IDENTIFICATION OF IDURONATE-2-SULFATASE (IDS) IN MOUSE PANCREATIC ISLETS

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ABSTRACT

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, the 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, beta and alpha clonal cells, being localized intracellularly in lysosomes. The transcriptional expression of *Ids* in mouse islets increased with glucose in a dose-dependent manner (11.5 % at 5.5 mM; 40.2 % at 11.1 mM; 88 % at 16.7 mM* and 179 % at 24.4 mM*; * p<0.01 compared to secretion at 3mM glucose). This increase was not produced by glyceraldehyde (1 mM) or 6-deoxyglucose (21.4 mM) and 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 islet cell viability decrease was observed in mouse islets carrying lentiviral contracts compared to the controls. However, insulin content was reduced (35 % versus controls, p<0.001) in islets infected with *IDS* antisense construct, whilst the secretory response to glucose of those islets was maintained. Inhibition of IDS by antisense infection lead to an increase lysosomal size and a high rate of insulin granules degradation via the crinophagic route in pancreatic beta 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 normal pathway of lysosomal degradation of secretory peptides and is likely to be essential to maintain pancreatic beta-cell function.

**Keywords:** perlecan; pancreatic islets; islet amyloid polypeptide; insulin content and secretion; lysosomes; apoptosis
INTRODUCTION

The human iduronate-2-sulfatase (IDS) gene contains nine exons spread over approximately 25 Kb and 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 has been studied in transfected fibroblasts. The enzyme (E.C. 3.1.6.13) is synthesized as two precursor forms of M\(_r\) 76000 and 90000; 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 polypeptides of M\(_r\) 55000 and 45000 (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 IDS gene has been described (19), which provoke a defect in the enzymatic activity leading to the accumulation of partially degraded glycosaminoglycans in lysosomes. This results in the development of the Hunter syndrome or mucopolysaccharidosis type II in man, which is a rare X-linked recessive lysosomal storage disorder (18,28).

IDS is a lysosomal enzyme involved in the degradation of glycosaminoglycans. Its activity is based on the removal the 2-sulfate group of the L-iduronate 2-sulfate units of dermatan sulfate proteoglycan 3 (DSPG3) and heparan sulfate proteoglycan 2 (HSPG2) (17). The HSPG2, also known as perlecan, has been identified in islet cells (20) and is thought to be involved in formation of pancreatic amyloid deposits described in type 2 diabetes (43), however the overproduction of 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) (7,39). In addition, perlecan, localized in the basement membrane of capillaries, may contribute to
amyloid deposition in Alzheimer’s disease, in familial amyloidoses, in prion diseases (22,35,38), and in the pathogenesis of diabetes complications (6). Furthermore, recent studies have demonstrated that heparin and perlecan can bind the human N-terminal pro-IAPP molecule acting as a potent enhancer of IAPP fibril formation in vitro (3,30).

Preliminary findings using differential display procedures have detected that the IDS gene is preferentially expressed in pancreatic islets relative to exocrine tissue (11). However now pieces of information are published related to the identification of IDS in islets cells. In this sense, the present work is focused to examine the expression and the main determination of metabolic regulation of the Ids gene in mouse pancreatic islets.
RESEARCH DESIGN AND METHODS

Mouse pancreatic islet isolation and culture

Mouse pancreatic islets were isolated from CD-1 male mice aged 2 months using a modification of the procedure originally described by Lacy and Kostianovsky (23). Briefly, the pancreas was cannulated and digested with collagenase (Roche Diagnostics GmbH, Mannheim, Germany) and islets were purified from the exocrine tissue through discontinuous Histopaque density gradients.

Groups of 100 islets were handpicked under a stereomicroscope, transferred to Petri dishes and precultured for 18 h in RPMI 1640 medium (Gibco-BRL, Life Technologies Inc., Paisley, UK) supplemented as described previously (14). Afterwards, the islets were transferred to 10 ml of new RPMI medium supplemented with a range of test agents, as described in the figure legends, for a further 24 h in the same conditions.

Gene expression analyses

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

After digestion with DNase (Gibco-BRL, Paisley, UK) for 15 min at room temperature, cDNAs were synthesized by using MMLV-reverse transcriptase (Promega, Madison, USA) 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, Madison, WI, USA). Twenty ml of the PCR products was examined by electrophoresis on 2 % agarose gel after electrophoresis.
Quantitative real-time RT-PCR reactions of IDS, Ids, insulin, Iapp, perlecan and ribosomal protein S18 (Rps18) were performed. The primers and probes used (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) to generate multiplex PCR. Serial dilutions of partial cDNA sequence of gene targets were used to acquire the standard curve in each experiment. In every assay, runs were independently repeated two times and a negative control was included. Data analysis was performed using the SDS Software Version 2.1 (Applied Biosystems). Afterwards, the Rps18 was used as a reference against which the expression level of the transcript genes of interest was normalized.

**Subcellular location of iduronate-2-sulfatase**

*Plasmid construction.* Human IDS cDNA (GenBank No. NM 000202) was generated by PCR using the PfuI enzyme (Stratagene, Amsterdam), and the primers IDS/NheI: 5'-ATCTAGCTAGCGAAATGCCGCCACC-3' and IDS/XhoI: 5'-CCGCTCGAGACAACTGGAAAAGATCTCCAC-3', were designed to create NheI and XhoI sites at 5' and 3'-tails, respectively. The NheI-XhoI fragment of the resulting PCR product was inserted into the NheI-XhoI sites of pEGFP-N1 vector (Clontech, Palo Alto, California, USA).

*Cell culture and transfection.* MIN6 (cell line derived from an insulinoma of transgenic mice) and alpha-TC1 cells (cell line derived from a pancreatic adenoma created in transgenic mice) were grown on tissue culture testplate in Dulbecco’s Modified Eagle’s Medium (Bio Whittaker Europe, Verviers Belgium) supplemented with 15 % fetal calf serum (FCS) (Gibco-BRL), penicillin (100 U/ml), streptomycin
(100 ug/ml) and 2 ul beta-mercaptoethanol (5 uM) (Gibco-BRL). Cells were transfected with the *IDS*-pEGFP-N1 and pEGFP vector by Lipofectamineplus kit (Gibco-BRL) according to the manufacturer’s protocol.

**Immunofluorescence.** Co-localization of IDS with lysosomal enzymes was performed using the lysosome-associated membrane proteins (LAMP-2) polyclonal antibody (Santa Cruz Biotechnology, Inc.) and an anti-goat IgG TRITC Conjugate (Sigma, Saint Louis, MO, USA) as a second antibody. Fluorescence images were analyzed with a Leica TCS NT confocal scanning laser microscope.

**Lentiviral vector constructions and infection**

To determine the effects of over expression of the *IDS* gene, cells were infected with the gene in lentiviral vectors. This transduction system permits the efficient integration of genes into the genome of non-dividing cells (10). The strategy of plasmid constructs described above was followed to clone sense and antisense *IDS* cDNA into the *MluI*-XhoI sites of pHR-CMV vector (kindly provided by B. Thorens, University of Lausanne). The oligonucleotides used were *MluI/IDS* sense 5’-AATCGACGCCTGAATGCGCCACC-3’; *XhoI/IDS* sense 5’-ACCGCTCGAGTCAACAACTGG-3’, *MluI/IDS* antisense 5’-TCGACGCTGATCAACAACTGG-3’ and *XhoI/IDS* antisense 5’-ACCGCTCGAGAAATGCACGCCACC-3’. The envelope plasmid pMDG and the packaging plasmid pCMVDR8.7 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 assay.
(Innogenetics, Belgium). The islets were infected with lentiviral particles (20 iu/beta 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 islets expression of *IDS* was determined by quantitative real-time RT-PCR.

**Insulin secretion and content**

Following lentivirus infections, groups of 8 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 minutes at 37°C in a shaking water bath. The supernatants were then stored at −20°C until assayed for insulin radioimmunoassay (CIS Biointernational, Gif-Sur-Yvette, France) (detection limit, 30 pM; intra- and inter-assay CV, 6 and 8 % respectively). For the determination of insulin content, the same islets were disintegrated by sonication at 4°C in 0.5 ml acid-ethanol solution (75 % ethanol, 1.5 % 10 mM HCl).

**Analysis of islet cell viability**

*Isolation of single mouse islet cells.* After lentiviral vector infection, single mouse islet cells were obtained by digesting fifty mouse islets in 2 ml of PBS containing 0.125 mg/ml of 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 additional 5 min on ice to allow islets to sediment. Then, supernatant containing the single cells were removed and placed in 1 ml of FCS (Gibco-BRL). In order 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 ul of binding buffer (100 mM Hepes (pH 7.4), 1.5 mM NaCl, 50
mmM KCl, 10 mM MgCl₂, 18 mM CaCl₂). For each experimental condition, 200 ul of cells were unlabelled and used to define the threshold of detection in fluorescence-activated cell sorting (FACS) analysis. The other 200 ul of cell solution were double stained with annexin V-phycoerythrin (PE) and 7-amino-actinomycin-D (7-AAD) by using Annexin V-PE Apoptosis Detection Kit I (Becton Dickinson, San Jose, CA, USA) following manufacturer’s instructions. Thereafter, cells were analyzed on a fluorescence-activated cell sorter (FACS Calibur, Becton Dickinson) through the Cell Quest Software (Becton Dickinson). FACS gating based on forward and side scatter of out of 10⁵ cells were included for analysis. Every run included positive and negative control samples for cell cytotoxicity. Negative cells for both annexin V and 7-AAD were considered as viable cells, early apoptotic cells were annexin V positive, whereas apoptotic or necrotic cells were both negative for annexin V and 7-AAD.

**Electron microscopy**

Lentiviral infected islets were briefly rinsed with phosphate-buffered (PB) and fixed with fresh 4 % paraformaldehyde/1 % gluteraldehyde in 0.1 M PB pH 7.4, for 1 h at room temperature. Islets were postfixed with OsO₄ in 0.1 M PB 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 Company Ltd, Berkshire, England), followed by two changes of undiluted LR White at room temperature. The infiltration was continued with fresh LR White overnight at 4°C, and after a final change to fresh resin, polymerization under vacuum at 55°C for 48 h completed the embedment. After separation of the Thermonox coverslip from the embedded cells, 80-100 nm cross-sections were cut and mounted on 150-mesh nickel girde supported with carbon-coated parlodion. Immunolocalization of IAPP in the islets
were performed by treating the sections with a polyclonal antibody against IAPP (kindly provided by A. Clark, University of Oxford). Antisera 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 a JEOL (EM 15007) electron microscope.

**Data presentation and statistical analysis**

Results are expressed as the mean ± SEM for the number of experiments stated. Results are reported relative to the expression in the presence of 3 mM glucose or in non-infected islets, which are taken as 100 %. Data were assessed by use of nonparametric Wilcoxon Test or the one-sample Student’s test. Differences were considered significant when p<0.05.
RESULTS

Expression of iduronate-2-sulfatase

We examined the gene expression of \textit{Ids} in islets and exocrine mouse tissue by RT-PCR analysis. \textit{Ids}, insulin and \textit{lapp} 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 \textit{lapp} genes were used as positive controls for pancreatic islets and amylase gene for exocrine tissue. The housekeeping gene ribosomal protein S9 (\textit{Rps9}) was used as internal control. We also detected specific expression of \textit{Ids} in mouse pancreatic islet cell lines MIN6 and alpha-TC1 (Fig. 1B).

Intracellular localization of iduronate-2-sulfatase

The subcellular localization of the IDS protein C-terminally tagged with the enhanced green fluorescence protein (IDS-EGFP-N1) was studied in transiently transfected pancreatic beta cells (MIN6). The fusion protein was detected mainly in the lysosomes of all transfected cells (Fig. 2A) as shown by co-localization with the TRITC labeled lysosomal proteins, LAMP-2 (Fig. 2B, C). MIN6 cells expressing the green fluorescent protein alone (GFP) showed uniform labeling of the nucleus (data not shown).

Effect of glucose metabolism on iduronate-2-sulfatase and perlecan mRNA levels

To determine whether \textit{Ids} and perlecan gene expression are regulated by metabolic signals we investigated the effect of glucose on \textit{Ids} and perlecan mRNA levels using the quantitative real-time RT-PCR technique in mouse pancreatic islets cultured in different conditions for 24 hours.
When islets were cultured in different concentrations of glucose, the levels of *Ids* mRNA increased, in a dose-dependent manner (Fig. 3A): 11.5 % at 5.5 mM; 40.2 % at 11.1 mM; 88 % at 16.7 mM and 179 % at 24.4 mM glucose. The increases at 16.7 mM and 24.4 mM glucose were statistically significant when compared to islets cultured at 3 mM glucose (p<0.01).

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

*Iapp*, insulin and perlecan gene expressions were analyzed in the same samples. Whereas *Iapp* and insulin gene expressions increased with glucose (Fig. 3B, 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 4A shows the *IDS* mRNA levels in infected islets. The islet infection with lentivirus-EGFP did not affect *IDS* expression. However when we infected with the *IDS* sense construct the gene expression increased 70 % in comparison with non-infected islets (p<0.05) and the islets infected with the *IDS* anti-sense construct showed a 43 % reduction in the *IDS* gene expression in relation to non-infected 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 pfu/ml (Table 2) was
determined. The insulin content and secretory response were unaffected by the 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) in comparison to non-
infected 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 conformation (see Table 2). Any of the infected lentiviral
constructions create a cytotoxic effect, namely apoptosis or necrosis, compared to the
uninfected islet cells (Table 2).

Phenotypic analysis of transfected islets

The phenotype of infected pancreatic islets was studied by electron microscope
analysis where immunogold labeling of IAPP were used to localize lysosomes and
secretory granules. Electron microscope images reveals reactivity against IAPP in the
lysosomes and secretory granules of all infected islets and controls (Fig. 5). No
morphological cell changes were observed in either non-infected 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, it could be also observed a higher rate of
insulin granules degradation through crinophagic lysosomes (Fig. 7).
DISCUSSION

In this study we demonstrate the presence of \textit{Ids} in alpha and beta pancreatic cells. Our findings confirm the results of previous preliminary reports that detected the presence of \textit{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). In order to confirm the specific localization in islet cells and avoid contamination of islet extracts with fibroblasts, expression of \textit{Ids} was identified by PCR analysis in pure clonal cells such as MIN6 (cell line derived from an insulinoma of transgenic mice) and alpha-TC1 cells (from a pancreatic adenoma created in transgenic mice). \textit{IDS} was identified at intracellular sites in lysosomes by transfecting MIN6 cells with the green fluorescent protein construct (IDS-EGPF), 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 beta cell is not known, but it could be involved in the degradation of HSPG2 (perlecan), which has been identified in pancreatic islet cells (42). Recent studies have demonstrated that pancreatic islet beta-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 vascularised 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, since it leads to progressive deterioration and death by apoptosis of beta cells (15). Perlecan binds to the N-terminal site of the human pro-IAPP, which enhances IAPP fibril formation in vitro (20). Furthermore, several studies have identified the N-terminal region of pro-IAPP in
amyloid deposits of diabetic pancreas (5,40). The role of IDS in islet amyloid formation is unclear, however, partly due to the difficulty of measurement of IDS enzymatic activity in beta cells.

IDS expression is regulated by signals from glucose metabolism, since expression is dependent on glucose concentrations and phosphorylation and glycolysis. However, glucose did not affect the expression of perlecan. A decrease of perlecan mRNA was observed in adipocytes, and glomerular epithelial cells from rat and diabetic mice cultured at high glucose conditions (21,31,33). Such differences in regulation of enzyme and substrate could be attributed to the differences in cell type, or that the transcriptional and or post-transcriptional mechanism 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 glucose-stimulated insulin secretion. However, inhibition of IDS expression resulted in decreased cellular insulin content. Lysosomal activity in beta-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 result in increased lysosomal size and increased degradation of insulin granules via the crinophagic pathway, as it has been also proposed (34). However, if IDS is impaired it does not necessarily mean that other lysosomal activities, 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, since 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.
ACKNOWLEDGEMENTS

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cytokine-induced apoptosis while preserving in vitro and in vivo control of insulin secretion.


FIGURE LEGENDS

FIG. 1. RT-PCR analysis demonstrated that the *Ids* gene expression was specifically located in islets cells. mRNA from mouse islets and exocrine tissue extracts (A) and from MIN6 and alpha-TC1 clonal cells (B). RT-PCR product of *Ids, Iapp, Ins, Amy, Gcg* and *Rps9* are shown. Each PCR amplification was made without cDNA (CN), with reverse transcription sample (+) and, as a control, the sample without transcriptase enzyme (-). The sizes of specific bands are expressed in Table 1. *Ids, Iapp* and *Ins* mRNA are expressed in islet tissue but not in exocrine cells where *Amy* is specifically expressed. *Ids* is expressed in the clonal islet cells, beta cells (MIN6) and alpha cells (alpha-TC1).

FIG. 2. Localization of IDS-EGFP to the lysosomal compartment in transfected MIN6 beta cells. (A) EGFP localization of IDS in the cytoplasmatic compartment; (B) The localization of LAMP-2, a lysosomal marker protein visualized with TRITC coupled antibodies (red); (C) Co-localization of LAMP-2 and IDS-EGFP (orange) by superimposition of both images.

FIG. 3. Effect of glucose, various glucose analogues and inhibitors on (A) *Ids* mRNA (B) *Iapp* mRNA, (C) *Ins* mRNA and (D) *Hspg2* 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), 24.4 mM (G24) glucose, G3 + 1 mM glyceraldehyde (GL), G24 + 21.4 mM mannoheptulose (MH) and G3 + 21.4 mM 6-deoxyglucose (6DG). Glucose (G16, G24) but not other agents significantly (p<0.05) increased expression of *Ids, Ins* and *Iapp*, the effects of glucose were blocked by mannoheptulose. *Hspg2* expression
(D) was unaffected by glucose. The mRNA levels are normalized to *Rps18* mRNA expression and relative expressed. The results are expressed as the mean ± SEM of six islet preparations examined in duplicate. *, p<0.05; **, p<0.01 vs G3.

**FIG. 4.** Analysis of *IDS* mRNA expression by using quantitative real-time RT-PCR in non-infected mouse pancreatic islets and islets infected with *EGFP, IDS* sense and *IDS* antisense lentivirus constructions. The mRNA levels are normalized to *Rps18* mRNA expression and relative expressed. *IDS* expression was significantly increased with *IDS* sense transfection and decreased in *IDS* antisense infected islets (*, p<0.05). The results are expressed as the mean ± SEM, n=3.

**FIG. 5.** Electron micrograph of beta cell of a pancreatic islet. Immunogold labeling for IAPP in lysosomes and secretory granules. (A) non infected islets; (B) islets infected with *IDS* sense; (C) islets infected with *IDS* antisense.

**FIG. 6.** Electron micrograph of beta cell of a pancreatic islet infected with *IDS* antisense. Note a large lysosomes containing several vacuoles.

**FIG. 7.** Electron micrograph of beta cell of a pancreatic islet infected with *IDS* antisense. Crinophagic bodies.
- IDS in pancreatic islets -

**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>
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<th>PCR product (bp)</th>
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<tr>
<td>IDS</td>
<td>5'-CCCACAGCTCCTCCTCCA-3'</td>
<td>5'-TGFCAGGTCCTCCTGCCAGTG3'</td>
<td>5'-ATGCGTTTGCGCAGCAAGCAGTG3'</td>
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<td>Hspg2</td>
<td>5'-GGCCGGGCTCAGTGT-3'</td>
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*Ids*, iduronidate 2-sulfatase; *Iapp*, islet amyloid polypeptide; *Ins*, insulin; *Amy*, amylase; *Gcg*, glucagon; *Rps9*, ribosomal protein S9
- IDS in pancreatic islets -

**TABLE 2** Insulin content, insulin secretion (ng/islet-90 min$^{-1}$) 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>
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<td>Secretion</td>
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<tr>
<td>Control$^*$</td>
<td>79.10±4.6</td>
<td>0.73±0.13</td>
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<tr>
<td>Lenti-EGFP</td>
<td>72.55±4.11</td>
<td>0.75±0.15</td>
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<tr>
<td>Lenti-IDS sense</td>
<td>72.57±5.5</td>
<td>0.72±0.15</td>
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<tr>
<td>Lenti-IDS antisense</td>
<td>51.18±3.1$^\dagger$</td>
<td>0.77±0.20</td>
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</tbody>
</table>

$^*$, Uninfected islets are used as controls; $^+$, $p < 0.05$ relative to islets cultured in 5.5 mM glucose; $\dagger$, $p < 0.001$ relative to islets cultured in 5.5 mM glucose; $^\ddagger$, $p < 0.01$ relative to islets cultured in 5.5 or 16.7 mM glucose.
A

<table>
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<tr>
<th>Protein</th>
<th>CN</th>
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<th>Exocrine</th>
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<tbody>
<tr>
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<td>Iapp</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ins</td>
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B

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<tr>
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</tbody>
</table>
- IDS in pancreatic islets -
- IDS in pancreatic islets -

A. $Ids$ mRNA levels

B. $lapp$ mRNA levels

C. $Ins$ mRNA levels

D. $Hspg2$ mRNA levels
IDS mRNA levels

% Relative control

Non-infected  EGFP  IDS sense  IDS antisense

IDS mRNA levels

*
- IDS in pancreatic islets -
- IDS in pancreatic islets -