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Glucose and Streptozotocin Stimulate p135 O-Glycosylation in Pancreatic Islets

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Streptozotocin has been widely used to create animal models of diabetes. Structurally, streptozotocin resembles *N*-acetylglucosamine, with a nitrosourea group corresponding to the acetate present in *N*-acetylglucosamine. Streptozotocin has recently been shown to inhibit *O*-GlcNAc-selective *N*-acetyl-β-D-glucosaminidase, which removes O-linked *N*-acetylglucosamine from proteins. Compared to other cells, β-cells express much more of the enzyme *O*-GlcNAc transferase, which catalyzes addition of O-linked *N*-acetylglucosamine to proteins. This suggests why β-cells might be particularly sensitive to streptozotocin. In this report, we demonstrate that both streptozotocin and glucose stimulate O-glycosylation of a 135 kD β-cell protein. Only the effect of glucose, however, was blocked by inhibition of fructose-6-phosphate amidotransferase, suggesting that glucose acts through the glucosamine pathway to provide UDP-*N*-acetylglucosamine for p135 O-glycosylation. The fact that both glucose and streptozotocin stimulate p135 O-glycosylation provides a possible mechanism by which hyperglycemia may cause streptozotocin-like effects in β-cells and thus contribute to the development of type 2 diabetes.

Early in the course of type 2 diabetes, pancreatic β-cell function is sufficient such that in many patients, oral hypoglycemic agents are adequate to compensate for increased insulin resistance (1). As type 2 diabetes progresses, however, β-cells lose their capacity to produce sufficient amounts of insulin to control the blood glucose level and patients become increasingly hyperglycemic (2). It has been suggested that the hyperglycemia itself may cause damage to β-cells (3–5). The exact mechanism by which an increased concentration of glucose may affect β-cells, however, is not completely elucidated.

One metabolite of glucose that has been proposed to mediate adverse effects of hyperglycemia is glucosamine (2-amino-2-deoxyglucose) (6, 7). Glucosamine is a product of glucose metabolism and is synthesized from fructose-6-phosphate by the apparently unique and rate limiting enzyme, glutamine: fructose-6-phosphate amidotransferase (GFAT) (8–10). This metabolic step provides the substrate UDP-N-acetylglucosamine (UDP-GlcNAc) for glycoprotein synthesis. Quantitatively, most glycosylation occurs on proteins destined for export from the cell or for the plasma membrane of the cell. In eukaryotic cells, however, there is a cytoplasmic form of glycosylation that involves O-linkage of the monosaccharide, N-acetylglucosamine (GlcNAc), to proteins at serine or threonine residues (11–19). An enzyme responsible for this form of protein modification, O-linked N-acetylglucosamine transferase (OGT), has recently been characterized, and its cDNA has been cloned (20). Studies on the tissue distribution of O-GlcNAc transferase have indicated that OGT mRNA, although ubiquitous, is particularly abundant in the pancreatic β-cell (21).

Interestingly, an analog of N-acetylglucosamine called streptozotocin (STZ) has been used for the past few decades to create animal models of diabetes (22). A nitrosourea group is present at a position that corresponds to the acetate in N-acetylglucosamine (Fig. 1). A single dose of 50–100 mg/kg of streptozotocin administered to a rat causes death of most of the β-cells and development of diabetes. Very recently, streptozotocin has been shown to act by inhibiting the enzyme O-GlcNAc-selective N-acetyl-β-D-glucosaminidase (O-GlcNAcase), which cleaves O-linked N-acetylglucosamine off protein (21, 23). These data suggest why the β-cell, with its elevated level of O-GlcNAc transferase, may be particularly vulnerable to streptozotocin.

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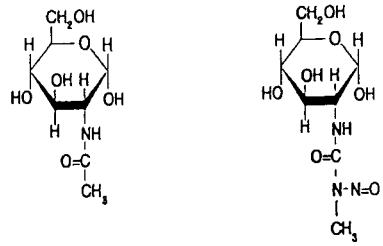

FIG. 1. The chemical structures of *N*-acetylglucosamine (GlcNAc) and streptozotocin (STZ).

In this report, we demonstrate, using isolated rat islets, that glucose, glucosamine, and streptozotocin all stimulate O-glycosylation of a single 135 kD β-cell protein. The effect of glucose, but not that of glucosamine or streptozotocin was blocked by inhibition of glutamine: fructose-6-phosphate amidotransferase (GFAT), suggesting that glucose acts through the glucosamine pathway to provide more UDP-N-acetylglucosamine substrate for OGT to attach to p135. The effect of glucose on p135 O-glycosylation was reversible while that of streptozotocin was not, indicating that streptozotocin irreversibly inactivated β-cell O-GlcNAcase. Experiments performed in clonal β-cell lines demonstrated an inability of β-cells grown in culture to regulate increased p135 O-glycosylation in response to glucose, glucosamine, or streptozotocin, indicating a fundamental difference between β-cells grown in culture and actual β-cells in islets. Together, these data provide strong evidence that glucose and streptozotocin act through a common pathway of p135 O-glycosylation that is present only in primary β-cells in islets. The observation that both glucose and streptozotocin stimulate p135 O-glycosylation provides a possible mechanism by which hyperglycemia may cause streptozotocin-like effects in β-cells and thus contribute to the development of type 2 diabetes.

EXPERIMENTAL PROCEDURES

Islet isolation. In a typical experiment, islets were isolated aseptically from 3–4 male Sprague-Dawley rats. Islets were isolated using Hanks' Balanced Salt Solution (HBSS) and Ficoll supplemented with an additional 5.5 mM glucose, 1 mM L-glutamine, penicillin (25 U/ml) and streptomycin (25 U/ml). During surgery, the common bile duct of each pancreas was cannulated, and the pancreas was inflated with 20 ml of HBSS. The distended pancreas was excised, and lymph nodes, fat, blood vessels and bile ducts were removed under a stereo dissecting microscope. The tissue was chopped extensively and rinsed 5–6 times with HBSS. The tissue was digested with collagenase P (3 mg/ml tissue) at 37°C for 3–4 min. Digested tissue was rinsed 3–4 times with HBSS, and islets were purified on a discontinuous Ficoll gradient consisting of 27%, 23%, 20.5%, and 11% Ficoll in (25 mM) HEPES-HBSS buffer. Islets were harvested from the gradient and washed once with HBSS. Islets were washed 6 times in Kreb's-HEPES buffer (25 mM HEPES, pH 7.40, 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$) supplemented with 0.1% bovine serum albumin, 3 mM glucose, and 1 mM L-glutamine. This isolation procedure typically provided 400–500 islets per rat, which were then used as described below.

Incubation of islets for insulin secretion and protein O-glycosylation. Freshly isolated islets were placed into siliconized 16 × 100-mm round bottom, screw cap tubes and pre-incubated for 30 min in 1 ml of Krebs'-HEPES buffer. For each experiment, 100 islets were placed into each tube. The pre-incubation and all subsequent incubations were performed at 37°C under an atmosphere of 95% O_2/5% CO_2. After 30 min, the pre-incubation buffer was aspirated, and islets were incubated with Krebs'-HEPES buffer supplemented with the appropriate secretagogue. At the end of the incubation period, the entire supernatant was removed for insulin radioimmunoassay. Islets were processed for subsequent immunoprecipitation as described below. For each experiment, insulin secretion in the control sample was set at 100% and all other results were expressed as percentages of control.

β-TC3 cell line culture. β-TC3 cells (passage 37) were obtained form the University of Pennsylvania Diabetes Center from Dr. D. Hanahan (University of California, San Francisco). β-TC3 cells were cultured in 10-cm dishes in the presence of RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (75 μg/ml), streptomycin (50 μg/ml), and 2 mM L-glutamine as previously described (24). Cells were trypsinized and subcloned weekly. Media were changed twice weekly and on the day prior to an experiment, and insulin responsiveness to glucose and carbachol was routinely monitored. Cells were used between passages 38 and 55.

Incubation of β-TC3 cells for insulin secretion and protein O-glycosylation. β-TC3 cells in 10-cm dishes were washed three times in Krebs'-HEPES buffer supplemented with 0.1% bovine serum albumin and 1 mM L-glutamine. Cells were pre-incubated in 5 ml of the same buffer for 30 min at 37°C under an atmosphere of 95% air/5% CO_2. After 30 min, the pre-incubation buffer was aspirated and cells were incubated at 37°C under an atmosphere of 95% air/5% CO_2 with 5 ml of Krebs'-HEPES buffer supplemented with the appropriate secretagogue. At the end of the incubation period, supernatant was removed for insulin radioimmunoassay. Cells left in the dishes were processed for subsequent immunoprecipitation as described below.

Processing of islets and β-TC3 cells for immunoprecipitation of O-glycosylated proteins. At the end of the experiment, all supernatant was removed from the tubes or dishes. One ml of ice-cold lysis buffer (50 mM HEPES, pH 7.40, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 20 mM NaF, 20 mM $Na_4P_2O_7$, 1 mM $NaVO_4$, 1 mg/ml bacitracin, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin) was added to each tube or dish. Cells were scraped on ice into 1.5 ml conical screw cap Eppendorf tubes. Borosilicate tubes containing islets were vortexed for 30 sec and placed on ice for 30 min. Islet samples were vortexed for an additional minute before transfer to 1.5-ml conical screw cap Eppendorf tubes. All subsequent immunoprecipitation steps were performed at 4°C. Samples were centrifuged at 10,000 × *g* for 15 min. The supernatant was transferred to a second 1.5-ml conical Eppendorf tube, and O-glycosylated proteins were immunoprecipitated for 2 h on a rocker with 2 μl of mouse monoclonal RL2 antibody, which selectively binds to O-glycosylated protein (11). After 2 h, 20 μl of protein A trisacryl beads (Pierce) preadsorbed with 20 μg of rabbit anti-mouse antibody (Sigma) were added to the tubes and the incubation was continued for an additional 2 h. At the end of the incubation, beads were washed once with Wash Buffer 1 (150 mM NaCl, 10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS) and once with Wash Buffer 2 (10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS). After the final washing step, 25 μl of 2× sample buffer (100 mM Tris, pH 6.80, 4% SDS, 20% glycerol, and 20 μg/liter

bromophenol blue) was added to each tube. Samples were vortexed for 30 sec, boiled for 5 min, and stored at −20°C prior to subsequent analysis.

Western blotting of samples. Samples were loaded onto 7.5% SDS-polyacrylamide gels. Colored molecular weight markers (Amersham) were run on each gel. Proteins were separated for 1 h at 175 V at room temperature using a Bio-Rad Mini-PROTEAN II dual slab cell. Proteins were transferred to ECL nitrocellulose paper (Amersham) for 1.5 h (100 V, 4°C). Nitrocellulose blots were blocked for 1 h at room temperature in blocking buffer (5% bovine serum albumin in 10 mM Tris, pH 7.40, 150 mM NaCl, 0.1% sodium azide, 0.05% Tween 20). After blocking, the blots were probed with RL2 antibody (1:1000 dilution in blocking buffer) for 1 h at room temperature. Blots were washed 6 times (5 min each) with TBST (10 mM Tris, pH 7.40, 150 mM NaCl, 0.05% Tween 20). After washing, blots were probed with horseradish peroxidase conjugated sheep anti-mouse antibody (Amersham) at a 1:1500 dilution in TBST for 1 h at room temperature. Blots were washed again as above and developed with ECL reagent (Amersham). After air-drying, blots were exposed to Bio-Max X-ray film (Kodak). For each experiment, the intensity of the control sample was set at 100% and all other results were expressed as percentages of control.

Data analysis. Films were photographed using a Sony digital camera, and intensities of bands present on films were quantitated using the program NIH Image. All results are expressed as the mean ± SEM, using the Windows-compatible version 2.98 of the program FigP (Biosoft, St. Louis, MO). Statistical analysis was performed using the same program. Data were analyzed by one-way analysis of variance followed by multiple comparisons between the means using the least significant difference test. A probability of $p < 0.05$ was considered to indicate statistical significance.

RESULTS

We first examined the effect of glucose, glucosamine, and streptozotocin on islet protein O-glycosylation. In other cell types such as BSC40 cells or vascular smooth muscle cells, treatment with glucose, glucosamine, or streptozotocin has been shown to stimulate the accumulation of O-glycosylated protein (21,23). In these cell types, however, non-specific increases in O-glycosylation have been observed only after the cells have been artificially starved of glucose overnight. In order to determine if increased protein O-glycosylation occurred in β-cells under physiologically relevant conditions, freshly isolated islets were incubated in Krebs'-HEPES buffer containing 3 mM glucose, 28 mM glucose, 15 mM glucosamine, or 5 mM streptozotocin. After incubation, subsequent immunoprecipitation was performed with RL2 antibody, which selectively binds to O-glycosylated protein (21,23). Following immunoprecipitation with RL2 antibody, Western blotting was performed with the same antibody.

As shown in Fig. 2, 28 mM glucose, 15 mM glucosamine, and 5 mM streptozotocin all dramatically increased O-glycosylation of the same 135 kD protein in islets. No change in O-glycosylation was detected in any other proteins. O-glycosylation of p135 was increased 417.1 ± 66.4% by glucose ($p < 0.05$ versus control), 581.0 ± 93.4% by glucosamine ($p < 0.05$

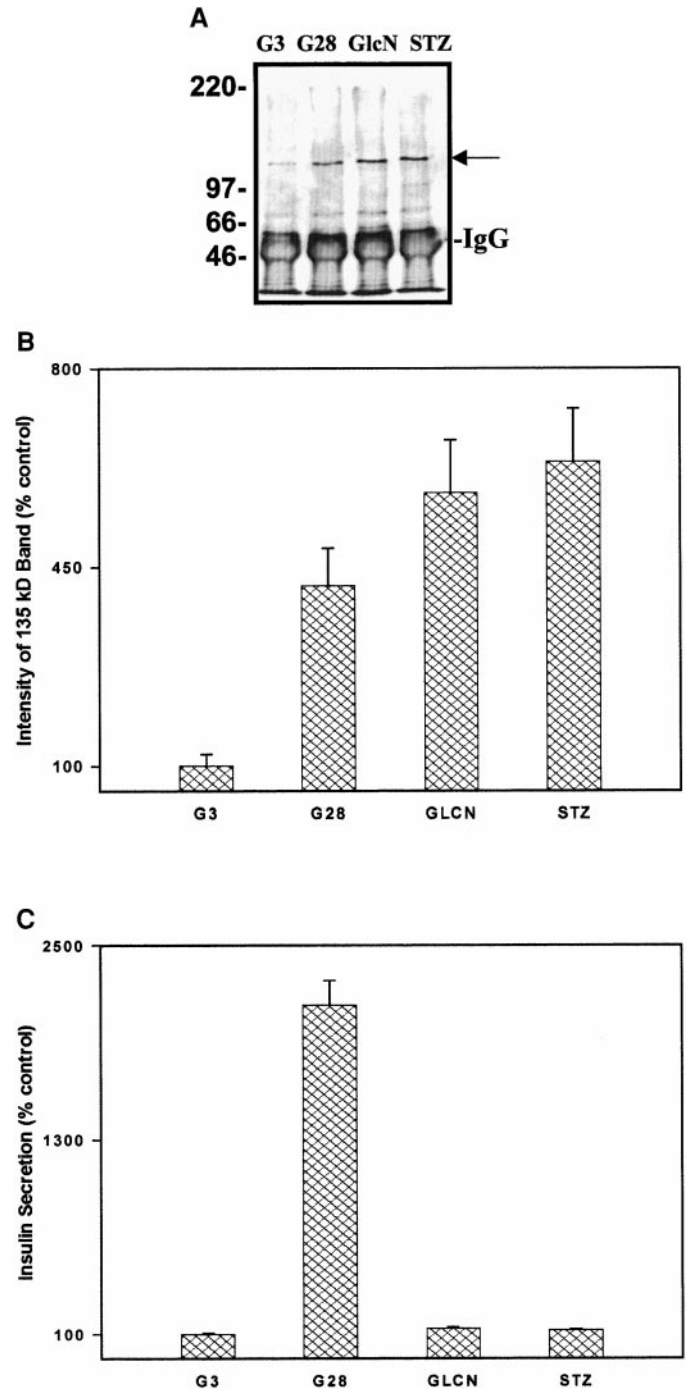

FIG. 2. Glucose, glucosamine, and streptozotocin stimulate O-glycosylation of p135 in islets. Isolated islets (100/condition) were incubated for 60 min with 3 mM glucose (G3), 28 mM glucose (G28), 15 mM glucosamine (GLCN), or 5 mM streptozotocin (STZ). At the end of the incubation period, the supernatant was removed for insulin measurement and O-glycosylated proteins were immunoprecipitated with RL2 antibody. Immunoprecipitated proteins were then separated and transferred to nitrocellulose for Western blotting with RL2 antibody. (A) Results from a representative experiment. (B) Results from A in which the intensity of the band is shown as the mean ± SE from 8 sets of observations. (C) Insulin secretion data corresponding to B.

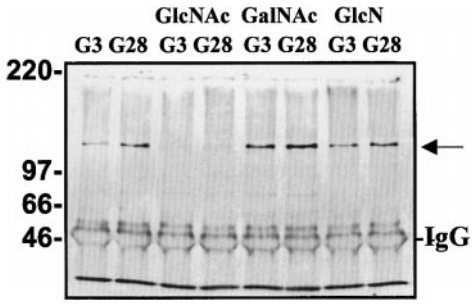

FIG. 3. *N*-Acetylglucosamine (GlcNAc) selectively blocks binding of RL2 antibody to p135. Islets (100/condition) were incubated for 60 min with either 3 mM glucose (G3) or 28 mM glucose following preincubation with 5 mM streptozotocin (STZ). At the end of the incubation period, O-glycosylated proteins were immunoprecipitated with RL2 antibody in the presence of 0.3 M *N*-acetylglucosamine (GlcNAc), 0.3 M *N*-acetylgalactosamine (GalNAc), or 0.3 M glucosamine (GlcN) and analyzed by Western blotting as described in Fig. 2.

versus control), and 636.0 ± 93.3% by streptozotocin ($p < 0.05$ versus control) after 60 min. Insulin secretion, in contrast, was stimulated only by 28 mM glucose (2129.4 ± 153.0%, $p < 0.05$ versus control). Neither streptozotocin nor glucosamine had any significant effect on insulin secretion compared to 3 mM glucose, suggesting that increased O-glycosylation of p135 was not acutely related to insulin release from the islets.

In order to confirm that RL2 binding was selective for O-GlcNAc modified protein, N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), and glucosamine itself were added during the RL2 immunoprecipitation step. As can be seen in Fig. 3, addition of GlcNAc completely inhibited immunoprecipitation of p135. In contrast, neither GalNAc nor glucosamine added at the same concentration as GlcNAc prevented RL2 antibody from immunoprecipitating the protein. Together with the data shown in Fig. 2 these results demonstrated that glucose, glucosamine, and streptozotocin selectively increased the O-linked N-acetylglucosamine content of p135 in islets.

The most likely explanation for the data shown in Figs. 2 and 3 was that glucose and glucosamine were providing more substrate for β-cell O-GlcNAc transferase (OGT) while streptozotocin was inhibiting β-cell O-GlcNAcase. In each case, the end result would be an increase in p135 O-glycosylation. In order to test this idea, islets were incubated with glucose, glucosamine, and streptozotocin in the presence and absence of 6-diazo-5-oxonorleucine (DON), a glutamine analog inhibitor of glutamine: fructose-6-phosphate amidotransferase (GFAT). As shown in Fig. 4, addition of DON inhibited the glucose-induced O-glycosylation of p135, suggesting that glucose acts through the glucosamine pathway to provide more substrate for OGT. In contrast, glucosamine was still able to stimulate p135 O-glycosylation in the presence of DON, most likely because the addition of glucosamine bypasses GFAT. Likewise, streptozotocin was also able to increase p135 O-glycosylation in the presence of DON, in agreement with a previous report that streptozotocin increases protein O-glycosylation by inhibiting removal of O-linked GlcNAc from protein (21).

In order to investigate further streptozotocin-induced p135 O-glycosylation, islets were pre-incubated in the presence or absence of streptozotocin and then stimulated with either 3 or 28 mM glucose. Following this stimulation, islets were then incubated for an additional 30 min in 3 mM glucose. As Fig. 5 demonstrates, O-glycosylation of p135 is reversible when islets are stimulated with 28 mM glucose in the absence of streptozotocin and then exposed to low glucose for an additional 30 min. In contrast after exposure to streptozotocin, increased O-glycosylation of p135 is not readily reversible after stimulation with either 3 or 28 mM glucose, suggesting that streptozotocin may act by irreversibly inhibiting β-cell O-GlcNAcase.

The above data suggested that increased p135 O-glycosylation in response to glucose (and glucosamine and streptozotocin) may be an important intracellular pathway in β-cells. To investigate whether this pathway might also be active in clonal β-cell lines, O-glycosylation in response to glucose, glucosamine, and streptozotocin was examined in the β-TC3 insulinoma cell line. β-TC3 cells have previously been shown to secrete insulin in response to stimulation with glucose and carbachol. Importantly, β-TC3 cells also undergo increased tyrosine phosphorylation of a 125 kD protein in response to glucose, similar to that observed in isolated islets (24). As Fig. 6 shows, however, β-TC3

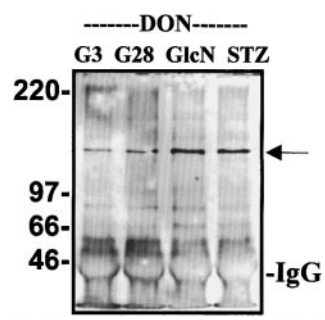

FIG. 4. The GFAT inhibitor 6-diazo-5-oxonorleucine (DON) inhibits glucose but not glucosamine or streptozotocin-induced p135 O-glycosylation. Islets (100/condition) were incubated for 60 min with 3 mM glucose (G3), 28 mM glucose (G28), 15 mM glucosamine (GLCN), or 5 mM streptozotocin (STZ) in the presence or absence of 100 μM of the glutamine:fructose-6-phosphate (GFAT) inhibitor 6-diazo-5-oxonorleucine (DON). At the end of the incubation period, O-glycosylated proteins were immunoprecipitated and analyzed by Western blotting as described in Fig. 2. Results shown are representative of duplicate sets of observations.

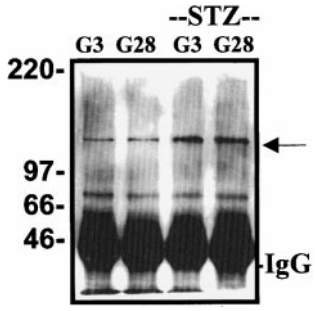

FIG. 5. Glucose-induced p135 O-glycosylation is reversible while streptozotocin-induced p135 O-glycosylation is irreversible. Islets (100/condition) were preincubated for 30 min with either 3 mM glucose or 5 mM streptozotocin (STZ). Islets were then incubated for 30 min with either 3 mM glucose (G3) or 28 mM glucose (G28). Afterward, all islets were incubated in 3 mM glucose for an additional 30 min. O-glycosylated proteins were immunoprecipitated and analyzed by Western blotting as described in Fig. 2. Results shown are representative of 4 sets of observations.

cells fail to undergo increased O-glycosylation in response to glucose, glucosamine, or streptozotocin. In addition, the pattern of protein O-glycosylation observed in β-TC3 cells was much different than that seen in islets. Whereas islets contained a single responsive O-glycosylated protein (p135), β-TC3 cells contained numerous O-glycosylated proteins that failed to undergo any change in their level of O-glycosylation in response to glucose, glucosamine, or streptozotocin. Similar results were also obtained with an additional β-cell line (HIT cells, data not shown). Together, these findings suggested that regulated p135 O-glycosylation may be an important characteristic that separates actual primary β-cells from β-cell lines grown in culture.

DISCUSSION

The results shown above demonstrate that glucose, glucosamine and streptozotocin act through a common pathway in β-cells of pancreatic islets by selectively increasing O-glycosylation of a 135 kD protein. Importantly, the phenomenon of acutely increased p135 O-glycosylation in response to glucose, glucosamine and streptozotocin appears to be specific for islets. Regulated p135 O-glycosylation was not found to occur in clonal insulinoma cell lines, indicating that the pathway may be a characteristic that distinguishes actual β-cells from clonal β-cell lines.

Based on the results obtained when islets were incubated in the presence and absence of the glutamine: fructose-6-phosphate amidotransferase (GFAT) inhibitor 6-diazo-5-oxonorleucine (DON), glucose and glucosamine appear to increase p135 O-glycosylation by providing more UDP-N-acetylglucosamine (UDP-GlcNAc) substrate for the enzyme O-GlcNAc transferase (OGT) to attach to p135. This substrate-driven change, at least in the case of glucose, appears to be reversible. In the case of streptozotocin, however, the O-GlcNAcase enzyme that cleaves O-linked N-acetylglucosamine (O-GlcNAc) off p135 appears to be irreversibly inhibited and O-glycosylated protein accumulates. Therefore, in order for streptozotocin to increase O-GlcNAc on p135, O-GlcNAc must be added and removed cyclically. In the absence of high extracellular glucose, the steady-state O-GlcNAc content of p135 is low, while high glucose shifts the equilibrium to the more highly O-glycosylated form. Streptozotocin appears to trap p135 in the highly O-glycosylated form.

Streptozotocin (Fig. 1) has a chemical structure that resembles N-acetylglucosamine (GlcNAc) but contains a nitrosourea group that can release a molecule of nitric oxide (22). This has led several investigators to suggest that streptozotocin may exert its effects on β-cells by acting as an alkylating agent or nitric oxide donor (25–28). In light of the fact the O-GlcNAcase enzyme contains a free sulfhydryl group in its reactive site (29) and that the N-acetylglucosamine analog streptozotocin can act as a nitric oxide donor, it seems possible that streptozotocin might be acting as a suicide substrate for the enzyme. In this scenario, streptozotocin would compete with O-linked N-acetylglucosamine (O-GlcNAc) for the enzyme O-GlcNAcase. Once streptozotocin binds to the O-GlcNAcase, it could release a molecule of nitric oxide that might react with a free sulfhydryl (SH) group in the active site of the enzyme, thus S-nitrosylating and irreversibly inactivating the enzyme (30). A similar mechanism of S-nitrosylation has been hypothesized for the tyrosine phosphatase inhibitor dephostatin, which competes with the natural substrate of tyrosine phosphatase enzyme and can release a molecule of nitric oxide that reacts with a free sulfhydryl group in the active site of the enzyme, thus inactivating it (31–33).

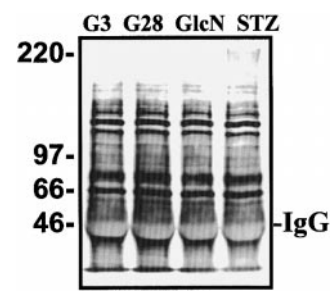

FIG. 6. Glucose, glucosamine, and streptozotocin fail to stimulate p135 O-glycosylation in β-TC3 cells. β-TC3 cells grown in culture in 10-cm dishes were preincubated for 30 min in 0 mM glucose. Following preincubation, the cells (1 dish/condition) were incubated for 60 min with 0 mM glucose (G0), 28 mM glucose and 0.5 mM carbachol (G28), 15 mM glucosamine (GLCN), or 5 mM streptozotocin (STZ). At the end of the incubation period, O-glycosylated proteins were immunoprecipitated and analyzed by Western blotting as described in Fig. 2.

The identity of the 135 kD protein whose O-glycosylation state increases after streptozotocin-induced inactivation of the O-GlcNAcase enzyme in islets is not known and is under intense investigation. As evidenced by the insulin secretion data, O-glycosylation of p135 does not appear to be involved in short-term regulation of insulin secretion. It is possible, however, that the pathway may play a role in long-term glucose sensing by the β-cell.

Regardless of the necessity of p135 O-glycosylation for insulin secretion, this pathway may be of critical importance in β-cells, where glucosamine has been proposed as a metabolite of glucose that mediates maladaptive changes in response to prolonged hyperglycemia (34–37). In other cell types, such as insulin sensitive cells, exposure to both glucose and glucosamine has been shown to increase insulin resistance (38–43). The observation that inhibition of GFAT blocks the effect of glucose on insulin sensitivity suggests that glucose may increase insulin resistance through the glucosamine pathway (44, 45). These studies indicate that glucosamine may play a role in energy homeostasis, but the exact mechanism by which glucosamine exerts its effects is not completely understood, as evidenced by a recent report showing that glucosamine may act in 3T3-L1 adipocytes by decreasing ATP levels (46).

Recent data, however, confirm that glucose and streptozotocin can both act *in vivo* by increasing β-cell protein O-glycosylation as detected by RL2 staining of histologic sections of pancreas (21). In light of this, p135 O-glycosylation in islets may be important since β-cells have been shown to contain much more O-GlcNAc transferase (OGT) than any other cell type (21). The data presented above demonstrate that both glucose and streptozotocin can increase O-glycosylation of the same target protein in β-cells. As a result, these data provide a possible mechanism by which hyperglycemia may cause streptozotocin-like effects in β-cells and thus contribute to the development of type 2 diabetes. Thus, p135 O-glycosylation may play a role in the progression of type 2 diabetes in humans as well as in the development of streptozotocin-induced diabetes in animals.

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