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Increased O-GlcNAc transferase in pancreas of rats with streptozotocin-induced diabetes

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Abstract

Aims/hypothesis. Streptozotocin (STZ), a chemically reactive analogue of *N*-acetylglucosamine, induces necrosis of the beta cells, resulting in diabetes mellitus. Glucose-induced insulin resistance is mediated by increased activity of the hexosamine pathway. We aimed to examine the regulation of O-GlcNAc transferase expression and activity in the normal and streptozotocin diabetic pancreas.
Methods. Rats were made diabetic by an injection of streptozotocin (65 mg/kg). The expression of O-GlcNAc transferase protein was examined by immunoblot analysis. Activity of O-GlcNAc transferase was assayed by the incorporation of [^{3}H]GlcNAc into the synthetic peptide. Localization of O-GlcNAc transferase was done by immunohistochemistry. The change of O-GlcNAc modification of proteins was examined by immunoblot analysis.
Results. In the STZ-induced diabetic pancreas, a severe loss of beta cells was observed, whereas alpha cells had increased in number. The diabetic pancreas showed an increase in the expression of O-GlcNAc transferase at the protein level and the O-GlcNAc transferase activity in it was increased significantly ($p < 0.05$). An increase in the immunostaining intensity in the cytoplasm of islet beta cells was also observed in the diabetic pancreas, whereas exocrine cells and islet cells other than beta cells showed little change in immunostaining intensity. The pancreas of STZ-diabetic rats showed a 3.1-fold increase in total cellular O-GlcNAc-modified proteins.
Conclusion/interpretation. These findings indicate that O-GlcNAc transferase plays an important part in the modulation of O-GlcNAc concentrations in the pancreas and suggest that the increase in O-GlcNAc modification of the proteins correlates closely with diabetes. [Diabetologia (2000) 43: 1239–1247]

Keywords O-GlcNAc transferase, pancreas, streptozotocin, diabetes, immunohistochemistry, rat.

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Abbreviations: DAPI, 4',6'-Diamidine-2-phenylindole hydrochloride; dpm, disintegrations per min; GFAT, glutamine:fructose-6-phosphate amidotransferase; O-GlcNAc, serine(threonine)-*O*-linked *N*-acetylglucosamine; UPPGlcNAc, uridine diphospho-*N*-acetylglucosamine; OGTase, O-GlcNAc transferase; STZ, streptozotocin; HRP, horseradish peroxide; FITC, fluorescein isothiocyanate conjugate.

There are many nuclear and cytoplasmic proteins that are modified by a single *O*-linked *N*-acetylglucosamine (O-GlcNAc) moiety at serine or threonine residues [1, 2]. We proposed that this O-GlcNAc modification (termed O-GlcNAcylation) is a regulatory modification, analogous to phosphorylation, that is, capping of the potential phosphorylation sites [1, 3, 4]. O-GlcNAc transferase (OGTase: EC2.4.1), an enzyme responsible for O-GlcNAcylation of proteins, has been characterized [5] and its cDNA has been cloned [5, 6]. O-GlcNAc transferase mRNA is highly expressed in the pancreas [6]. We previously showed that OGTase and O-GlcNAc are abundant in almost all pancreatic cells and are also abundant

in the cells of the islets of Langerhans, especially in the alpha cells [7]. We speculate that O-GlcNAcylation of proteins by OGTase participates in the glucose-sensing mechanism in the pancreas.

The hexosamine biosynthesis pathway plays an important part in the induction of insulin resistance [8–14]. In this pathway, glutamine: fructose-6-phosphate amidotransferase (GFAT) has an integral role in the development of insulin resistance. The OGTase enzyme uses cytoplasmic uridine diphospho-*N*-acetylglucosamine (UDP-GlcNAc), which is synthesized in the hexosamine biosynthetic pathway [15, 16]. Animals with streptozotocin (STZ)-induced diabetes and hyperglycaemia show greatly increased concentrations of hexosamine metabolites including UDP-GlcNAc [11]. This increase in hexosamine biosynthesis could stimulate OGTase activity, inducing O-GlcNAc modification (O-GlcNAcylation) of proteins that are involved in the glucose transport system or in granule secretion. Recent kinetic analyses have confirmed that OGTase is exquisitely regulated by UDP-GlcNAc concentration across a broad range, from low micromolar to millimolar concentrations [17]. It is therefore likely that the effects of hyperglycaemia result from abnormal O-GlcNAcylation. In this study, the effects of streptozotocin (STZ) diabetes on the expression and activity of OGTase and on O-GlcNAcylation were investigated in the rat pancreas.

Materials and methods

Rat pancreas. The animal study was approved by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee (IACUU). The Spraque-Dawley rats (CD strain, male, body weight 150–200 g) were obtained from the Charles River Laboratory (Wilmington, Mass., USA). Streptozotocin (STZ, 65 mg/kg body weight; Sigma, St. Louis, Mo., USA) was freshly dissolved in 50 mmol/l citrate buffer, pH 4.5, and injected intraperitoneally into rats fasted overnight. Control rats received an injection of citrate buffer alone.

Antibodies. Rabbit polyclonal anti-OGTase antibody (AL-25, purified IgG) was generated against a purified recombinant 110,000-M_r subunit of OGTase that was expressed in *E. coli* [5]. The AL-25 antibody recognizes both 110,000-M_r and 78,000-M_r subunits of OGTase and both subunits are immunoprecipitated by it. Moreover, OGTase enzymatic activity is precipitated by AL-25 [4]. Mouse monoclonal anti-O-GlcNAc antibody (HGAC 85) [18] was generously provided by Dr. N.S. Greenspan (Case Western Reserve University, Cleveland, Ohio, USA). We obtained Cy3 or horseradish peroxide (HRP)-conjugated donkey anti-rabbit IgG antibody and fluorescein isothiocyanate conjugate (FITC) or alkaline phosphatase (ALPase)-conjugated donkey anti-mouse IgG from Jackson Immunoresearch (West Grove, Pa., USA). Mouse monoclonal anti-insulin antibody and anti-glucagon antibody were purchased from Sigma, and rabbit polyclonal anti-insulin and glucagon antibodies were obtained from Novocastra Laboratories (Newcastle, UK).

Western blot analysis. Crude protein extracts were prepared from pancreata dissected from either normal or STZ-diabetic Sprague-Dawley rats (male, body weight 150–200 g). The pancreas was homogenized in a homogenization buffer [20 mmol/l TRIS-HCl (pH 7.4), 5 mmol/l EDTA, 5 mmol/l EGTA, 1 mmol/l dithiothreitol (DTT), 2 mmol/l phenylmethylsulphonyl fluoride (PMSF), protease inhibitor cocktail 1 and 2 (1:1000 dilution)] [19]. When the O-GlcNAc modification of proteins was examined, *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)-amino-*N*-phenylcarbamate [PUGNAc, a generous gift from Oxford GlycoSciences (Oxford, UK)] was added to the homogenization buffer (final concentration, 0.1 mmol/l) to inhibit the activity of endogenous O-GlcNAc-*N*-acetylglucosaminidase (O-GlcNAcase), the enzyme responsible for the removal of O-GlcNAc from proteins [20, 21]. The proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Purified rabbit polyclonal IgG AL-25 (1:5000) or mouse monoclonal anti-O-GlcNAc antibody (HGAC 85, 1:1000 dilution) was used as a primary antibody and anti-rabbit or anti-mouse IgG coupled to horseradish peroxidase (Amersham Pharmacia, Piscataway, N.J., USA), as the secondary antibody (1:20,000 dilution). The horseradish peroxidase activity was detected by enhanced chemiluminescence (ECL) as described by the manufacturer (Amersham). The intensity of protein bands of normal and diabetic rat pancreas was quantified by scanning densitometry.

OGTase activity assay. Normal and STZ-diabetic rat liver, eye, heart, spleen and pancreas were homogenized in a Dounce homogenizer with buffer [10 mmol/l TRIS-HCl (pH 7.5), 10 mmol/l $MgCl_2$, 1 mmol/l EDTA and 1 mmol/l PMSF] and sonicated. The insoluble material was pelleted by centrifugation at 27,500 *g* for 30 min and discarded. The supernatant was made 30 % saturated with ammonium sulphate. The supernatants were allowed to sit overnight on ice, and the resulting precipitate was collected by centrifugation at 12,000 *g* for 20 min. The supernatants were discarded, and the pellets were resuspended in buffer (20 mmol/l TRIS-HCl, pH 7.8, 20 % glycerol). The insoluble material was removed by centrifugation at 12,000 *g* for 15 min, and the final supernatant was used as the source of enzyme.

The OGTase assays were done as described previously [22]. The reaction mixture for the standard assay contained 50 mmol/l sodium cacodylate, (pH 6.0), 150 μg of the synthetic peptide YSDSPSTST, 2.5 mmol/l 5'-adenosine monophosphate and 18.5 kBq of UDP-[6-^{3}H]GlcNAc. The reaction was started by the addition of enzyme and continued for 30 min at 20 °C. The reaction was stopped by the addition of 50 mmol/l formic acid and then the mixture was loaded onto a 0.5-ml SP-Sephadex (SP-C25–120, Sigma) column equilibrated with the same buffer. The column was washed with 50 mmol/l formic acid and the peptides were eluted with 0.5 mol/l NaCl. Incorporation of [^{3}H]GlcNAc into the peptide was quantified by liquid scintillation spectrophotometry.

Immunostaining for light microscopic observation. Specimens were fixed in 4 % formaldehyde in 0.1 mol/l phosphate buffer (pH 7.3) for 1 h at 4 °C, immersed in 2.3 mol/l sucrose-phosphate-buffered saline (PBS) for 1 h at 4 °C, and then frozen with liquid nitrogen. Semithin-frozen sections of 1–2 μm thickness were cut, washed with PBS and treated for 10 min with 1 % bovine serum albumin (BSA) in PBS. The sections were then incubated with AL-25 (1:250) or with pre-immune rabbit IgG (1:250) for 1 h at room temperature, washed with PBS and subsequently incubated for 1 h with Cy3-conjugated donkey anti-rabbit IgG antibody (1:1000) (Jackson Immunoresearch). The sections were then incubated with the mouse

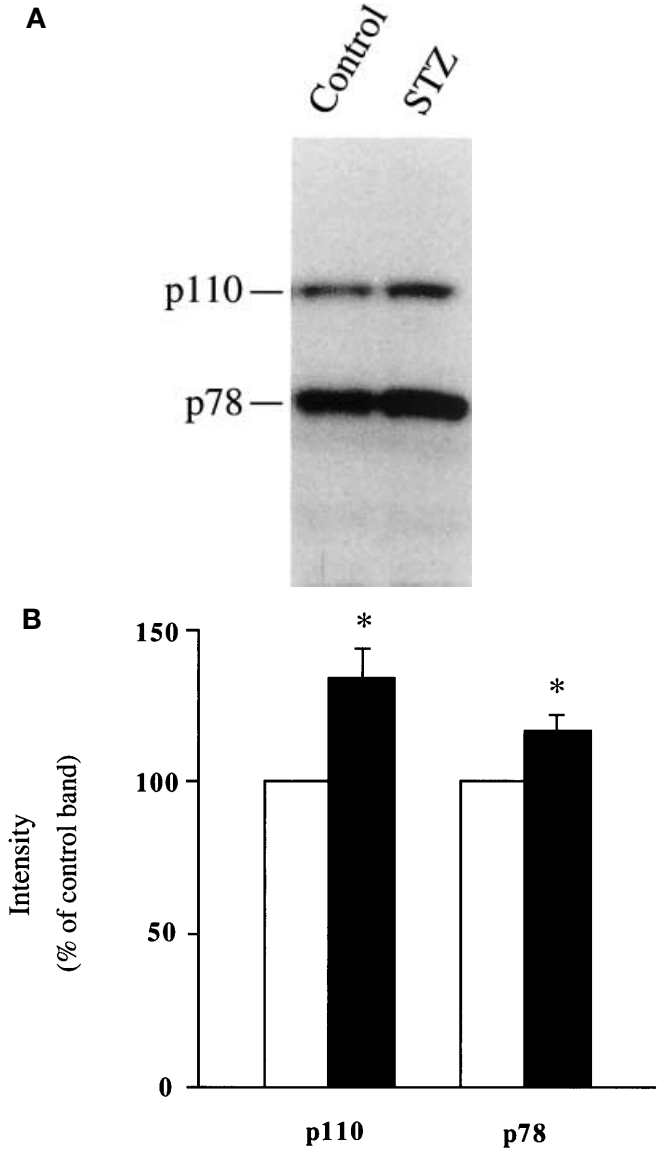

Fig. 1 A, B. Immunoblot analysis of normal and streptozotocin-diabetic pancreata lysate by use of anti-OGTase antibody. **A** The normal or STZ-diabetic pancreas was lysed. The same amount of protein is loaded per track in the normal and STZ-diabetic pancreas lysates and electrophoresed on a 7.5 % polyacrylamide gel and immunoblotted with AL-25. The positions of the p110 and p78 subunits of OGTase are indicated. The figure is representative of three experiments. **B** The intensity of bands obtained from homogenates of normal (□) and diabetic (■) rat pancreas was quantified by scanning densitometry. In the STZ-diabetic pancreas, the intensity of both p110 and p78 was significantly increased over the normal value. The intensity of each band (p110 and p78) was expressed as the mean percentages of the control ± standard error of the band intensity ratio. *$p < 0.05$ vs control. Experiments were done in triplicate

monoclonal anti-insulin (1:1000) or anti-glucagon (1:1000) antibodies (Sigma) for 1 h at room temperature, washed with PBS and incubated for 1 h with FITC-conjugated donkey anti mouse IgG (Jackson Immunoresearch). Nuclei were stained with 4',6-diamidine-2-phenylindole hydrochloride (DAPI, Boehringer Mannheim, Indianapolis, Ind., USA). After a wash with PBS, the specimens were mounted in 90 % glycerol-0.1 mol/l TRIS-HCl buffer (pH 8.5) containing 0.5 mmol/l p-phenylene diamine, which prevents fading of fluorescence during microscopic examination, and observed under a Leica microscope equipped with an epifluorescence system and chilled 3CCD colour camera (C5810, Hamamatsu Photonics Systems, Bridgewater, N.J., USA). Quantification of immunostaining intensity in nuclear and cytoplasmic regions was done by use of the public domain NIH image program (developed at the National Health Institutes, Bethesda, Md., USA). The results (means ± SEM) represent duplicate measurements made in six separate experiments. The terms increase and decrease are applied only when the results were statistically significant (*t* test, $p < 0.05$).

Results

Immunoblot analysis of OGTase in the normal and diabetic pancreas with anti-OGTase antibody (AL-25). The AL-25 antibody recognized both the 110,000-M_r and 78,000-M_r subunits of OGTase (Fig. 1). The intensity of the 78,000-M_r band was stronger than that of the 110,000-M_r one. The density of protein bands obtained with homogenates of normal and diabetic rat pancreas was quantified by scanning densitometry (Fig. 1). The amount of both 110,000-M_r alpha subunit (p110) and 78,000-M_r beta subunit (p78) was significantly increased in the STZ diabetic pancreas ($p < 0.05$), because the same amount of protein is loaded per track.

Assay for OGTase activity in normal and STZ-diabetic rat pancreas. Rats were made diabetic using standard procedures, as follows. On days 5–7 after injection of STZ, the serum glucose concentration was assayed by the hexokinase and glucose-6-phosphate dehydrogenase method (Sigma). The rats were then killed to obtain their pancreatic tissue. Plasma glucose was increased ($p < 0.001$) in diabetic animals (25.65 ± 1.47 mmol/l, means ± SEM, $n = 8$) compared with controls (5.42 ± 1.34 mmol/l, $n = 6$). To examine the effect of STZ diabetes on the OGTase activity of pancreas and other tissues, the OGTase activities in pancreas, liver, heart, spleen and eye were assayed. The 30 % ammonium sulphate cytosolic pellets were assayed for enzymatic activity. There was no significant difference in the OGTase activity between normal [366.4 ± 24.5, 115 ± 11.2, 224.1 ± 13.9 means ± SEM disintegrations per min (dpm)/μg protein] and STZ-diabetic (336.7 ± 20.3, 116.8 ± 5.1, 208.6 ± 14.1 dpm/μg protein) rat liver, heart and spleen, respectively (Fig. 2). The OGTase activity of the diabetic rat pancreas (535.3 ± 17.6 dpm/μg protein) was, however, higher ($p < 0.05$) than that of the normal pancreas (199.3 ± 9.1 dpm/μg protein). The enzyme activity of the diabetic rat eye (134.55 ± 19.6 dpm/μg protein) was also higher ($p < 0.05$) than that of a normal eye (55.98 ± 14.6 dpm/μg protein).

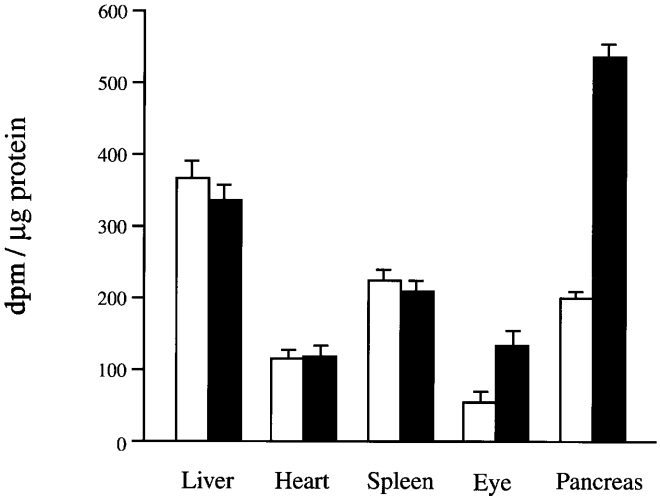

Fig. 2. OGTase activity (dpm/μg protein) in normal (□) and diabetic (■) rat liver, heart, spleen, eye and pancreas. Data represent the means ± SEM. ($n = 6$)

Changes in immunostaining of OGT in STZ diabetic pancreas. The distribution of OGTase in the pancreas was examined by immunofluorescence microscopy (Figs. 3, 4). The immunofluorescence intensity of nuclear and cytoplasmic regions of alpha and beta cells was quantified in the control and STZ diabetic pancreas (Fig. 5). The multiple staining method showed that the alpha cells of Langerhans islets of normal pancreas were enriched in OGTase, that is, intense staining was observed in the cytoplasm as well as in the nucleus of the alpha cells (Fig. 4). In the beta cells, intense staining was observed in the nucleus, whereas the cytoplasm was diffusely and weakly stained (Fig. 4). In the exocrine acinar cells, nuclei and the region containing zymogen granules were intensely stained with AL-25 (data not shown). In the Langerhans islets of STZ-diabetic pancreas, a significant decrease in the number of beta cells due to beta-cell necrosis was observed, whereas alpha cells increased in number (Fig. 4). The immunostaining in the cytoplasm of the beta cell of the STZ-diabetic rat increased in intensity, whereas the nucleus showed little change (Fig. 5). No change in the localization or immunostaining intensity of OGTase was, however, observed in alpha cells (Figs. 4, 5) or acinar cells (data not shown). In the control experiment in which the primary antibody was omitted or replaced with preimmune rabbit IgG instead of AL-25, no positive staining was observed (data not shown).

Effect of STZ diabetes on the O-GlcNAc modification of proteins in the pancreas. To study the effect of STZ diabetes on the O-GlcNAc modification, we extracted total proteins from normal and STZ-diabetic pancreata. Proteins were separated by SDS-gel electrophoresis and analysed by Western blotting with monoclonal anti-O-GlcNAc antibody (HGAC 85). Proteins extracted from the normal pancreata had a lower O-GlcNAc content (Fig. 6). In contrast, at least six major proteins [molecular weights (M_r) of 46,000, 37,000, 31,000, 15,000, 12,000, 9300] from the STZ-diabetic pancreata showed an increase in the amount of O-GlcNAc modification [The increases were (in folds) 3.13, 1.25, 1.32, 6.32, 4.45, 2.51 for each of the above proteins respectively, with an average increase of 3.1 fold].

Discussion

Because we showed previously that OGTase and O-GlcNAc are present abundantly both in the exocrine acinar cells and endocrine islet cells of the pancreas [7], as a next step, we examined the expression and the activity of the OGTase in the pancreas and the O-GlcNAcylation of total pancreatic proteins. In this study, an increase in OGTase expression and activity was found in the STZ-diabetic pancreas. The amount of enzyme activity was, however, not linearly dependent upon the amount of OGTase proteins, as we previously observed in various tissues [5]. This indicates that additional factors regulate the activity of OGTase [17]. Previous immunohistochemical studies of OGTase distribution in the pancreas showed a uniquely concentrated distribution of the O-GlcNAc modification [7]. Its localization both in nucleus and cytoplasm of all pancreatic cells, especially in the nucleus, suggests that it has a fundamental cellular function. In the nucleus OGTase catalyses the O-GlcNAcylation of many nuclear proteins, such as nuclear pore proteins, RNA polymerase II, transcription factors, c-myc oncoprotein, p53 tumour suppressor and oestrogen receptor [2, 23]. Another characteristic distribution of OGTase is its rich cytoplasmic localization in the exocrine acinar cells and islet cells. An immunoelectron microscopic study showed that in the exocrine acinar cells and islet cells OGT was localized around the secretory granules [7], suggesting that OGTase is involved in the process of secretion of granules. In this study a statistically significant increase in immunostaining intensity of OGTase was detected in the cytoplasm of islet beta cells in the STZ-diabetic pancreas whereas no change was observed in the exocrine acinar cells and other islets cells. The capacity to secrete insulin in STZ-diabetic rats is significantly reduced from the control in the isolated islets [24]. The OGTase enzyme could regulate granular secretion of beta cells in the STZ-diabetic pancreas by catalysing the O-GlcNAcylation of cytoskeletal proteins such as microtubule-associated proteins which are involved in the secretion or transport of granules/vesicles [25–29] and are heavily O-GlcNAcylated [30–33].

It has been shown that the hexosamine biosynthesis pathway mediates the glucose-induced desensiti-

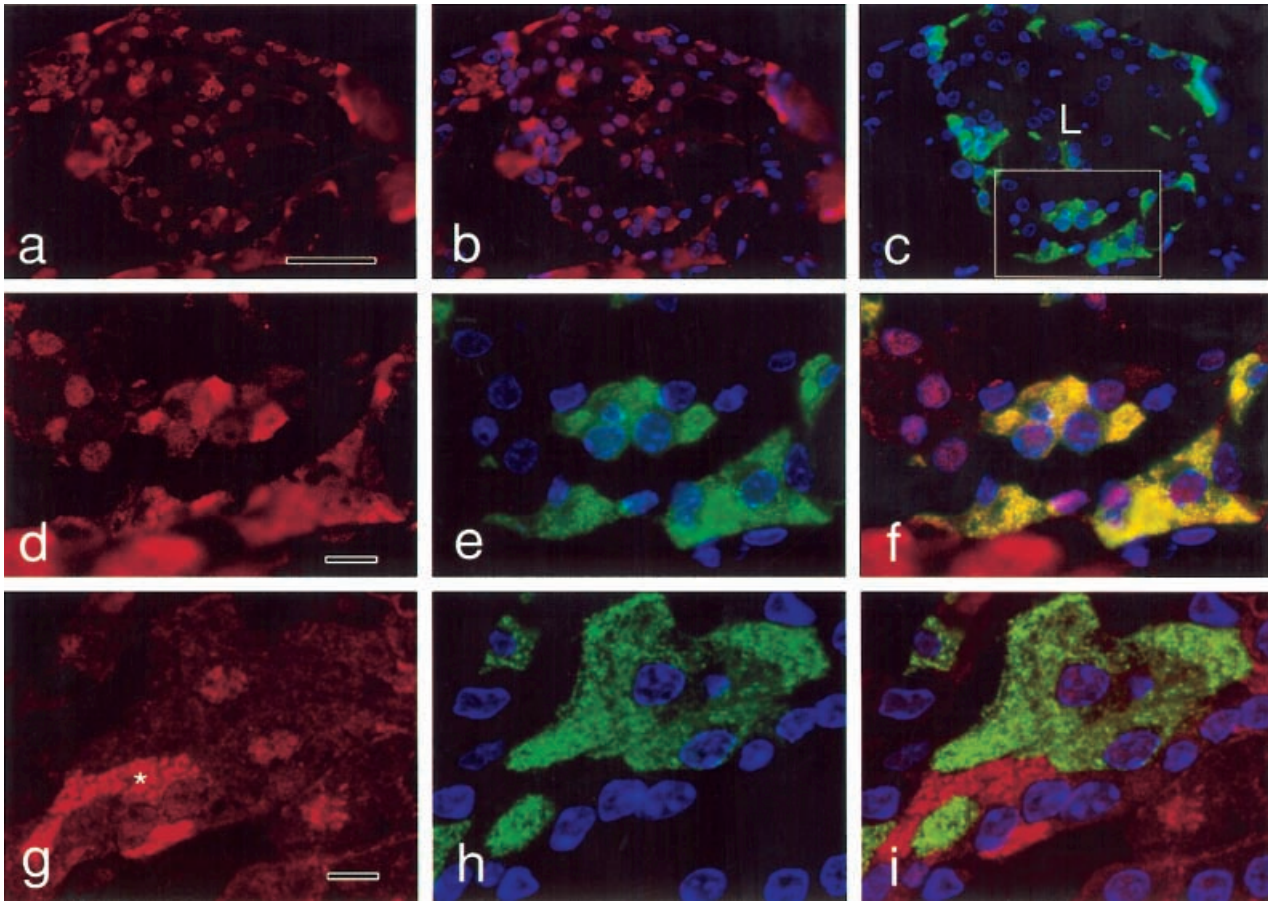

Fig. 3 a–i. Immunofluorescent distribution of anti-OGTase, anti-glucagon and anti-insulin reactivity in the Langerhans islet of normal rat pancreas. Pancreas was fixed with 4 % paraformaldehyde. Semithin frozen sections reacted with rabbit polyclonal anti-OGTase antibody (AL-25) and then with Cy3-donkey anti-rabbit IgG. The sections were subsequently incubated with mouse monoclonal anti-glucagon or insulin antibody, then with FITC-donkey anti-mouse IgG. Nuclei were stained with DAPI (blue). **a–c** Same optical field. **a** Distribution of AL-25 reactivity (red). **b** Double-exposure image for AL-25 and nucleus (blue). **c** Double-exposure image for glucagon (green) and nucleus (blue). The alpha cells are preferentially localized at the periphery of the Langerhans islet (L). **d–f** Same optical field. **d** Enlargement of **a**. **e** Enlargement of rectangle in **c**. **f** Triple-exposure image for AL-25, glucagon and nuclei. Intense signals are observed in both the cytoplasm and nucleus of the alpha cells. **g–i** Same optical field. **g** Distribution of AL-25 reactivity (red) in another optical field of an islet. **h** Double-exposure image for insulin (green) and nuclei (blue). **i** Triple-exposure image for AL-25, insulin and nuclei. Intense signals are observed in the nuclei of beta cells and in the cytoplasm of the cells that are thought to be alpha cells (asterisk). Bar in **a**, 50 μm. Bars in **d** and **g**, 10 μm

zation of the glucose transport system and induces insulin resistance [8–14]. The glutamine:fructose-6-phosphate amidotransferase (GFAT), which is a rate-limiting enzyme of the hexosamine pathway, plays an integral part in insulin resistance. Its activity is regulated coordinately by insulin, glucose and glutamine [34]. Overexpression of GFAT in the transgenic mouse induces insulin resistance [35] and inhibits glucose transporter 4 (GLUT-4) translocation from intracellular vesicles to the plasma membrane [36]. Diabetes induced by STZ increases the concentration of UDP-hexosamine in rat skeletal muscle [11]. The UDPGlcNAc is a final product of hexosamine synthesis. In the O-GlcNAcylation of proteins, OGTase uses cytoplasmic UDPGlcNAc that is synthesized in the hexosamine biosynthetic pathway [15, 16] and OGTase's activity is tightly regulated by the amounts of O-GlcNAc over the entire physiologic range of the sugar donor's concentration within cells [17]. Here we show that the OGTase activity in the pancreas and eye is increased in STZ-diabetic rats. Using anti-O-GlcNAc antibody for immunoblotting, we found that STZ-diabetic rats showed a 3.1-fold increase in total O-GlcNAcylated proteins in their pancreata. Several protein bands from STZ-diabetic rat pancreata had greater staining for O-GlcNAc than the corresponding bands from control pancreata. This increase in the staining for O-GlcNAc could be ascribed to: (1) increased O-GlcNAc-modification of pre-existing proteins, (2) increased synthesis of O-GlcNAcylated-protein, (3) the increased proportion of alpha cells or exocrine cells in the STZ-diabetic rat pancreas or (4) all

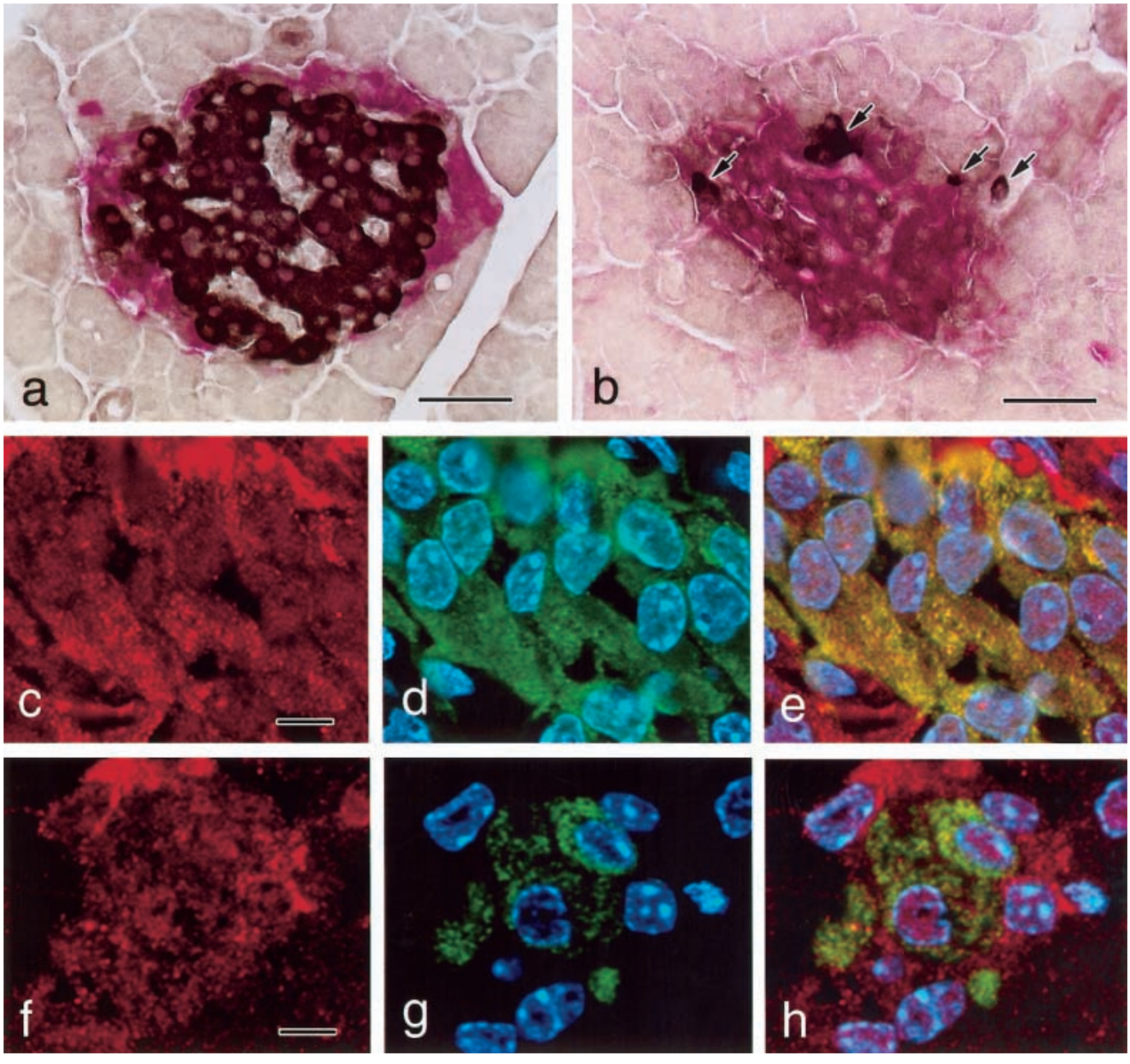

Fig. 4 a–h. Immunofluorescent distribution of anti-OGTase, anti-glucagon and anti-insulin reactivities in the Langerhans islets of STZ-diabetic rat pancreas. **a,b** Pancreas was fixed with 4% paraformaldehyde. Paraffin sections reacted with mouse monoclonal anti-insulin antibody and then with HRP-labelled secondary antibody. The signal (dark brown) of insulin was detected with 3,3'-diaminobenzidine (DAB) in the presence of $NiCl_2$. After the DAB reaction, the sections were incubated with rabbit polyclonal anti-glucagon antibody and then with alkaline phosphatase-labelled secondary antibody. The signal (red) of glucagon was detected with fast red. **a** In the islet of normal pancreas, the beta cells are located centrally, whereas the alpha cells lie peripherally. **b** In the islet of the STZ-diabetic pancreas, beta cells (arrows) are decreased in number, whereas alpha cells have increased in number. **c–h** The immunostaining was done as in Fig. 3. **c–e** Same optical field. **c** Distribution of AL-25 reactivity (red) in the alpha cells. **d** Distribution of glucagon reactivity (green) in the alpha cell. **e** Triple-exposure image for AL-25, glucagon and nuclei. **f–h** Same optical field. **f** Distribution of AL-25 reactivity (red). **g** Distribution of insulin reaction (green). **h** Triple-exposure image for AL-25, insulin and nuclei. Bars in **a** and **b**, 50 μm. Bars in **c** and **f**, 10 μm

three. Enhancement of OGTase activity in the diabetic pancreas implies an important role for OGTase in diabetes. This heightened activity could induce an increase in the O-GlcNAcylation of proteins that are involved in granule secretion and vesicle translocation. We posit that in the pancreas, O-GlcNAcylation of proteins by OGTase are involved in the normal glucose-sensing mechanism and in the peripheral tissues, OGTase is involved in the desensitization of the glucose uptake system by mediating O-GlcNAcylation of cytoskeletal proteins. These are the proteins that regulate the glucose transporter translocation from intracellular vesicle to the plasma membrane.

In our study no significant change of OGTase activity was observed in the liver, heart and spleen of STZ-diabetic rats, but OGTase activity increased in the eye and pancreas. In the liver the GFAT activities are similar in *ob*/*ob* mice and controls [13] but de-

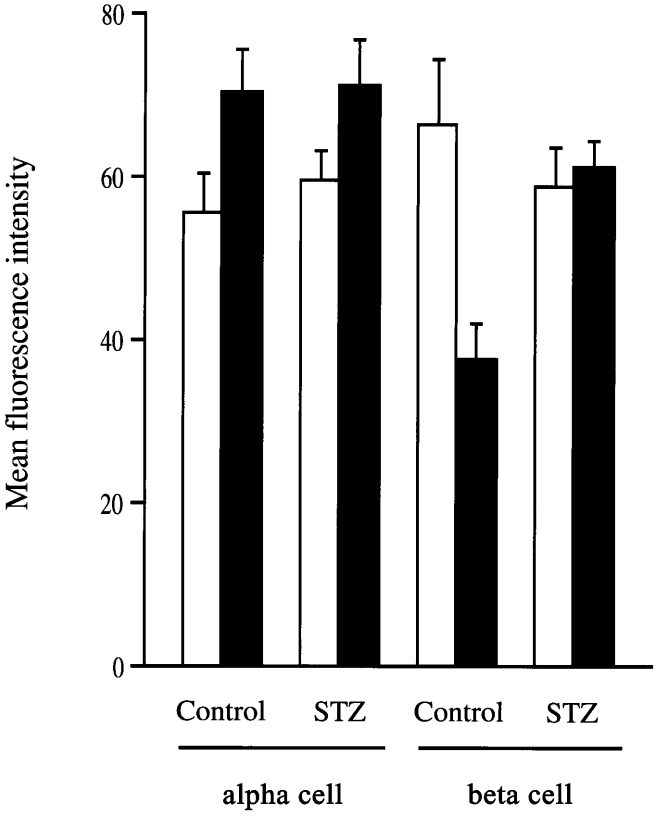

Fig. 5. Effect of STZ diabetes on the OGTase immunoreactivity of islet cells. Graphical quantification of the OGTase immunoreactivity in nucleus and cytoplasm of alpha and beta cells. Symbols: (□) nucleus, (■) cytoplasm. In the alpha cells, there was little change in the immunoreactivity. STZ diabetes resulted in an increase in immunoreactivity in the cytoplasm of beta cells. Data represent the means ± SEM in six independent experiments

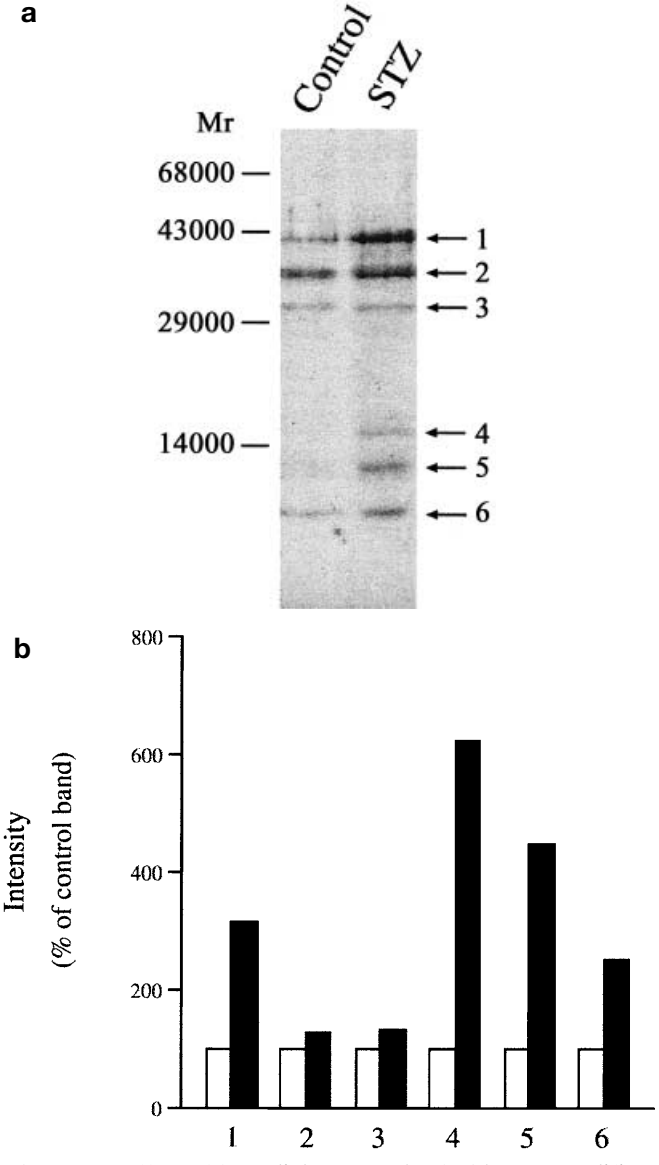

Fig. 6 a, b. Effect of STZ diabetes on the O-GlcNAc modification of proteins in pancreas. **a** The total proteins from control and STZ diabetic pancreas were separated by SDS gel electrophoresis and analysed by western blotting using monoclonal anti-O-GlcNAc antibody (HGAC85 as a probe). **b** The intensity of bands (1–6 in **a**) was measured by a scanning densitometer. The intensity of each band is plotted as the percentage of the intensity of the corresponding control band. The figure is representative of three similar experiments. Symbols: (□) control, (■) STZ

crease in STZ-diabetic rats [11]. The OGTase activity seems to be differently regulated in each tissue. Each tissue consists of heterogeneous cells, therefore OGTase activity could be different in each cell type and respond differently to diabetes [11]. The expression of tissue-specific forms of OGTase is one mechanism by which the activity or substrate specificity of the enzyme could be regulated [5].

Streptozotocin is a glucosamine analogue that specifically destroys islet beta cells [37, 38]. In the STZ-diabetic pancreas, necrosis of beta cells occurs and severe degranulation is observed in the beta cells [39–41]. Beta cells do not, however, completely necrotize. Limited proliferation derived from pre-existing precursor beta cells occurs about 4 days after STZ is given to rats [42]. The beta cells or beta-cell lines that express glucose transporter 2 (GLUT-2) show enhanced transport of STZ [43]. Meanwhile, kidney, liver and small intestine, all of which also express GLUT-2 [44], are less susceptible to STZ. Streptozotocin has been shown to induce DNA strand breaks or free radicals [45–48]. The mechanism of STZ-induced beta-cell toxicity is, however, not clearly understood [46, 49–52]. Because STZ is a weak inhibitor of O-GlcNAcase (IC_{50} = ~ 2.5 mmol/l, Parker and Hart, unpublished), the enzyme responsible for O-GlcNAc removal, it has been proposed that inhibition of O-GlcNAcase induces the accumulation of O-GlcNAcylated proteins, thus contributing to selective beta cell apoptosis by STZ [53, 54]. Our data indicates that OGTase plays a part in the modulation of O-GlcNAc concentrations in the beta cells

of STZ-diabetic pancreas. Recently, it was shown that STZ and glucose increase the O-GlcNAcylation of beta-cell proteins, especially 135,000-M_r protein [53, 55, 56]. In the absence of hyperglycaemia, STZ itself does not alter the O-GlcNAcylation in beta cells [56]. Although glucose induces the rapid and reversible accumulation of O-GlcNAc in beta cells, STZ-induced accumulation of O-GlcNAc is irreversible even after normoglycaemia is re-established by insulin infusion [56]. This indicates that the removal of O-GlcNAc modification was blocked by STZ. Furthermore, transgenic mice in which GFAT antisense is expressed to impair glucosamine synthesis are resistant to the diabetogenic effect of STZ [56]. Thus, the O-GlcNAcylation of proteins in the beta cells could play a part in the development of diabetes induced by STZ and glucose. Of note STZ-induced accumulation of O-GlcNAc in the beta cells is implicated in beta cell apoptosis. Our study showed that more O-GlcNAcylated proteins, measured in total pancreatic proteins, are present in STZ-diabetic rats than in control rats [55, 56] and that the amount of the OGTase is stimulated in STZ-diabetic rats. Thus we think that STZ has effects not only on the islet cells but also on the exocrine cells.

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