

The Expression of Hormone-Sensitive Lipase in Clonal β -Cells and Rat Islets Is Induced by Long-Term Exposure to High Glucose

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Hormone-sensitive lipase (HSL) is expressed and enzymatically active in β -cells and has been proposed to be involved in the generation of the lipid-derived signal that seems to be necessary for glucose-stimulated insulin secretion. In this study, we investigated whether the expression of HSL in INS-1 cells and in rat islets is affected by exposure to high glucose concentrations. Incubation of INS-1 cells in 25 mmol/l glucose for 16 and 32 h induced HSL protein expression twofold, whereas no effect was observed after 4 and 8 h of incubation. The HSL activity, defined as the diglyceride lipase activity inhibited by anti-rat HSL antibodies, constituted ~25% of total diglyceride lipase activity and was induced to a similar extent as HSL protein levels. The glucose effect at 16 h on HSL protein expression level was confirmed in freshly isolated rat islets. Exposure of INS-1 cells to different glucose concentrations for 16 h showed that the inductive effect on HSL protein levels was maximum at 20 mmol/l glucose (2- to 2.5-fold). Northern blot analysis demonstrated a more than threefold elevation of HSL mRNA levels. The induction was blocked by actinomycin D, and the half-life of the transcript seemed to be unchanged by high glucose, suggesting a transcriptional nature of the glucose effect on HSL gene expression. The nonmetabolizable glucose analog 2-deoxyglucose, which has no mitogenic effect, induced HSL ~1.3-fold, whereas mannose was similar to glucose, stimulating HSL expression 1.7- to 2-fold. The results suggest that HSL is involved in the β -cell responses to hyperglycemia and also in generating the lipid signal that is needed in stimulus-secretion coupling. *Diabetes* 50:2225-2230, 2001

It is known that an important event underlying glucose-stimulated insulin secretion (GSIS) is an increase in the intracellular ATP/ADP ratio, resulting in closure of ATP-sensitive K^+ channels, depolarization of the cell membrane, and activation of voltage-sensitive Ca^{2+} channels, and leads to a rise in intracellular

Ca^{2+} concentration (1,2). However, accumulating data suggest that other, postglycolytic signaling pathways are involved in GSIS, and in recent years it has become clear that a lipid-derived signal is of importance (3-7). However, the exact nature of the lipid signal, as well as the mechanism whereby it affects the insulin secretory process, is not known. Among candidates for the signal are malonyl-CoA, long-chain acyl CoA, and diacylglycerol (8-10). It has been shown that if β -cells are depleted of their triglyceride stores, then GSIS is blunted, which further emphasizes the role of the β -cell lipid signals for GSIS (11). Conversely, islets that overstore triglycerides, for instance, islets of Zucker rats and *ob/ob* and *db/db* mice, are dysfunctional, possibly because of an incapability to generate the lipid-mediated signal (12). In fact, several adaptive changes occur at the level of lipid metabolism in response to long-term exposure of β -cells to high glucose concentrations, which might be of relevance for the altered GSIS after glucose exposure. These changes include increased triglyceride and phospholipid synthesis, decreased fatty acid oxidation, and induction of lipogenic enzymes (13). Therefore, lipid signals seem to be required for normal GSIS and to also be involved in the adaptation to long-term glucose exposure. However, the mechanisms of the glucose effects on β -cell lipid metabolism remain to be established.

One important regulator of cellular lipid homeostasis is hormone-sensitive lipase (HSL), which catalyzes the hydrolysis of stored triacylglycerol (14). The activity of HSL is hormonally regulated; cAMP-elevating agents, such as catecholamines, increase its activity via protein kinase A-mediated phosphorylation, whereas insulin prevents this activation, mainly via activation of phosphodiesterase 3B and thereby a reduction in cAMP levels (15,16). HSL is expressed in many tissues and was identified recently also in pancreatic β -cells (17). It was shown to account for a significant part of the lipase activity in these cells and on this basis was proposed to be involved in generating the lipid-derived signal that seems to be necessary for GSIS. In this study, we investigated the ability of glucose to regulate HSL expression. Both normal islets and the rat insulinoma cell line INS-1 were studied. We found that long-term (≥ 16 h) exposure of both INS-1 cells and rat islets to high concentrations of glucose induces the expression of HSL. The effect seemed to be transcriptional and to require phosphorylation of glucose to glucose-6-phosphate. The results support that HSL is involved in the β -cell adapta-

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GSIS, glucose-stimulated insulin secretion; HBSS, Hanks' balanced salt solution; HSL, hormone-sensitive lipase; PBS, phosphate-buffered saline.

tion to hyperglycemia and in the generation of lipid signals that are important for GSIS.

RESEARCH DESIGN AND METHODS

Materials. RPMI 1640 medium, fetal bovine serum, and streptomycin/penicillin were from Gibco BRL (Life Technologies, Gaithersburg, MD), and Hanks' balanced salt solution (HBSS), glucose, and glucose analogs were from Sigma Chemical (St. Louis, MO). Collagenase P was from Boehringer Mannheim (Mannheim, Germany). [32 P]dCTP, Hybond-N+, Hybond-C extra, horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody, and enhanced chemiluminescence reagents were from Amersham Pharmacia (Uppsala, Sweden) Biotech; and RNazol was from Nordic BioSite (Täby, Sweden); and ExpressHyb was from Clontech (Palo Alto, CA).

Animals. Male Sprague-Dawley rats that weighed ~225 g were purchased from Møllegaard A/S (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22°C) on a 12-h light-dark cycle with lights on at 06.00. The study was approved by the Animal Ethics Committee at Lund University.

Cell culture and incubation condition. INS-1 cells (a gift from Professor Claes Wolheim, Geneva, Switzerland) were grown as previously described (18) in RPMI 1640 medium containing 11.1 mmol/l glucose supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, 100 μ g/ml streptomycin, and 50 μ mol/l β -mercaptoethanol. The cells were cultured at 37°C in a humidified atmosphere (5% CO₂, 95% air). The cells were used at passage numbers 90–100. When cells reached 70–80% confluence, they were washed twice with phosphate-buffered saline (PBS) and then cultured in serum-free medium that contained different concentrations of glucose (or glucose analogs) for the indicated times.

Islet isolation and culture. Rat islets were isolated by the collagenase digestion technique. Briefly, 10 ml of cold HBSS supplemented with 1.0 mg/ml collagenase (Collagenase P, activity 1.52 units/mg) was injected into the bile duct, and the pancreas was filled retrogradely. The pancreas subsequently was removed and incubated for 24 min at 37°C. Islets were isolated after washing three times with HBSS. The islets were cultured overnight at 37°C in a humidified atmosphere (5% CO₂, 95% air) in RPMI 1640 medium supplemented with low or high concentrations of glucose (3.3 and 25 mmol/l, respectively), 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 μ g/ml streptomycin. After 16 h of incubation, the islets were washed twice with PBS and then lysed in SDS-PAGE sample buffer. Islet protein was measured using a commercial kit (BCA protein; Pierce, Rockford, IL).

Western blot analysis. After incubation of INS-1 cells and rat islets under different conditions, as indicated in the figure legends, cells were washed twice with PBS and then scraped with a rubber policeman into homogenization buffer (0.25 mol/l sucrose, 1 mmol/l dithioerythritol, 1 mmol/l EDTA, 10 μ g/ml leupeptin, 10 μ g/ml antipain, 1 μ g/ml pepstatin A). Homogenization was performed by passing through a 27-gauge needle five times. The protein concentration was measured in the homogenates using the BCA-protein kit (Pierce). A total of 100 μ g protein was separated by SDS-PAGE and electroblotted onto nitrocellulose membranes (Hybond-C extra). Western blot analysis was performed using an affinity-purified rabbit anti-rat HSL antibody, followed by a horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody and enhanced chemiluminescence reagents. The amount of HSL protein was quantified using a CCD camera (LAS 1000; Fuji, Tokyo, Japan).

Enzyme activity assays and immunoinhibition. Homogenates, prepared as for Western blot analysis, were assayed for diglyceride lipase activity using the mono-oleoyl-2-O-monooleylglycerol, a diacylglycerol analog, as substrate (19). One unit of enzyme activity corresponds to 1 μ mol of fatty acid released per minute at 37°C. Immunoinhibition was performed by preincubating the cell homogenates with chicken anti-rat HSL antiserum (dilution 1:10) or with preimmune chicken serum (dilution 1:10) for 1 h at room temperature.

Glycerol assay. Glycerol release from INS-1 cells was measured in medium samples with an automatic luminometric kinetic assay as described previously (20).

RNA isolation and Northern blot analysis. INS-1 cells were lysed with 2 ml of RNazol per 10-cm dish, and total cellular RNA was isolated according to the manufacturer's instructions. Thirty micrograms of RNA was denatured and electrophoresed on a 1% agarose gel that contained 2.2 mol/l formaldehyde and then transferred to nylon membranes (Hybond-N+). The blots were probed with a full-length rat adipocyte HSL cDNA labeled with [32 P]dCTP using ExpressHyb according to the manufacturer's instructions. Membranes were analyzed and quantified by digital imaging (Fujix Bas 2000; Fuji). For correction for differences in RNA loading on the gel, HSL mRNA levels were normalized to those of 18S rRNA, quantified through hybridization to a 32 P-end-labeled oligonucleotide (21).

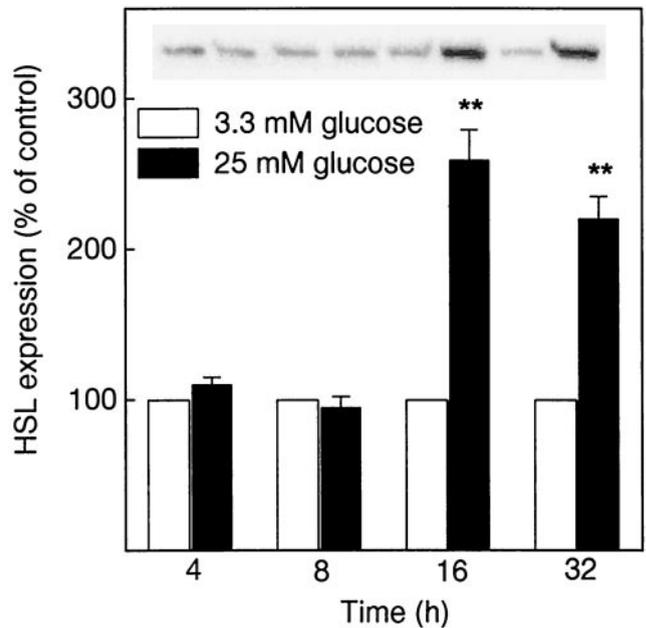


FIG. 1. Time course of HSL protein induction by high glucose. INS-1 cells were cultured in 3.3 or 25 mmol/l glucose for 4, 8, 16, or 32 h. The cells were homogenized, and HSL was quantified in aliquots corresponding to equal amounts of total protein by Western blot analysis using an affinity-purified polyclonal anti-rat HSL antibody. The HSL protein levels at high glucose are expressed as a percentage of the levels at low glucose for each time point. Values are expressed as means \pm SE for three independent experiments, in which each condition was analyzed in triplicates. ** $P < 0.01$.

HSL mRNA stability. The effect of glucose on HSL mRNA stability was determined by addition of actinomycin D, a potent inhibitor of RNA polymerase II, to the cell culture medium. After an overnight preincubation of INS-1 cells in serum-free RPMI 1640 with 11 mmol/l glucose, cells were switched to 25 or 3.3 mmol/l glucose and 4 μ g/ml actinomycin D. Cells were harvested after 2, 4, and 10 h of incubation, and mRNA was extracted and quantified by Northern blot analysis (see above).

Statistics. Values are expressed as means \pm SE. Differences between individual data were compared with Student's unpaired t test or by one-way analysis of variance, followed by Tukey's test for individual comparisons. The critical probability used to determine significance was $P < 0.05$.

RESULTS

Glucose augments the expression of HSL in INS-1 cells and in islets. The effect of glucose on HSL expression in β -cells was studied by incubating INS-1 cells in 3.3 or 25 mmol/l glucose for 4, 8, 16, and 32 h. Western blot analysis of total cell homogenates revealed that after 4 and 8 h, there was no difference between high and low glucose, whereas after 16 and 32 h, the HSL protein was induced more than twofold ($259 \pm 20\%$) at high compared with low glucose concentrations (Fig. 1). The HSL activity, defined as the diglyceride lipase activity that was inhibited by neutralizing anti-rat HSL antibodies, showed the same degree of induction by high glucose concentrations (Table 1). Total diglyceride lipase activity, however, was induced much less, suggesting that lipase activity that is not accounted for by HSL is not increased by high glucose concentrations (Table 2). Preincubation with anti-rat HSL antibodies reduced the diglyceride lipase activity by 25%, which is similar to our previous findings (17).

To determine the dose dependence for the glucose-inducing effect on HSL expression, we incubated INS-1 cells with 1, 5, 10, 15, 20, 25, and 30 mmol/l glucose for

TABLE 1
Effect of glucose on HSL activity in homogenates of INS-1 cells

Glucose (mmol/l)	Time (h)			
	4	8	16	32
3.3	100	100	100	100
25	103 ± 1	97 ± 3	193 ± 12**	286 ± 13**

Homogenates of INS-1 cells were assayed for diglyceride lipase activity. One unit of enzyme activity corresponds to 1 μ mol of fatty acid released per minute at 37°C. Immunoinhibition was performed by preincubating triplicates of cell homogenates from each incubation with chicken anti-rat HSL antiserum (dilution 1:10) or with preimmune chicken serum (dilution 1:10) for 1 h at room temperature. The HSL activity is defined as the diglyceride lipase activity that was inhibited by neutralizing anti-HSL antibodies. The activity in cells cultured with 3.3 mmol/l glucose was set to 100%. The values in the table are means \pm SE from one experiment with quadruplicates of each condition. The HSL activity was analyzed in triplicate. ** P < 0.01.

16 h. HSL expression was induced at all concentrations between 10 and 30 mmol/l, with maximum effects observed at 20–25 mmol/l (Fig. 2).

To examine the effect of high glucose concentration on HSL mRNA levels, we performed Northern blot analysis. Incubation of INS-1 cells for 16 h at 25 mmol/l glucose resulted in more than threefold higher levels of the 3.1-kb HSL mRNA found in these cells (17) compared with cells incubated at 3.3 mmol/l glucose (Fig. 3). No difference was observed for 18S rRNA levels, used to correct for differences in RNA loading on the gel. To determine whether induction of HSL mRNA levels can occur in the absence of transcription and whether glucose exposure affects the half-life of HSL mRNA, we incubated INS-1 cells at high (25 mmol/l) or low (3.3 mmol/l) glucose concentrations in the presence of 4 μ g/ml actinomycin D. RNA was extracted at different time points and quantified by Northern blot analysis. These experiments demonstrated that actinomycin D inhibited the induction of HSL mRNA by high glucose and, furthermore, that the half-life of the HSL mRNA in INS-1 cells is short (<10 h) with no difference between high and low glucose conditions (data not shown). Consequently, glucose induction of HSL mRNA seems to not be the result of increased stability of HSL mRNA but rather the result of an increased transcription of the HSL gene.

To confirm in normal islet tissue the effect of glucose observed on HSL expression in INS-1 cells, we incubated freshly isolated rat islets for 16 h in RPMI 1640 medium

TABLE 2
Effect of glucose on total lipase activity in homogenates of INS-1 cells

Time (h)	3.3 mmol/l glucose (mU/mg protein)	25 mmol/l glucose (mU/mg protein)	% Induction
4	4.00 \pm 0.13	4.10 \pm 0.05	103
8	3.60 \pm 0.08	3.93 \pm 0.12	109
16	3.94 \pm 0.10	4.90 \pm 0.08***	125
32	3.68 \pm 0.11	5.62 \pm 0.06***	153

Homogenates of INS-1 cells were assayed for diglyceride lipase activity. One unit of enzyme activity corresponds to 1 μ mol of fatty acid released per minute at 37°C. The values in the table are means \pm SE from one experiment with quadruplicates of each condition. The HSL activity was analyzed in triplicate. *** P < 0.001.

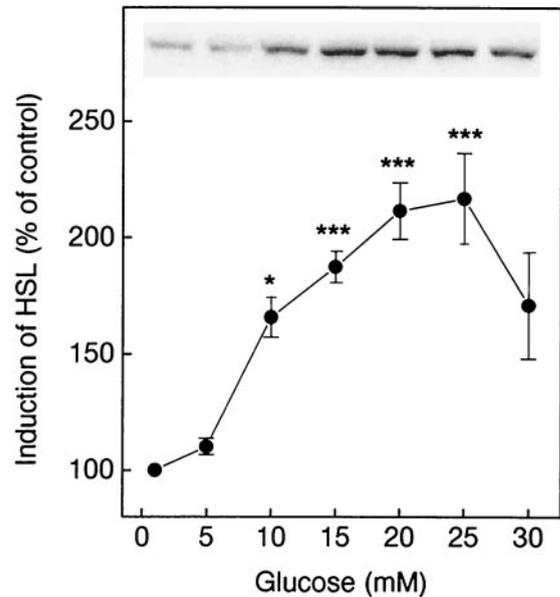


FIG. 2. HSL protein expression in INS-1 cells exposed to different glucose concentrations. INS-1 cells were cultured under serum-free conditions in RPMI 1640 medium supplemented with different concentrations of glucose ranging from 1 to 30 mmol/l for 16 h. A total of 100 μ g total protein was analyzed by Western blot. The values are expressed as percentage of induction compared with 1 mmol/l glucose and represent the means \pm SE of four independent experiments in which each condition was analyzed in triplicate. * P < 0.05, *** P < 0.001, one-way analysis of variance, F = 11.3, P < 0.001.

supplemented with 10% serum and 3.3 or 25 mmol/l glucose. Western blot analysis showed a twofold induction of HSL protein at the higher glucose concentration (Fig. 4). Similar induction of HSL protein was seen in INS-1 cells when the incubation was performed in the presence of serum (data not shown). Two isoforms of HSL were found

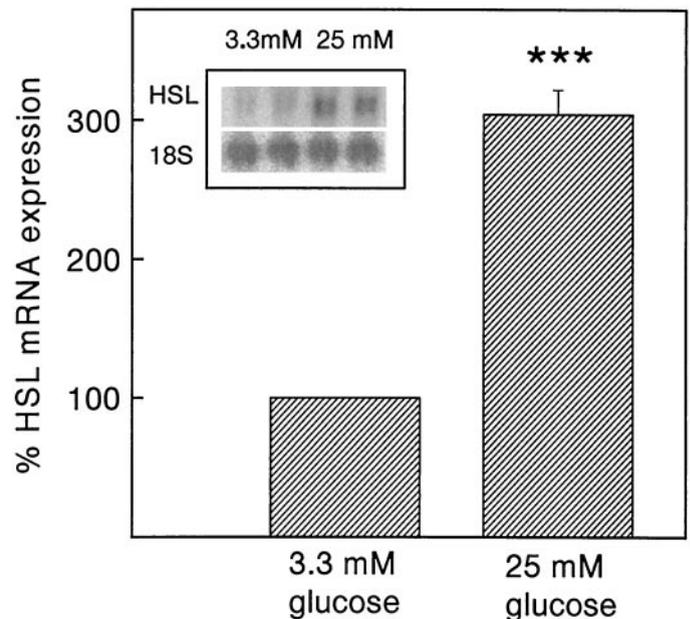


FIG. 3. Effect of glucose on the expression level of HSL mRNA. After incubation of INS-1 cells for 16 h with 3.3 and 25 mmol/l glucose, respectively, total RNA was extracted and the HSL mRNA was quantified by Northern blot analysis. The experiment is representative of four independent experiments in which each condition was analyzed in duplicate. The values represent means \pm SE. *** P < 0.001.

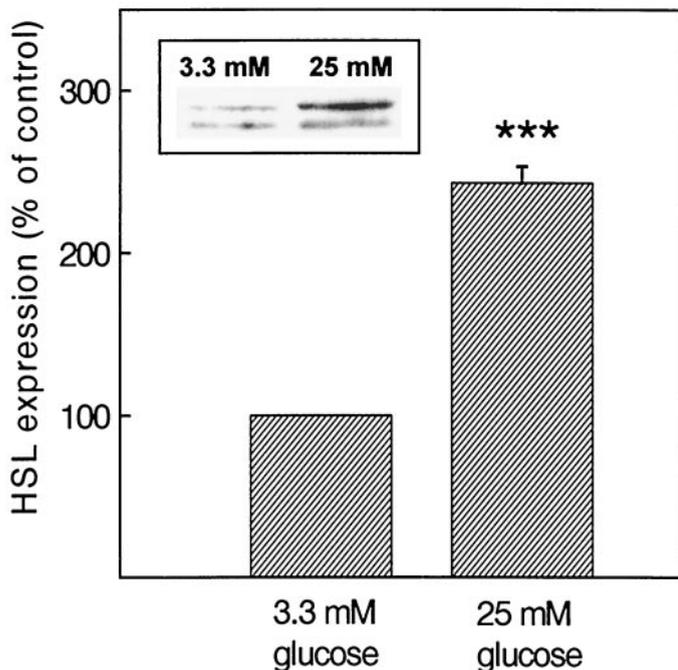


FIG. 4. Induction of HSL protein expression by high glucose in rat islets. Freshly isolated islets were incubated for 16 h in RPMI 1640 medium supplemented with 3.3 or 25 mmol/l glucose, followed by quantification of HSL protein by Western blot analysis. Each lane corresponds to equal amounts of islet protein. The experiment is representative of four independent experiments in which each condition was analyzed in duplicate. The values represent means \pm SE. *** $P < 0.001$.

in lysates from rat islets, which is in agreement with an earlier study (17). Both isoforms of HSL were induced at high concentrations of glucose, although not to the same extent. At 3.3 mmol/l glucose, the two isoforms were expressed at equal levels, whereas at 25 mmol/l glucose, the level of expression of the 89-kDa isoform was 50% higher than that of the 84-kDa form.

Lipolytic activity is increased in INS-1 cells cultured in high glucose. To confirm that the induction of HSL by glucose correlated with an increased mobilization of tri-glyceride stores, the release of glycerol, which is an end product in lipolysis, was measured. After 16 h of incubation of INS-1 cells with 3.3 or 25 mmol/l glucose, medium samples were collected from each well and glycerol was measured. The accumulated glycerol was several-fold higher in cells that were incubated with high glucose compared with low glucose (Fig. 5). Cells that were incubated with high glucose concentrations released 17.5 ± 1.5 nmol glycerol/mg protein during the 16-h incubation, whereas cells that were incubated with 3.3 mmol/l glucose released only 1.0 ± 0.05 nmol glycerol/mg protein.

Glucose needs to be metabolized to induce HSL expression. To gain insight into the mechanism of HSL induction by glucose, we investigated the effect of various sugars on the HSL expression. INS-1 cells were incubated in RPMI medium containing 3.3 mmol/l glucose and 21.7 mmol/l of one of various glucose analogs. Cells that were challenged with 25 mmol/l D-glucose showed a significant increase ($223 \pm 14\%$) in HSL protein expression (Fig. 6). Mannose, which is metabolized in β -cells and a potent insulin secretagogue, also increased the expression level of HSL ($179 \pm 9\%$), whereas fructose had no effect.

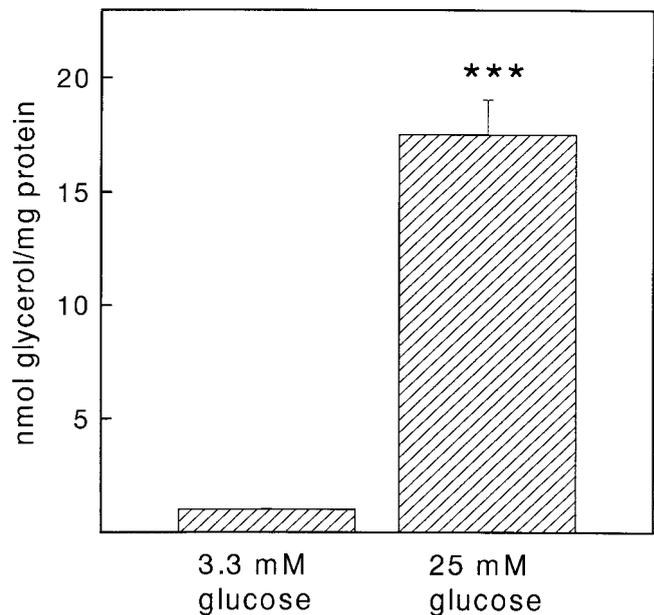


FIG. 5. Lipolysis measurements in INS-1 cells that were incubated with high and low concentrations of glucose. Cells were cultured with 3.3 or 25 mmol/l glucose for 16 h, after which the glycerol accumulated in the medium was measured. Data shown are means \pm SE of six samples of each condition. *** $P < 0.001$.

2-Deoxyglucose, which is phosphorylated by glucokinase but not metabolized further, was able to stimulate HSL expression significantly ($130 \pm 4\%$), albeit to a lower

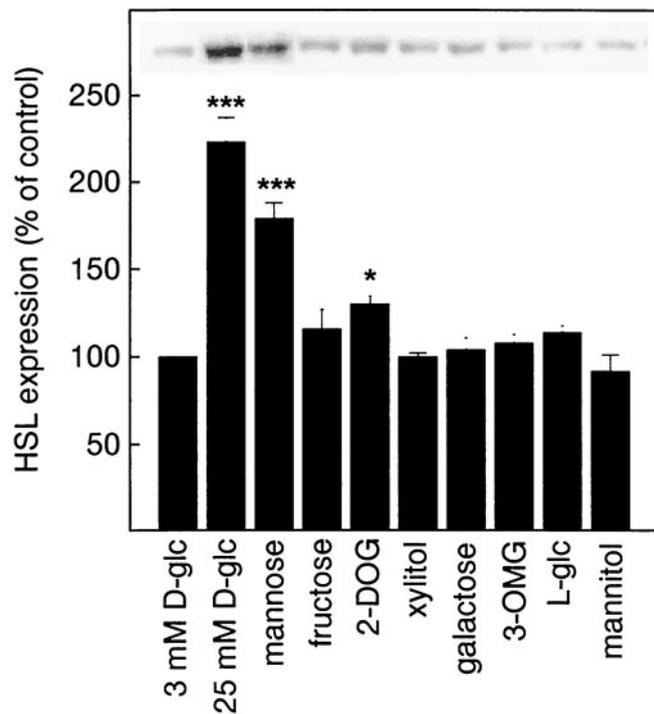


FIG. 6. Effect of various glucose analogs on HSL protein expression. INS-1 cells were cultured for 16 h in RPMI 1640 medium with 3.3 mmol/l glucose and 21.7 mmol/l of one of the following substances: mannose, fructose, 2-deoxyglucose (2-DOG), xylitol, galactose, 3-orthomethyl-glucose (3-OMG), L-glucose, and mannitol. After the incubation, aliquots of the cell homogenates corresponding to equal amounts of total protein were analyzed by SDS-PAGE and Western blot. The values are expressed as means \pm SE of four different experiments in which each condition was analyzed in triplicate. * $P < 0.05$; *** $P < 0.001$.

extent. Galactose and 3-orthomethylglucose, two sugars that are transported into the cell but not metabolized, had no effect on HSL expression. L-Glucose and mannitol did not induce HSL expression, showing that the effect of the sugars was not due to changes in osmolarity. Using Northern blot analysis, we investigated the effect of mannose and 2-deoxyglucose at the mRNA level. The result showed that mannose and 2-deoxyglucose induced HSL mRNA, mannose being as effective as glucose, whereas 2-deoxyglucose was less effective (data not shown). Taken together, the results suggest that glucose-6-phosphate is an important mediator of the glucose effect on HSL, although a contribution by additional mediators, generated through metabolization of glucose beyond the glucokinase step, cannot be excluded.

DISCUSSION

This study shows that long-term (≥ 16 h) exposure of INS-1 cells and islets to high glucose concentrations causes a concentration-dependent induction of HSL expression. The induction, which is paralleled by an increased lipolysis, is seen at the level of enzyme activity, protein, and as mRNA expression, suggesting that the effect is mediated via increased transcription of the HSL gene. This proposal is supported by the results of actinomycin D decay assays, which showed no significant effect of high glucose on HSL mRNA stability. Mannose and 2-deoxyglucose also induced HSL protein expression in the INS-1 cells, whereas other tested glucose analogs had no effect. The stimulation of HSL expression by 2-deoxyglucose was smaller than the effects seen with glucose and mannose, which may be due to the toxic effects of this analog. Consequently, glucose-6-phosphate may be the effector in stimulating HSL expression. However, the existence of additional mediators, generated through metabolization of glucose beyond the glucokinase step or generated as a result of early mitogenic events induced by glucose, i.e., activation of signal transduction pathways involving mitogen-activated protein kinase, protein kinase C, and Ca^{2+} -dependent kinases, cannot be excluded and needs to be investigated further.

The regulation of HSL expression by glucose has been studied previously in adipocytes. With the murine adipocyte cell line 3T3-F442A as a model, deprivation of glucose was found to decrease HSL mRNA and HSL activity levels by a factor of 2–3 (22). In another study in which primary rat adipocytes were used as a model, glucose in combination with insulin was found to increase the levels of basal lipolysis as well as HSL protein expression (23). Glucose alone, however, had no effect. The reason for the discrepancy between the two studies is not known but could be attributed to the different experimental models. Glutamine has been shown not to be required for the glucose effect on induction of basal lipolysis in adipocytes, indicating that the hexosamine pathway is not involved in mediating the effect (23). Glucose analogs have not been tested in the adipocyte models, making it difficult to draw any conclusions about how the effect of glucose on HSL expression is mediated.

In agreement with what has been described previously, we observed two different HSL isoforms in isolated islets: one 84-kDa form and one 89-kDa form (17). INS-1 cells, however, express only the larger isoform. This may reflect

either that β -cells express the 89-kDa isoform whereas other islet cell types express the 84-kDa form or that there is a difference between normal β -cells and INS-1 cells. Previous reverse transcriptase–polymerase chain reaction data indicated that the 84-kDa isoform in islets is identical to the main isoform expressed in adipocytes (17). In this study, we found that glucose induces both isoforms, although not to the same extent. Induction of both isoforms also was observed in adipocytes (23), suggesting that in both islets and adipocytes, there is a coordinated regulation of the two HSL isoforms by glucose. The possibility that the 84-kDa protein in islets represents a proteolytic fragment of the larger isoform cannot be excluded.

Because we found that incubation of cells or islets with high glucose for 16 h was required to induce HSL expression, whereas no effect of glucose was evident after 8 h of incubation, it is possible that the glucose effect on HSL is of major relevance during long-term exposure to high glucose. Chronically elevated glucose levels are known to alter β -cell secretory function, as evidenced in studies that showed sensitization to glucose with a markedly augmented secretion at low glucose concentrations and a lack of response to high glucose concentrations after prolonged exposure of β -cells to high glucose (24,25). The mechanisms underlying these adaptive changes of the β -cell to a hyperglycemic environment are only partly understood but may involve altered expression of a number of glucose-regulated genes, in particular glycolytic enzymes and other proteins involved in glucose metabolism. GLUT2 as well as downstream glycolytic enzymes, such as phosphofruktokinase isozymes, glyceraldehyde-3-phosphate dehydrogenase, and L-pyruvate kinase, have been shown to be induced in the β -cell line INS-1 (26,27). Glucokinase activity has been found to be increased without altered mRNA levels (28). Insulin gene transcription and proinsulin mRNA translation increase in response to long-term exposure to high glucose levels (29). The massive deposition of glycogen has been proposed to contribute to the sensitization to glucose at low concentrations by channeling glucose to the glycolytic pathway (26). Long-term exposure of INS-1 cells to high glucose also has been found to induce lipogenic enzymes such as fatty acid synthase, acetyl-CoA carboxylase, and malic enzyme (13). Our present study suggests that the induction of HSL expression is part of the adaptive process to a long-term hyperglycemic environment. HSL was recently found to be expressed in β -cells and proposed to be involved in generating lipid-derived signals that are necessary for stimulus-secretion coupling (17). It is of interest that the dose-response relationship between glucose concentration and HSL expression (see Fig. 2) displays a marked glucose effect within the range of hyperglycemic levels, which correspond to the levels seen in patients with type 2 diabetes. It is suggested that the adaptive response to long-term glucose exposure in HSL expression evolves for the generation of lipid signals needed for the augmentation of insulin secretion to normalize the hyperglycemia. The nature of the lipid-derived signals is unknown, but among suggested candidates are malonyl CoA, long-chain acyl CoA, and diglycerides. Long-chain acyl CoA thereby might be the effector signal, augmenting the exocytosis of

insulin, whereas malonyl CoA is more of a regulatory signaling molecule, because it inhibits carnitine palmitoyl-transferase 1, leading to inhibition of fatty acid oxidation with a resulting accumulation of long-chain acyl CoA in the cytosol (8,30). Other candidate lipid-derived signals are fatty acid derivatives that act as ligands for the peroxisomal proliferating activated receptors, which may regulate genes of importance for the β -cell adaptation to hyperglycemia.

We found evidence of increased lipolytic activity after exposure of β -cells to a high glucose concentration because glycerol release was stimulated under such conditions. Thus, increased HSL expression and activity may contribute to an increased flux of all potential lipid-signaling molecules, through hydrolysis of the increased triglyceride stores. Noteworthy, fatty acid oxidation is decreased as β -cells are exposed to high concentrations of glucose (13), which is in agreement with the notion that simultaneous increase in triglyceride deposition and lipolysis mainly serves the purpose of providing an increased flux of lipid-signaling molecules. The concerted induction of lipogenic and lipolytic enzymes thus is triggered by the increased glycolytic flux and may be part of the mechanism behind the glucose sensitization and altered secretory function observed upon prolonged exposure of β -cells to high glucose concentrations. It may be speculated that the β -cell adaptation to long-term glucose exposure fails in type 2 diabetes, initiating the progressive islet dysfunction. If so, then pharmacological means to augment and support the glucose-induced HSL expression might be a new avenue for preservation of islet function in type 2 diabetes.

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REFERENCES

- Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163–214, 1986
- Newgard CB, McGarry JD: Metabolic coupling factors in pancreatic beta-cell signal transduction. *Annu Rev Biochem* 64:689–719, 1995
- Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997 (published erratum appears in *Diabetes* 46:536, 1997)
- Boden G: Free fatty acids, insulin resistance, and type 2 diabetes mellitus. *Proc Assoc Am Physicians* 111:241–248, 1999
- Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44:863–870, 1995
- Dobbins RL, Chester MW, Stevenson BE, Daniels MB, Stein DT, McGarry JD: A fatty acid-dependent step is critically important for both glucose- and non-glucose-stimulated insulin secretion. *J Clin Invest* 101:2370–2376, 1998
- McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
- Corkey BE, Glennon MC, Chen KS, Deeney JT, Matschinsky FM, Prentki M: A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic beta-cells. *J Biol Chem* 264:21608–21612, 1989
- Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE: Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J Biol Chem* 267:5802–5810, 1992
- Prentki M, Corkey BE: Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45:273–283, 1996
- Koyama K, Chen G, Wang MY, Lee Y, Shimabukuro M, Newgard CB, Unger RH: Beta-cell function in normal rats made chronically hyperleptinemic by adenovirus-leptin gene therapy. *Diabetes* 46:1276–1280, 1997
- Milburn JL Jr, Hirose H, Lee YH, Nagasawa Y, Ogawa A, Ohneda M, Beltran del Rio H, Newgard CB, Johnson JH, Unger RH: Pancreatic beta-cells in obesity. Evidence for induction of functional, morphologic, and metabolic abnormalities by increased long chain fatty acids. *J Biol Chem* 270:1295–1299, 1995
- Roche E, Farfari S, Witters LA, Assimacopoulos-Jeannet F, Thumelin S, Brun T, Corkey BE, Saha AK, Prentki M: Long-term exposure of beta-INS cells to high glucose concentrations increases anaplerosis, lipogenesis, and lipogenic gene expression. *Diabetes* 47:1086–1094, 1998
- Langin D, Holm C, Lafontan M: Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc Nutr Soc* 55:93–109, 1996
- Anthonsen MW, Ronnstrand L, Wernstedt C, Degerman E, Holm C: Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J Biol Chem* 273:215–221, 1998
- Degerman E, Belfrage P, Manganiello VC: Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *J Biol Chem* 272:6823–6826, 1997
- Mulder H, Holst LS, Svensson H, Degerman E, Sundler F, Ahren B, Rorsman P, Holm C: Hormone-sensitive lipase, the rate-limiting enzyme in triglyceride hydrolysis, is expressed and active in beta-cells. *Diabetes* 48:228–232, 1999
- Asfari M, Janjic R, Meda P, Li G, Halban PA, Wollheim CB: Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 130:167–178, 1992
- Østerlund T, Danielsson B, Degerman E, Contreras JA, Edgren G, Davis RC, Schotz MC, Holm C: Domain-structure analysis of recombinant rat hormone-sensitive lipase. *Biochem J* 319:411–420, 1996
- Hellmer J, Arner P, Lundin A: Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal Biochem* 177:132–137, 1989
- Chan YL, Gutell R, Noller HF, Wool IG: The nucleotide sequence of a rat 18 S ribosomal ribonucleic acid gene and a proposal for the secondary structure of 18 S ribosomal ribonucleic acid. *J Biol Chem* 259:224–230, 1984
- Raclot T, Dauzats M, Langin D: Regulation of hormone-sensitive lipase expression by glucose in 3T3-F442A adipocytes. *Biochem Biophys Res Commun* 245:510–513, 1998
- Botton LM, Green A: Long-term regulation of lipolysis and hormone-sensitive lipase by insulin and glucose. *Diabetes* 48:1691–1697, 1999
- Leahy JL, Bonner-Weir S, Weir GC: Beta-cell dysfunction induced by chronic hyperglycemia. Current ideas on mechanism of impaired glucose-induced insulin secretion. *Diabetes Care* 15:442–455, 1992
- Bonner-Weir S: Regulation of pancreatic beta-cell mass in vivo. *Recent Prog Horm Res* 49:91–104, 1994
- Roche E, Assimacopoulos-Jeannet F, Witters LA, Perruchoud B, Yaney G, Corkey B, Asfari M, Prentki M: Induction by glucose of genes coding for glycolytic enzymes in a pancreatic beta-cell line (INS-1). *J Biol Chem* 272:3091–3098, 1997
- Marie S, Diaz-Guerra MJ, Miquerol L, Kahn A, Iynedjian PB: The pyruvate kinase gene as a model for studies of glucose-dependent regulation of gene expression in the endocrine pancreatic beta-cell type. *J Biol Chem* 268:23881–23890, 1993
- Liang Y, Najafi H, Smith RM, Zimmerman EC, Magnuson MA, Tal M, Matschinsky FM: Concordant glucose induction of glucokinase, glucose usage, and glucose-stimulated insulin release in pancreatic islets maintained in organ culture. *Diabetes* 41:792–806, 1992
- Philippe J: Structure and pancreatic expression of the insulin and glucagon genes. *Endocr Rev* 12:252–271, 1991
- Corkey BE, Deeney JT, Yaney GC, Tornheim K, Prentki M: The role of long-chain fatty acyl-CoA esters in beta-cell signal transduction. *J Nutr* 130:299S–304S, 2000