Identification of iduronate-2-sulfatase in mouse pancreatic islets

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Identification of iduronate-2-sulfatase in mouse pancreatic islets. Am J Physiol Endocrinol Metab 287: E983–E990, 2004. First published May 18, 2004; doi:10.1152/ajpendo.00528.2003.—The lysosomal enzyme iduronate-2-sulfatase (IDS) is expressed in pancreatic islets and is responsible for degradation of proteoglycans, such as perlecan and dermatan sulfate. To determine the role of IDS in islets, expression and regulation of the gene and localization of the enzyme were investigated in mouse pancreatic islets and clonal cells. The IDs gene was expressed in mouse islets and ß- and α-clonal cells, in which it was localized intracellularly in lysosomes. The transcriptional expression of IDs in mouse islets increased with glucose in a dose-dependent manner (11.5, 40.2, 88, and 179% at 5.5, 11.1, 16.7, and 24.4 mM, respectively, P < 0.01 for 16.7 and 24.4 mM glucose vs. 3 mM glucose). This increase was not produced by glyceraldehyde (1 mM) or 6-deoxyglucose (21.4 mM) and was blocked by the addition of mannoheptulose (21.4 mM). Neither insulin content nor secretory response to glucose (16.7 mM) was altered in mouse islets infected with lentiviral constructs carrying the IDs gene in sense orientation. Furthermore, no decrease in islet cell viability was observed in mouse islets infected with lentiviral constructs compared with controls. However, insulin content was reduced (35% vs. controls, P < 0.001) in islets infected with IDS antisense construct, while the secretory response of those islets to glucose was maintained. Inhibition of IDS by antisense infection led to an increase in lysosomal size and a high rate of insulin granule degradation via the crinophagic route in pancreatic β-cells. We conclude that IDS is localized in lysosomes in pancreatic islet cells and expression is regulated by glucose. IDS has a potential role in the normal pathway of lysosomal degradation of secretory peptides and is likely to be essential to maintain pancreatic β-cell function.

perlecan; islet amyloid polypeptide; insulin content and secretion; lysosomes; apoptosis; β-cell

THE HUMAN IDURONATE-2-SULFATASE (IDS) gene contains nine exons spread over ~25 kb and is located on the Xq28 chromosome band. The IDS full-length cDNA encodes a protein of 550 amino acids (12, 41, 42). Biosynthesis and processing of IDS have been studied in transfected fibroblasts. The enzyme (EC 3.1.6.13) is synthesized as two 76,000- and 90,000-molecular-weight precursor forms; subsequent modification of N-linked sugar residues, with the addition of mannose 6-phosphate, targets newly synthesized IDS to lysosomal compartments, where, after proteolytic cleavage, it is converted to mature 55,000- and 45,000-molecular-weight polypeptides (13, 25). Murine IDS cDNA, which encodes 564 amino acid residues, has also been cloned and characterized (36). The coding sequence of the murine gene has 85% identity to the human gene, and the amino acid sequence is 89% identical. In addition, molecular defects in the IDS gene, which provoke a defect in the enzymatic activity leading to the accumulation of partially degraded glycosaminoglycans in lysosomes, have been described (18). This results in the development in humans of Hunter syndrome or mucopolysaccharidosis type II, which is a rare X-linked recessive lysosomal storage disorder (19, 28).

IDS is a lysosomal enzyme involved in the degradation of glycosaminoglycans. Its activity is based on the removal the sulfate group of the iuronate-2-sulfate units of dermatan sulfate proteoglycan 3 and heparan sulfate proteoglycan 2 (HSPG2) (17). HSPG2, also known as perlecan, has been identified in islet cells (20) and is thought to be involved in the formation of pancreatic amyloid deposits described in type 2 diabetes (43); however, overproduction of the perlecan core protein is insufficient to lead to amyloidosis (16). Glycosaminoglycans, including perlecan, are components of extracellular amyloid deposits formed from islet amyloid polypeptide (IAPP) (7, 40). In addition, perlecan, localized in the basement membrane of capillaries, may contribute to amyloid deposition in Alzheimer’s disease, familial amyloidoses, prion diseases (22, 35, 38), and pathogenesis of diabetes complications (6). Furthermore, recent studies have demonstrated that heparin and perlecan can bind the human NH2-terminal pro-IAPP molecule, acting as a potent enhancer of IAPP fibril formation in vitro (3, 30).

Preliminary studies using differential display procedures have found that the IDS gene is preferentially expressed in pancreatic islets relative to exocrine tissue (11). However, some information related to the identification of IDS in islets cells has been published. In this sense, the present work is focused on examining the expression and the main determination of metabolic regulation of the IDS gene in mouse pancreatic islets.

MATERIALS AND METHODS

Mouse Pancreatic Islet Isolation and Culture

Mouse pancreatic islets were isolated from 2-mo-old CD-1 male mice by a modification of the procedure originally described by Lacy and Kostianovsky (23). Briefly, the pancreas was cannulated and digested with collagenase (Roche Diagnostics, Mannheim, Germany), and islets were purified from the exocrine tissue through discontinuous Histopaque density gradients. Groups of 100 islets were hand picked under a stereomicroscope, transferred to petri dishes, and precultured for 18 h in RPMI 1640 medium (GIBCO-BRL Life Technologies, Paisley, UK) supple-

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http://www.ajpendo.org 0193-1849/04 $5.00 Copyright © 2004 the American Physiological Society E983
Gene Expression Analyses

Total RNA from mouse pancreatic islets was extracted using QuickPrep Total RNA Extraction Kit (Amersham Pharmacia Biotech) and from the exocrine tissue by the guanidine isothiocyanate method (4).

After digestion with DNase (GIBCO-BRL) for 15 min at room temperature, cDNAs were synthesized using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) following the manufacturer’s instructions. These cDNAs were used as the PCR template for reactions set up in the presence of specific primers (Table 1), dNTPs, and Taq DNA polymerase (Promega). Twenty milliliters of the PCR products were examined by electrophoresis on 2% agarose gel after electrophoresis.

Quantitative real-time RT-PCR of IDS, I, insulin, Iapp, perlecan, and ribosomal protein S18 (Rps18) was performed. The primers and probes (Table 1) were selected with Primer Express software (Applied Biosystems). Amplifications were carried out using Prism 7900HT Sequence Detection System (Applied Biosystems) containing 5 ng of cDNA template per sample and the endogenous detection probe TaqMan 18S rRNA (Applied Biosystems). Then the Rps18 was used as a reference against which the expression level of the transcript genes of interest was normalized.

Subcellular Location of IDS

Plasmid construction. Human IDS cDNA (GenBank accession no. NM_000202) was generated by PCR using the PfdI enzyme (Stratagene, Amsterdam, The Netherlands), and the primers IDS/Nhe-ATCTAGCTAGCGAAATGCCGCCACC-3 (5’/H9251 from a pancreatic adenoma created in transgenic mice) were grown on Primo SV CMV vector (kindly provided by B. Thorens, University of Lausanne). The oligonucleotides were as follows: 5’-AATTCGACGCGGTAAATGCCGCCACC-3’ (sense) and 5’-TCGACGCGGTCAAGATCCGGACC-3’ (antisense) for MluI/IDS and 5’-ACCCTGCTGTAAGAATCCGGACC-3’ (sense) and 5’-ACCCTGCTGTAAGAATCCGGACC-3’ (antisense) for XhoI/IDS. The enolase plasmid pMDG and the packaging plasmid pmCMVDR8.7 were described previously (10) (supplied by B. Thorens). Virus stocks were prepared as previously described (26, 27) by transient cotransfection of three plasmids into 293T cells. The medium was collected after 48 h and filtered through a 0.45-mm pore-size filter and concentrated by ultracentrifugation. The viral titer was calculated by quantification of the p24 content by ELISA (Innogenetics). The islets were infected with lentiviral particles (20 IU/β-cell) for 4 h and then cultured in the corresponding medium for 48 h. To assess the efficiencies of the lentiviral vector infection of mouse islet, expression of IDS was determined by quantitative real-time RT-PCR.

Insulin Secretion and Content

After lentivirus infections, groups of eight mouse islets were placed in 1 ml of bicarbonate-buffered solution with 5 mg/ml BSA in the presence of 5.5 and 16.7 mM glucose and incubated for 90 min at 37°C in a shaking water bath. The supernatants were then stored at −20°C until radioimmunoassay for insulin (CIS Biointernational, Gif-sur-Yvette, France; detection limit = 30 pm, intra- and interassay coefficients of variation = 6 and 8%, respectively). For the determination of insulin content, the islets were sonicated at 4°C in 0.5 ml of acid-ethanol solution (75% ethanol and 1.5% 10 mM HCl).

Table 1. Primer and probe sequences used for RT-PCR and quantitative real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>TaqMan Probe</th>
<th>PCR Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDS</td>
<td>5’-GCCACAGCCCTCCTCTTGCA-3’</td>
<td>5’-CTTCACTTCTCGGAGTGC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>90</td>
</tr>
<tr>
<td>Ids</td>
<td>5’-GCCATCCACTCCCACTCTG-3’</td>
<td>5’-TGCAGCTGCTTTGCTCTTA-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>201</td>
</tr>
<tr>
<td>Iapp</td>
<td>5’-GCCCTCACTCCCACTCTG-3’</td>
<td>5’-TGCAGCTGCTTTGCTCTTA-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>101</td>
</tr>
<tr>
<td>Hspg2</td>
<td>5’-GCCACACTGAGGTTGGAAGCT-3’</td>
<td>5’-GACCTGCAAGCAGATCTGTC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>73</td>
</tr>
<tr>
<td>Ins</td>
<td>5’-GCCACACTGAGGTTGGAAGCT-3’</td>
<td>5’-GACCTGCAAGCAGATCTGTC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>106</td>
</tr>
<tr>
<td>Ics</td>
<td>5’-GCCACACTGAGGTTGGAAGCT-3’</td>
<td>5’-GACCTGCAAGCAGATCTGTC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>101</td>
</tr>
<tr>
<td>Rs9</td>
<td>5’-GCCACACTGAGGTTGGAAGCT-3’</td>
<td>5’-GACCTGCAAGCAGATCTGTC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>404</td>
</tr>
<tr>
<td>Amy</td>
<td>5’-TGAGCTTCTGCTGCTGTCTGGA-3’</td>
<td>5’-GGGAGGCGGCTGTCTGCTGTCTGGA-3’</td>
<td>5’-GGGAGGCGGCTGTCTGCTGTCTGGA-3’</td>
<td>537</td>
</tr>
<tr>
<td>Gcg</td>
<td>5’-GCCACAGCCCTCCTCTTGCA-3’</td>
<td>5’-CTTCACTTCTCGGAGTGC-3’</td>
<td>5’-ATGCAGTTTGGGCAAGAGCAGTG-3’</td>
<td>443</td>
</tr>
</tbody>
</table>

IDS and Ids, human and murine iduronate-2-sulfatase; Iapp, islet amyloid polypeptide; Hspg2, heparan sulfate proteoglycan 2; Ins, insulin; Amy, amylose; Gcg, glucagon; Rs9, ribosomal protein S9.
Analysis of Islet Cell Viability

Isolation of single mouse islet cells. After lentiviral vector infection, single mouse islet cells were obtained by digesting 50 mouse islets in 2 ml of PBS containing 0.125 mg/ml trypsin and 0.05 mg/ml EDTA (GIBCO-BRL) at 37°C. The cell suspension was cycled for 5 min at 37°C and for an additional 5 min on ice to allow islets to sediment. Then the supernatant containing the single cells was removed and placed in 1 ml of fetal calf serum (GIBCO-BRL). To obtain additional single islet cells, the digestion process was repeated a maximum of four times.

Fluorescence-activated cell sorting. Isolated islet cells were pelleted, washed, and resuspended in 400 μl of binding buffer [in mM: 100 HEPES (pH 7.4), 1.5 NaCl, 50 KCl, 10 MgCl₂, and 18 CaCl₂]. For each experimental condition, 200 μl of cells were unlabeled and used to define the threshold of detection in fluorescence-activated cell sorting (FACS) analysis. The other 200 μl of cell solution were double stained with annexin V-phycocerythin (PE) and 7-aminoactinomycin D (7-AAD) by using Annexin V-PE Apoptosis Detection Kit I (Becton Dickinson, San Jose, CA) following the manufacturer’s instructions. Thereafter, cells were analyzed on a FACS Calibur (Becton Dickinson) with Cell Quest software (Becton Dickinson). FACS gating based on forward and side scatter out of 100,000 cells was included for analysis. Every run included positive and negative control samples for cell cytotoxicity. Negative cells for annexin V and 7-AAD were considered viable cells, early apoptotic cells were annexin V positive, and apoptotic or necrotic cells were negative for annexin V and 7-AAD.

Electron Microscopy

Lentiviral-infected islets were brieﬂy rinsed with phosphate buffer (PB) and ﬁxed with fresh 4% paraformaldehyde-1% glutaraldehyde in 0.1 M PB, pH 7.4, for 1 h at room temperature. Islets were postﬁxed with OsO₄ in 0.1 M PB for 1 h at 4°C. Islets for immunolabeling were dehydrated through ascending series of ethanol to 80% and inﬁltrated with 1:1 80% ethanol-LR White hydrophilic acrylic resin (London Resin, Berkshire, UK); then undiluted LR White was changed twice at room temperature. The inﬁltration was continued with fresh LR White overnight at 4°C, and, after a ﬁnal change to fresh resin, embedding was completed by polymerization under vacuum at 55°C for 48 h. After separation of the Thermonox coverslip from the embedded cells, 80- to 100-nm cross sections were cut and mounted on a 150-mesh nickel grid supported with carbon-coated parlodion. Immunolocalization of IAPP in the islets was performed by treatment of the sections with a polyclonal antibody against IAPP (kindly provided by A. Clark, University of Oxford). Antiseria binding sites were identiﬁed with protein A-conjugated gold particles (15 nm; BB International). The sections were stained for tissue contrast with uranyl acetate and lead citrate. They were viewed in an electron microscope (model EM 15007, JEOL).

Data Presentation and Statistical Analysis

Values are means ± SE. Results are reported relative to expression in the presence of 3 mM glucose or in noninfected islets, which are taken as 100%. Data were assessed by use of the nonparametric Wilcoxon’s test or the one-sample Student’s t-test. Differences were considered signiﬁcant when P < 0.05.

RESULTS

Expression of IDS

We examined the gene expression of Ids in islets and exocrine mouse tissue by RT-PCR analysis. Ids, insulin, and Iapp mRNA were speciﬁcally expressed in mouse pancreatic islets (Fig. 1A), but no signal was identiﬁed in exocrine tissue, which was positive for amylase. To conﬁrm the purity and speciﬁcity of the tissue samples, insulin and Iapp genes were used as positive controls for pancreatic islets and amylase gene for exocrine tissue. The housekeeping gene ribosomal protein S9 (Rps9) was used as internal control. We also detected speciﬁc expression of Ids in the mouse pancreatic islet cell lines MIN6 and α-T1 (Fig. 1B).

Intracellular Localization of IDS

The subcellular localization of the IDS protein COOH-terminally tagged with the enhanced green ﬂuorescence protein (IDS-EGFP-N1) was studied in transiently transfected pancreatic β-cells (MIN6). The fusion protein was detected mainly in the lysosomes of all transfected cells (Fig. 2A), as shown by colocalization with the tetramethylrhodamine isothiocyanate-
Fig. 2. Localization of IDS-EGFP to the lysosomal compartment in transfected MIN6 β-cells. A: enhanced green fluorescence protein (EGFP) localization of IDS in cytoplasmatic compartment. B: localization of lysosomal-associated membrane protein (LAMP)-2, a lysosomal marker protein visualized with tetramethylrhodamine isothiocyanate (TRITC)-coupled antibodies (red). C: colocalization of LAMP-2 and IDS-EGFP (orange) by superimposition of A and B.

Fig. 3. Effect of glucose, various glucose analogs, and inhibitors on Ids (A), Iapp (B), Ins (C), and heparan sulfate proteoglycan 2 (Hspg2, D) mRNA expression in mouse islets. Islets were incubated for 24 h in the presence of 3 mM (G3), 5.5 mM (G5), 11.1 mM (G11), 16.7 mM (G16), and 24.4 mM (G24) glucose, G3 + 1 mM gyceraldehyde (GL), G24 + 21.4 mM mannoheptulose (MH), and G3 + 21.4 mM 6-deoxyglucose (6DG). Glucose (G16 and G24), but not other agents, significantly (P < 0.05) increased expression of Ids, Iapp, and Hspg2; effects of glucose were blocked by mannoheptulose. Hspg2 expression (D) was unaffected by glucose. mRNA levels are normalized to Rps18 mRNA expression. Values are means ± SE of 6 islet preparations examined in duplicate. *P < 0.05; **P < 0.01 vs. G3.
labeled lysosomal protein LAMP-2 (Fig. 2, B and C). MIN6 cells expressing the green fluorescent protein (GFP) alone showed uniform labeling of the nucleus (data not shown).

Effect of Glucose Metabolism on IDS and Perlecan mRNA Levels

To determine whether *Ids* and perlecan gene expressions are regulated by metabolic signals, we investigated the effect of glucose on *Ids* and perlecan mRNA levels by using the quantitative real-time RT-PCR technique in mouse pancreatic islets cultured in different conditions for 24 h.

When islets were cultured in different concentrations of glucose, the levels of *Ids* mRNA increased in a dose-dependent manner (Fig. 3A): 11.5, 40.2, 88, and 179% at 5.5, 11.1, 16.7, and 24.4 mM glucose, respectively. The increases at 16.7 and 24.4 mM glucose were statistically significant compared with islets cultured at 3 mM glucose (*P* < 0.01).

To evaluate the effects of glucose metabolism on the expression of the *Ids* gene, we tested the effects of different glucose analogs and blocking agents (Fig. 3A). Neither glycerol (1 mM), which is incorporated into the glycolytic pathway, nor 6-deoxyglucose (21.4 mM), a glucose analog that is not phosphorylated by glucokinase, was able to produce an increase of the *Ids* mRNA levels. Mannose (21.4 mM), a glucokinase inhibitor, completely blocked the effect of glucose (24 mM) on *Ids* mRNA levels.

Table 2. Insulin content, insulin secretion, and cell viability of mouse islets infected with EGFP, *IDS* sense, and *IDS* antisense lentivirus

<table>
<thead>
<tr>
<th>Glucose in Culture</th>
<th>5.5 mM</th>
<th>16.7 mM</th>
<th>Cell Viability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content, ng/islet</td>
<td>Secretion, ng/islet⁻¹×90 min⁻¹</td>
<td>Content, ng/islet</td>
</tr>
<tr>
<td>Control</td>
<td>79.10±4.6</td>
<td>0.73±0.13</td>
<td>82.68±4.8</td>
</tr>
<tr>
<td>Lenti-EGFP</td>
<td>72.55±4.11</td>
<td>0.75±0.15</td>
<td>72.44±5.1</td>
</tr>
<tr>
<td>Lenti-IDS sense</td>
<td>72.57±5.5</td>
<td>0.72±0.15</td>
<td>74.67±4.9</td>
</tr>
<tr>
<td>Lenti-IDS antisense</td>
<td>51.18±3.1</td>
<td>0.77±0.20</td>
<td>44.14±3.1‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Uninfected islets were used as controls. EGFP, enhanced green fluorescent protein. *P* < 0.05 vs. 5.5 mM glucose. †P < 0.001 vs. 5.5 mM glucose; ‡P < 0.001 vs. 5.5 or 16.7 mM glucose.
Iapp, insulin, and perlecan gene expressions were analyzed in the same samples. Whereas Iapp and insulin gene expressions increased with glucose (Fig. 3, B and C), there was no change of perlecan expression under any of the conditions (Fig. 3D).

**Insulin Content and Secretion of Transfected Islets**

To explore the role of IDS in mouse islets, islets were infected with lentivirus containing IDS constructs in sense and antisense orientation. Figure 4 shows the IDS mRNA levels in infected islets. The islet infection with lentivirus-EGFP did not affect IDS expression. However, when we infected the islets with the IDS sense construct, the gene expression increased 70% compared with noninfected islets (P < 0.05), and the islets infected with the IDS antisense construct showed a 43% reduction in the IDS gene expression in relation to noninfected islets (P < 0.05).

To determine the effects of IDS overexpression on islet function, insulin content and secretion of mouse islets infected with the lentiviruses at 20 plaque-forming units/ml (Table 2) were determined. The insulin content and secretory response were unaffected by infection with lentivirus-EGFP. The insulin content of islets infected with the antisense IDS construct was reduced by 35% (P < 0.001) and 46% (P < 0.001) compared with noninfected islets cultured at 5.5 and 16.7 mM glucose, respectively. The increased insulin-secretory response of islets to high glucose (16.7 mM) was not affected by the presence of IDS in antisense or sense conformations (Table 2). Any of the infected lentiviral constructions creates a cytotoxic effect, namely, apoptosis or necrosis, compared with the uninfected islet cells (Table 2).

**Phenotypic Analysis of Transfected Islets**

The phenotype of infected pancreatic islets was studied by electron-microscopic analysis where immunogold labeling of IAPP was used to localize lysosomes and secretory granules. Electron-microscopic images reveal reactivity against IAPP in the lysosomes and secretory granules of all infected islets and controls (Fig. 5). No morphological cell changes were observed in noninfected islets (Fig. 5A) or islets infected with IDS sense (Fig. 5B). However, islets infected with IDS antisense presented modified cellular phenotype (Fig. 5C), characterized by an increase in lysosomal rate and size (Fig. 6). Interestingly,
a higher rate of insulin granule degradation through crinophagic lysosomes could also be observed (Fig. 7).

DISCUSSION

In this study, we demonstrate the presence of Iids in α- and β-pancreatic cells. Our findings confirm the results of previous preliminary reports that detected the presence of IDS in several tissues, including pancreatic cells, by differential display and Northern blot analysis (12, 24). IDS is a component of lysosomes in fibroblasts (22). To confirm the specific localization in islet cells and avoid contamination of islet extracts with fibroblasts, expression of Ids was identified by PCR analysis in pure clonal cells such as MIN6 (a cell line derived from an insulinoma of transgenic mice) and α-TC1 cells (from a pancreatic adenoma created in transgenic mice). IDS was identified at intracellular sites in lysosomes by transfection of MIN6 cells with the GFP construct (IDS-EGFP), which is in agreement with previous studies on other cell types, such as fibroblast and neuronal cells (8, 22, 38).

The role of IDS activity in the pancreatic β-cell is not known, but it could be involved in the degradation of HSPG2 (perlecan), which has been identified in pancreatic islet cells (41). Recent studies have demonstrated that pancreatic islet β-cells synthesize and secrete predominantly HSPGs, and these proteoglycans bind to human amylin (32). Perlecan is a member of the HSPG family that is present in the basement membrane of several vascularized organs, and it has been implicated in complications such as diabetic nephropathy (17). Perlecan is also a component of amyloid deposits described in type 2 diabetes (13), together with other proteins such as apolipoprotein E (37) and the main component, the IAPP amylin. The accumulation of islet amyloid in the pancreas is pathogenic in diabetes, because it leads to progressive deterioration and death by apoptosis of β-cells (15). Perlecan binds to the NH2-terminal site of the human pro-IAPP, which enhances IAPP fibril formation in vitro (20). Furthermore, several studies have identified the NH2-terminal region of pro-IAPP in amyloid deposits of diabetic pancreas (5, 39). The role of IDS in islet amyloid formation is unclear, however, partly because of the difficulty of measurement of IDS enzymatic activity in β-cells.

IDS expression is regulated by signals from glucose metabolism, as expression is dependent on glucose concentrations and phosphorylation and glycosylation. However, glucose did not affect the expression of perlecan. A decrease of perlecan mRNA was observed in adipocytes and in glomerular epithelial cells from rat and diabetic mice cultured in high-glucose conditions (21, 31, 33). Such differences in regulation of enzyme and substrate could be attributed to the differences in cell type or the fact that the transcriptional and/or posttranscriptional mechanisms of IDS and perlecan are regulated on different time scales. The same pattern of regulation of IAPP has been previously described by our group (14, 29).

Overexpression of IDS had no effect on glucose-stimulated insulin secretion. However, inhibition of IDS expression resulted in decreased cellular insulin content. Lysosomal activity in β-cells includes degradation of insulin granules that are not directed to the secretory pool. This process is known as crinophagy (9). Proteolytic enzymes, including cathepsin, degrade insulin, IAPP, and other granule peptides (1). Inhibition of lysosomal enzymes results in increased lysosomal size and population (2). The inhibition of the lysosomal enzyme IDS by antisense transfection results in increased lysosomal size and increased degradation of insulin granules via the crinophagic pathway, as has been also proposed (34). However, if IDS is impaired, it does not necessarily mean that other lysosomal activities, such as those for protein degradation, are inhibited. Direct studies of intracellular insulin degradation are needed to clarify this question. With this restriction in mind, we suggest that the decreased insulin content in antisense transfected islets is due to the effect of IDS decrease on the lysosomal activity. The insulin-secretory response, however, was not affected by enzyme inhibition, because the release of insulin would be expected to be maintained in this short period of time.

In conclusion, we have demonstrated that IDS is localized to lysosomes and normally expressed in mouse pancreatic islets. Expression is regulated by signals from glucose metabolism. Lentivirus-mediated inhibition of IDS expression in pancreatic islets decreased the insulin content via the crinophagic pathway.

REFERENCES


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