v* 1.0 ± 0.1, n = 6, NS) weeks. These results indicate that a 6-week exposure of rat islets to supraphysiologic glucose concentrations decreases insulin mRNA levels without affecting GLUT2 and glucokinase gene expression. We conclude that the phenomenon of glucose toxicity decreasing insulin gene expression is not restricted to transformed cells, and might provide insight into the mechanisms by which chronic hyperglycemia adversely affects β-cell function.

CHRONIC HYPERGLYCEMIA has been postulated to contribute to defective β-cell function in type 2 diabetes.[1] Normalization of blood glucose levels in type 2 diabetic patients restores glucose-induced insulin secretion.[2,3] Similarly, insulin secretion in rodent models of diabetes is recovered upon normalization of blood glucose.[1] The biochemical mechanisms by which glucose affects β-cell function have been studied in insulin-secreting cell lines, and involve, at least in part, alterations in insulin gene expression. Chronic exposure of HIT-T15 cells to 11.1 mmol/L glucose for over 30 passages leads to a marked decrease in insulin gene transcription, which is not observed when the cells are cultured in 0.8 mmol/L glucose.[4,5] These abnormalities are associated with defective DNA binding of two transcription factors involved in the control of insulin gene transcription by glucose, the homeodomain protein STF-1 (PDX-1/IDX-1/IUF-1)[6] and the activator of the glucose-sensitive C1 element.[7] In the insulin-secreting cell line βTC-6, chronic exposure to elevated glucose concentrations is associated with decreased binding of the C1 activator, with no change in STF-1 binding.[8] These observations indicate that chronic exposure of β cells to elevated glucose concentrations affects insulin gene expression. However, the phenotype of immortalized cells is different from that of primary β cells, and marked differences in glucose regulation of insulin gene transcription between cell lines and primary β cells have been recently reported.[9] The demonstration that the observations in transformed cells are relevant to the pathophysiology of β-cell dysfunction in type 2 diabetes therefore requires similar studies in primary islets. In addition, it has not been determined whether chronic exposure to high glucose specifically alters insulin gene expression, or whether other β-cell genes are affected as well.

This prompted us to assess whether exposure of isolated rat islets to supraphysiologic glucose concentrations for up to 6 weeks in culture leads to decreased insulin gene expression. We also investigated the effects of elevated glucose concentrations on GLUT2 and glucokinase gene expression.

MATERIALS AND METHODS

Animals

Male Wistar rats (250 to 300 g) were purchased from Charles River France. Animals were housed on a 12-hour light/dark cycle with free access to water and standard laboratory chow (UAR, Epinay sur Orge, France).

Rat Islet Isolation

Pancreata were digested by intraductal injection of 5 mL collagenase type XI (Sigma, Saint Quentin Fallavier, France; 0.5 mg/mL in Hank's buffered saline solution [HBSS]) and stationary incubation as previously described.[10] Islets were purified on a bilayer HBSS/Histopaque 1077 (Sigma) gradient and cultured overnight in Ham's F10 (Sigma, France) supplemented with 1% Ultroser (Sépracor, France), 100 UI/mL penicillin, 100 mg/mL streptomycin (GIBCO BRL, France), and 11.1 mmol/L glucose in a humidified atmosphere of 5% CO_2:95% air at 37°C.

Rat Islet Culture

For short-term experiments, islets were cultured in the presence of either 2.8 or 16.7 mmol/L glucose for 24 hours. For long-term experiments, batches of approximately 300 islets were cultured in the

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presence of 5.6 or 16.7 mmol/L glucose for 2, 4, or 6 weeks. The osmolarity of the lower glucose condition was adjusted by adding 11.1 mmol/L mannitol. The media were changed once per week. Islets from both conditions were subcultured in 16.7 mmol/L glucose for 48 hours before each study to eliminate the acute effects of glucose on gene expression.

Measurement of mRNA Levels

Total RNA was extracted according to the method of Chomczynski and Sacchi.[11] Total RNA (500 ng) was reverse-transcribed by random priming using Avian Myeloblastosis Virus reverse transcriptase (RT) (first-strand DNA synthesis; Amersham, Les Ulis, France) according to the manufacturer's instructions. Control samples were tested in the absence of RT. cDNA fragments were amplified by polymerase chain reaction (PCR) using Taq polymerase (GIBCO BRL) in the presence of 1.5 mmol/L $MgCl_2$ and 0.1 mmol/L dNTP in a Crocodile III thermocycler (Appligène Oncor, Illkirch, France). PCR conditions and primer sequences are indicated in Table 1. Aliquots (5 μL) of the PCR were tested on a 2% agarose gel. Gels were stained with ethidium bromide. Signals were quantified by scanning densitometry using NIH Image 1.56 software.

Insulin, GLUT2, or glucokinase mRNA levels were compared between islets cultured in two glucose concentrations using semiquantitative PCR. This method, based on the coamplification of a target cDNA and a control cDNA in the same reaction, allows for comparison of the amount of amplification products between samples. It first requires that the products of target and control genes can be easily resolved on an agarose gel. The control genes used for insulin, GLUT2, and glucokinase are indicated in Table 1. Semiquantitative PCR also requires that the amplification stops during the exponential phase for both fragments of target and control cDNA. These conditions were defined for each pair of cDNAs. First, for a fixed amount of template, we determined the range of cycles of amplification yielding a linear increase in the intensity of the product. Second, for a fixed number of cycles, we verified that increasing the amount of template does not change the ratio of target product to control product (data not shown).

Expression of Data and Statistics

Data are presented as the mean ± SE for the ratio of target mRNA levels to control mRNA levels. The levels in islets cultured in low glucose were normalized to 1.0 by dividing each individual value by the mean level of the group. Similarly, the levels in islets cultured in high glucose were normalized by dividing each individual value by the mean level in islets cultured in low glucose. Related samples were compared by Student's paired *t* test. A *P* level less than .05 was considered significant.

RESULTS

Short-Term Effects of Glucose on Insulin, GLUT2, and Glucokinase mRNA Levels

To verify that semiquantitative RT-PCR allows detection of variations in gene expression in rat islets, we evaluated the acute effects of glucose on insulin, GLUT2, and glucokinase gene expression. Isolated rat islets were cultured for 24 hours in either 2.8 or 16.7 mmol/L glucose. Insulin mRNA levels in islets cultured in high glucose were increased 2.8- ± 0.5-fold compared with levels in islets cultured in 2.8 mmol/L glucose (n = 5, $P < .005$; Fig 1A). GLUT2 mRNA levels were increased 1.8- ± 0.3-fold (n = 5, $P < .05$; Fig 1B). In contrast, no effect of glucose was observed on glucokinase gene expression (1.1- ± 0.1-fold increase, n = 4, NS; Fig 1C). These results showing an acute stimulatory effect of glucose on insulin and GLUT2 but not glucokinase mRNA levels are in accordance with previously published data,[12-14] and validate the use of semiquantitative RT-PCR for evaluating variations of gene expression in isolated rat islets.

Long-Term Effects of Glucose on Insulin, GLUT2, and Glucokinase mRNA Levels

To assess the effects of chronic exposure to high glucose concentrations on gene expression, islets were cultured for 2, 4, or 6 weeks in either 5.6 or 16.7 mmol/L glucose. After 2 weeks of culture, insulin mRNA levels were significantly higher in islets cultured in 16.7 mmol/L glucose versus 5.6 mmol/L glucose (1.8 ± 0.1 *v* 1.0 ± 0.1, respectively, n = 4, $P < .05$; Fig 2A). After 4 weeks of culture, insulin mRNA levels from both groups were identical (0.9 ± 0.1 in high glucose *v* 1.0 ± 0.2 in normal glucose, n = 4, NS; Fig 2A). In contrast, insulin mRNA levels were decreased approximately twofold in islets cultured in 16.7 mmol/L glucose (0.6 ± 0.1 *v* 1.0 ± 0.2 in islets cultured in 5.6 mmol/L glucose, n = 6, $P < .05$; Fig 2A). After 2 weeks of culture, GLUT2 mRNA levels were higher in islets cultured in 16.7 mmol/L glucose versus 5.6 mmol/L glucose (1.5 ± 0.2 *v* 1.0 ± 0.2, n = 5, $P < .05$; Fig 2B). After 4 and 6 weeks of culture, GLUT 2 mRNA levels were identical in both groups (4 weeks, 1.0 ± 0.1 for 16.7 mmol/L *v* 1.0 ± 0.1 for 5.6 mmol/L, n = 3, NS; 6 weeks, 1.0 ± 0.1 for 16.7 mmol/L *v* 1.0 ± 0.1 for 5.6 mmol/L, n = 6, NS; Fig 2B).

No significant effect of glucose was found for the glucokinase message after 2, 4, and 6 weeks of culture (16.7 *v* 5.6

Table 1. Primer Sequences and PCR Conditions for Each Pair of Target and Control Genes

Target Gene	Reference	Control Gene	Reference	Denaturation, Annealing, Extension (°C)
Insulin (187 bp)	18	α-Tubulin (250 bp)	23	
5′:TGCCCAGGCTTTTGTCAAACAGCACCTT		5′:GCGTGAGTGTATCTCCATCCA		94, 52, 72
3′:CTCCAGTGCCAAGGTCTGAA		3′:GGTAGGTGCCAGTGCGAACTT		
GLUT2 (343 bp)	18	Cyclophylin (220 bp)		
5′:TTAGCAACTGGGTCTGCAAT		5′:ATGGTCAACCCCACCGTGTT		94, 57, 72
3′:GGTGTAGTCCTACACTCATG		3′:CGTGTGAAGTCACCACCCT		
Glucokinase (130 bp)	18	β-Actin (349 bp)	18	
5′:AAGGGAACTACATCGTAGGA		5′:CGTAAAGACCTCTATGCCAA		94, 57, 72
3′:CATTGGCGGTCTTCATAGTA		3′:AGCCATGCCAAATGTGTCAT		

Abbreviation: bp, base pair.

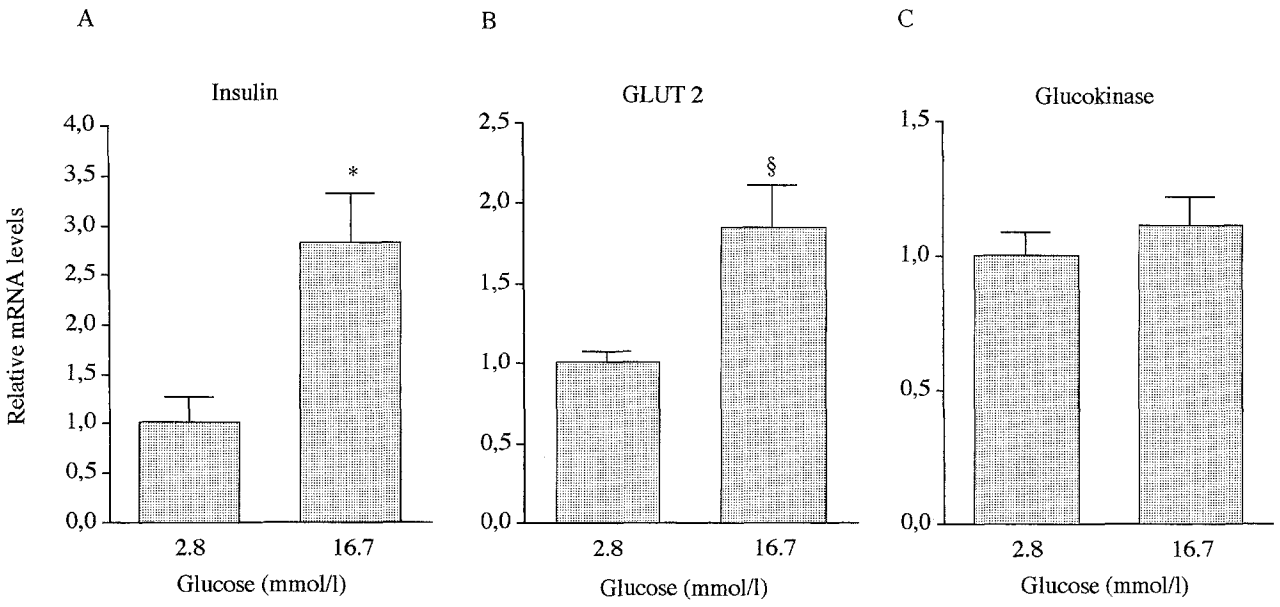

Fig 1. Short-term effects of glucose on insulin (A), GLUT2 (B), and glucokinase (C) gene expression. Islets were cultured for 24 hours in 2.8 or 16.7 mmol/L glucose. mRNA levels were then semiquantified by RT-PCR. Data are expressed as the target mRNA to control mRNA ratio and are normalized to the value in islets cultured in 2.8 mmol/L. *$P < .005$, §$P < .05$ *v* islets cultured in 2.8 mmol/L.

mmol/L glucose, 1.3 ± 0.4 *v* 1.0 ± 0.2, n = 4, NS, after 2 weeks; 0.9 ± 0.5 *v* 1.0 ± 0.3, n = 3, NS, after 4 weeks; 0.9 ± 0.2 *v* 1.0 ± 0.1, n = 6, NS, after 6 weeks; Fig 2C).

DISCUSSION

This study aimed to determine the effects of long-term exposure of isolated rat islets to supraphysiologic glucose concentrations on insulin, GLUT2, and glucokinase gene expression. Studies on gene expression using primary islets in culture are limited by the availability of tissue. The small amounts of total RNA recovered after 6 weeks of culture make it impossible to study gene expression by Northern analysis. We therefore used semiquantitative RT-PCR to compare mRNA levels between culture conditions. PCR conditions, particularly the number of cycles and amount of template, were chosen to allow comparative measurements. In addition, the method was vali-

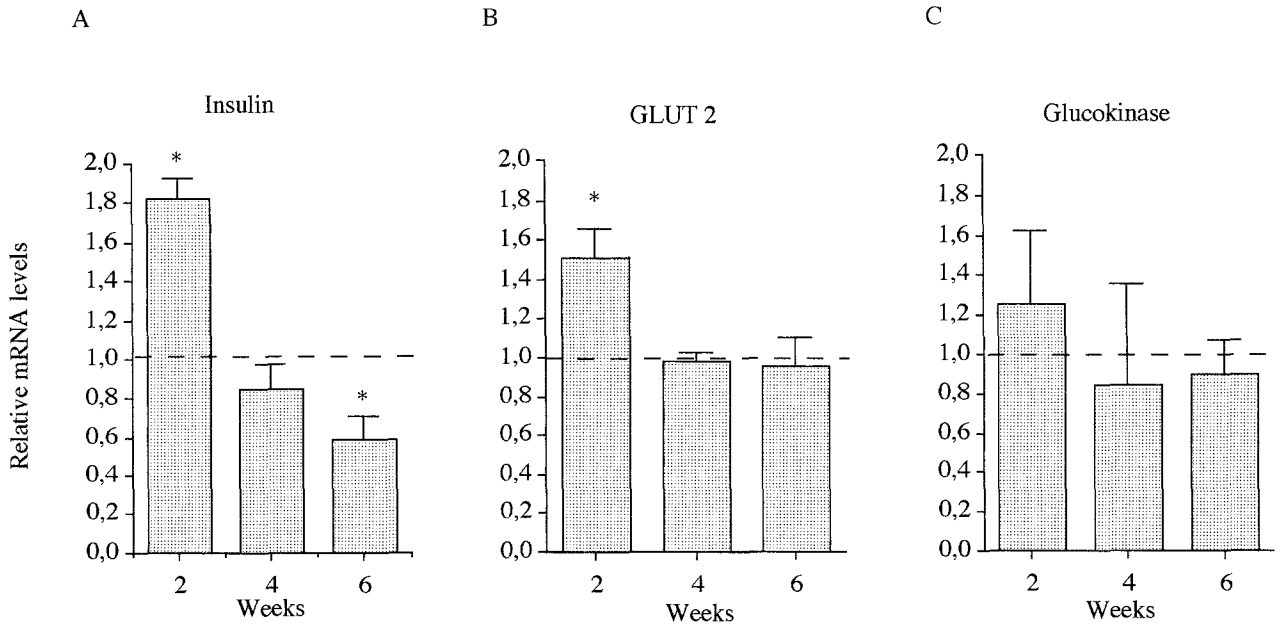

Fig 2. Long-term effects of glucose on insulin (A), GLUT2 (B), and glucokinase (C) gene expression. Islets were cultured in the presence of 5.6 or 16.7 mmol/L glucose for 2, 4, or 6 weeks. mRNA levels were then semiquantified by RT-PCR. Data are expressed as relative target mRNA levels in islets cultured in 16.7 mmol/L glucose, normalized to the levels in islets cultured in 5.6 mmol/L glucose (----). *$P < .05$ *v* islets cultured in 5.6 mmol/L.

dated by showing that acute exposure to high glucose stimulates insulin and GLUT2 but not glucokinase gene expression as previously reported.[12-14]

We found that insulin mRNA levels in islets cultured in 16.7 mmol/L glucose decrease during the culture period compared with islets cultured in 5.6 mmol/L glucose. After 2 weeks of culture, insulin mRNA levels are approximately twofold higher in islets cultured in high glucose, probably resulting from sustained stimulation of insulin gene expression. After 4 weeks of culture, insulin mRNA levels are identical in both groups. After 6 weeks of culture, the levels of insulin mRNA are about twofold lower in islets cultured in high glucose. Our results are in accordance with the observations in insulin-secreting cell lines,[4-8] and reinforce the hypothesis that chronic exposure of β cells to high glucose concentrations adversely affects insulin gene expression. In addition, our data provide evidence that toxic effects of glucose on insulin gene expression can be avoided by culturing islets in physiological glucose concentrations. We also demonstrate that a 6-week exposure of rat islets to high glucose concentrations does not affect GLUT2 gene expression. As expected, a stimulatory effect of glucose on GLUT2 gene expression was observed after a 24-hour culture. Accordingly, GLUT2 mRNA levels were higher in islets cultured in high glucose after 2 weeks. As observed for the insulin gene, GLUT2 mRNA levels were identical in both culture conditions after 4 weeks. After 6 weeks of culture, the levels of GLUT2 mRNA were identical in both groups, in contrast to the observations on the insulin gene. These results suggest that the deleterious effects of glucose on gene expression are specific for insulin, which was further confirmed by the observation that glucokinase mRNA levels are identical in both culture conditions throughout the whole culture period. Alternatively, we cannot exclude that a toxic effect of glucose on GLUT2 and glucokinase gene expression would be detected by a longer exposure to high glucose. However, we can infer that the decrease in insulin gene expression upon exposure to high glucose is not secondary to a defect in GLUT2 and/or glucokinase gene expression.

A point of concern in the interpretation of our data is the possibility that the observed decrease in insulin mRNA levels could be accounted for by changes in the relative proportion of different cell types within the islets. A selective loss of β cells could result in a diminished ratio of insulin to α-tubulin mRNA. The relative levels of glucokinase mRNA could be kept artifically constant by the corresponding increase in the proportion of α cells, because both α and β cells express glucokinase.[15] However, in this case, GLUT2 mRNA levels would be decreased as well, because GLUT2 is not expressed in α cells.[13,15] In contrast, we observed a selective decrease in insulin, not GLUT2, mRNA levels in islets cultured in high glucose for 6 weeks. This argument favors our suggestion that chronically elevated glucose specifically alters insulin gene expression.

Deleterious effects of glucose on insulin gene expression have been investigated in several rodent models of diabetes. Insulin mRNA levels decrease with age in diabetic *db/db* mice compared with nondiabetic controls.[16] Ninety percent pancreatectomy in the rat is associated with decreased insulin and GLUT2 gene expression.[17] Tokuyama et al[18] have shown reduced expression of several β-cell–specific genes, including insulin, GLUT2, and glucokinase, in the Zucker diabetic fatty rat. However, the conclusions from in vivo studies are complicated by confounding metabolic abnormalities associated with hyperglycemia. Alternatively, the use of isolated islets in vitro allows for investigating specifically the effects of glucose on insulin biosynthesis. Several studies have reported a decrease in the intracellular insulin content of human[19,20] or rat[21] islets cultured in elevated glucose concentrations, which was thought to be the consequence of prolonged overstimulation of insulin release. However, these experiments were conducted over relatively short periods (6 to 9 days) and insulin gene expression was not investigated. Tajiri et al[22] cultured normal rat islets in 38 mmol/L glucose for 6 weeks and did not detect any change in insulin mRNA levels as assessed by RNAse protection assay. The discrepancy between their results and our data might be related to differences in the subculture conditions during the 48 hours prior to RNA extraction. In our study, islets from both culture conditions were subcultured in 16.7 mmol/L glucose for 48 hours prior to RNA extraction, to eliminate the acute effects of glucose on insulin gene expression.

The mechanisms by which chronically elevated glucose adversely affects insulin gene expression have not been addressed in the present study. Determining whether the alterations in STF-1 and RIPE3b1 activity observed in insulin-secreting cells[6-8] are also relevant to primary islets requires further investigation.

In conclusion, this study uniquely demonstrates that chronic exposure of rat islets to supraphysiologic glucose concentrations decreases insulin gene expression, whereas neither the glucose responsive GLUT2 gene nor the glucose-unresponsive glucokinase gene were affected. These observations suggest that chronic hyperglycemia in type 2 diabetic patients contributes secondarily to β-cell dysfunction by adversely affecting the insulin gene, an effect that may be prevented by maintenance of normoglycemia.

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