Heparan Sulfate Proteoglycans Are Important for Islet Amyloid Formation and Islet Amyloid Polypeptide-induced Apoptosis*

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Background: Islet amyloid causes β cell death in T2D.

Results: Overexpression of heparanase reduced islet amyloid formation in cultured islets, and cells lacking surface associated HS were protected against IAPP-mediated toxicity.

Conclusion: IAPP fibrillation requires HSPG interaction for induction of apoptosis.

Significance: Inhibition of the HS and hIAPP interaction poses a potential intervention target to prevent β cell death in diabetes.

Deposition of β cell toxic islet amyloid is a cardinal finding in type 2 diabetes. In addition to the main amyloid component islet amyloid polypeptide (IAPP), heparan sulfate proteoglycan is constantly present in the amyloid deposit. Heparan sulfate (HS) side chains bind to IAPP, inducing conformational changes of the IAPP structure and an acceleration of fibril formation. We generated a double-transgenic mouse strain (hpa-hIAPP) that overexpresses human heparanase and human IAPP but is deficient of endogenous mouse IAPP. Culture of hpa-hIAPP islets in 20 mM glucose resulted in less amyloid formation compared to the IAPP structure and an acceleration of fibril formation. We generated a double-transgenic mouse strain (hpa-hIAPP) that overexpresses human heparanase and human IAPP but is deficient of endogenous mouse IAPP. Culture of hpa-hIAPP islets in 20 mM glucose resulted in less amyloid formation compared to the amyloid load developed in cultured islets isolated from littersmates expressing human IAPP only. A similar reduction of amyloid was achieved when human islets were cultured in the presence of heparin fragments. Furthermore, we used CHO cells and the mutant CHO pgsD-677 cell line (deficient in HS synthesis) to explore the effect of cellular HS on IAPP-induced cytotoxicity. Seeding of IAPP aggregation on CHO cells resulted in caspase-3 activation and apoptosis that could be prevented by inhibition of caspase-8. No IAPP-induced apoptosis was seen in HS-deficient CHO pgsD-677 cells. These results suggest that β cell death caused by extracellular IAPP requires membrane-bound HS. The interaction between HS and IAPP or the subsequent effects represent a possible therapeutic target whose blockage can lead to a prolonged survival of β cells.

Islet amyloid is present in almost all individuals with type 2 diabetes and in transplanted human islets. Islet amyloid poly-peptide (IAPP), the main amyloid component, forms intra- and extracellular aggregates, with an implication for the progressive β cell loss seen in these conditions, as reviewed in Ref. 1. The precise mechanism for amyloid deposition still needs to be clarified, but accumulating data suggest a common mechanism for amyloid toxicity (2) and that smaller aggregates (oligomers) formed during the earlier stage of amyloidogenesis constitute the principal toxic entity (2, 3). Mirzabekov et al. (4) have shown early that human IAPP oligomers can form ion-leaking pores, and fibril formation is accelerated dramatically along anionic lipid membranes (5). A cholesterol-dependent internalization of IAPP oligomers into β cells results in neutralization of cytotoxicity (6). Also, monomeric human IAPP can lead to increased fluidity and destabilization of the plasma membrane (7). Although five different hormone-producing cell types can be identified in the islets of Langerhans, extracellular IAPP deposits only affect β cells without damaging other cell types (8). This suggests a cellular property unique to β cells.

Several amyloid diseases exist, and they are classified on the basis of the specific protein that makes up the amyloid fibril. So far, more than 28 proteins have been identified to be able to form local or systemic amyloidosis in human (9). Beside the amyloid-specific protein, other components, such as serum amyloid P and proteoglycans, are always present in amyloid deposits, where both glycosaminoglycans (GAGs) (10, 11) and core proteins (12) have been identified. Heparan sulfate (HS) is found on cell membrane-associated syndecan and glypican and on perlecan and agrin present in the extracellular matrix (13), and HS dominates as the most frequently encountered GAG in

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¹ The abbreviations used are: IAPP, islet amyloid polypeptide; GAG, glycosaminoglycan; HS, heparan sulfate; hIAPP, human islet amyloid polypeptide; HG, high glucose; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DMso, dimethyl sulfoxide; ThT, thioflavin T; Z-LEHD-fmk, benzoylcarbonyl LEHD-fluoromethyl ketone; Z-LEHD-fmk, benzoylcarbonyl LEHD-fluoromethyl ketone; ANOVA, analysis of variance; CGRP, calcitonin gene related peptide; EYF, enhanced yellow fluorescent protein; ECFP, enhanced cyan fluorescent protein.

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amyloid deposits. The function of HS in amyloidogenesis is not clear, but accumulated information points to an important role during initiation of amyloid formation. Human IAPP, but not the non-amyloid forming rat IAPP (rIAPP) binds to perlecan isolated from Engelbreth-Holm-Swarm tumors (14). Also, iso-
lated β cell-associated heparan sulfate proteoglycan binds human IAPP, and no interaction occurs with rIAPP (15). A specific binding site for HS has been identified within the N-terminal processing site of human proIAPP (16), and binding of HS to monomeric proIAPP1–30 stimulates amyloid formation from this otherwise non-amyloid-forming peptide (17). Although binding of heparan sulfate proteoglycan to IAPP is mainly found with the monomeric form of IAPP, Watson et al. (18) showed that binding of heparin to IAPP or amyloid β depends on aggregation status and that binding requires mature fibrils. Also, chondroitin sulfate and keratan sulfate enhanced IAPP fibrillation (14), but with a significantly lower efficiency in comparison with HS.

Heparanase is a mammalian endoglycosidase that specifically cleaves HS chains (19), leading to reduced length of cell surface-bound and extracellular matrix-associated HS. Our earlier study showed that transgenic mice overexpressing human heparanase attenuated inflammatory induced AA amyloidosis (20). In the mouse, an organ-specific difference in human heparanase overexpression coincided with development of amyloid. Livers and kidneys with high levels of hepara-
nase overexpression showed little or no amyloid depositions, whereas spleens without heparanase expression displayed extensive deposits.

In this study, we aimed to investigate the effect of heparanase overexpression on IAPP aggregation and islet amyloid forma-
tion. A double-transgenic mouse overexpressing both human heparanase and human IAPP (hpa-hIAPP) was generated. Expression of heparanase did not alter glucose-stimulated insulin or IAPP release but led to a significant reduction of IAPP amyloid in islets cultured in 20 mM glucose. Culture of human islets in the presence of heparan fragments reduced islet amyloid, and this effect was influenced by fragment size.

**Experimental Procedures**

**Animals**—Double transgenic (tg) mice overexpressing hu-
man heparanase and hpa-hIAPP were generated by crossing human heparanase C57BL (21) with hIAPP FVB/N mice (22). Littermates expressing only hIAPP without concomitant expression of human heparanase were used as controls (hIAPP). Both hpa-hIAPP and hiAPP mice lack the gene for endogenous mouse IAPP shown previously to interfere in IAPP fibril formation (22). Animals were maintained at the animal facility at the Biomedical Centre, Uppsala University, and experiments were approved by the regional Animal Ethics Committee in Uppsala, Sweden.

**Islets**—Mice (9–13 weeks old) were sacrificed by cervical dis-
location. The pancreas was excised, and islets were isolated by collagenase digestion (Clostridium histolyticum, Sigma-Al-
drich, St. Louis, MO) as described previously (23). Mouse islets were cultured free-floating in RPMI 1640 culture medium con-
taining 11 mM glucose (Sigma-Aldrich) supplemented with 10% (v/v) FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 45 μM β-mercaptoethanol at 37 °C in 5% CO₂ and allowed to recover for at least 48 h post-isolation before being used in any experiment. Isolated human islets from brain-dead donors with beating hearts were provided by the Nordic Network for Clinical Islet Transplantation, Uppsala University. Human islets were cultured in CMRL 1066 medium containing 5.5 mM glu-
cose supplemented with 10% (v/v) FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM 1-glutamine.

**Islet Culture**—Islets were cultured for 19 days in culture media supplemented with glucose to yield a final concentration of 20 mM (high-glucose (HG)) to stimulate IAPP secretion and subsequent amyloid deposition. To study the effect of heparin chain length on amyloid load, mouse islets were cultured in HG medium with 4-, 8-, 12-, or 18-mers or full-length heparin cor-
responding to ~50-mers (150 nt if nothing else stated). Iso-
lated mouse islets were cultured in 11 mM glucose, and human islets were cultured in 5.5 mM glucose (normal glucose) for 19 days to determine amyloid development under unstimulated conditions. The medium was changed every second day. To confirm the diffusion of heparin into islets, they were cultured with 1 μM biotinylated full-length heparin for 3 h, followed by fixation in 4% paraformaldehyde and overnight incubation with Alexa Fluor 488-labeled streptavidin (Molecular Probes) diluted 1:250 in TBS at 4 °C.

**Quantification of Islet Amyloid Load and Apoptosis—**Islets were fixed in 4% paraformaldehyde and stained for amyloid with thioflavin S (0.125%, Sigma-Aldrich). Optical sections of islets were acquired with an EZ-C1 digital camera connected to a Nikon eclipse E600 microscope (Nikon, Kawasaki, Japan) with a Nikon C1 confocal unit using an argon 488-nm laser (Nikon). The volume of thioflavin S-stained regions and islet volume were determined with image analysis software (Imaris 7.6, Bit-
plane). Islets were restained with Mayer’s hematoxylin, and the number of apoptotic nuclei per islet area was determined (Imagej software, http://imagej.nih.gov/ij/). All quantifications were made in a blinded manner.

**Islet Hormone Release**—Analysis of hormone release was per-
formed on islets 3 days after isolation. Three samples of 10 islets per mouse were incubated in Krebs–Ringer bicarbonate buffer (114 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 4.3 mM NaOH, 2.5 mM CaCl₂) supplemented with 10 mM HEPES and 2 mg/ml bovine serum albumin (BSA) (KRBH-BSA) containing 1.7 mM glucose for the first hour fol-
ed by an incubation in 16.7 mM glucose for a second hour. Next, the islet triplicates were pooled, homogenized, and mixed with acid ethanol (180 mM HCl in 95% ethanol) to extract total islet insulin and IAPP content. Insulin was determined with rat insulin ELISA (Mercodia, Uppsala, Sweden) and IAPP with human amylin ELISA (Merck Millipore, Darmstadt, Germany). Western Blot Analysis—Isolated islets were rinsed in PBS and lysed by brief sonication and heating at 95 °C in 50 mM Tris-HCl (pH 6.8), 12% glycerol, 4% SDS, 10 mM dithiothreitol and 0.01% Coomassie Blue G-250. Islet extracts were separated by 6–12.5% Tricine SDS-PAGE and transferred onto a nitrocellu-
lose membrane (Hybond-ECL). Excess binding sites on the membrane were blocked in TBS with 0.1% Tween (TBS-T) and 5% (v/v) nonfat dry milk for 1 h and incubated overnight at 4 °C with rabbit antiserum 733 reactive against heparanase (24)
diluted 1:500 in TBS-T. Primary antibodies were detected with HRP-conjugated secondary antibodies (Dako, Glostrup, Denmark), and reactivity was visualized with chemiluminescence (Immobilon). Blots were incubated with guinea pig anti-insulin (Dako) as a loading control.

**Histological and Immunological Examination of Pancreas from hIAPP and hpa-hIAPP Mice**—Formalin-fixed, paraffin-embedded pancreas sections (10 μm) from hpa-hIAPP and hIAPP mice were deparaffinized and rehydrated, and antigens were exposed by heating in 25 mM sodium citrate (pH 7.2), followed by incubation in 0.4% Triton X-100. After overnight incubations with primary antibodies, 733 diluted 1:500, and guinea pig anti-insulin diluted 1:250 at 4 °C, reactivity was visualized with secondary antibodies conjugated to Alexa Fluor 546 (heparanase) and Alexa Fluor 488 (insulin) (Molecular Probes). Nuclei were counterstained with DAPI (Molecular Probes).

For β and α cell quantifications, pancreas sections were immersed in 0.3% H2O2 in TBS to block endogenous peroxidase, followed by incubation with guinea pig anti-insulin diluted 1:250 or mouse anti-glucagon (Abcam) diluted 1:100 overnight. Reactivity was visualized using HRP-conjugated anti-guinea pig (1:400) or Envision anti-mouse (Dako) and developed with 3,3′-diaminobenzidine. Sections were counterstained with Mayer’s hematoxylin, and the fraction of insulin-positive β cells and glucagon-positive α cells per islet were determined (ImageJ software). Formalin-fixed pancreas sections (10 μm) from 21-month-old hIAPP (n = 4) and hpa-hIAPP (n = 4) mice were stained for amyloid with Congo red and examined with a polarization microscope.

**Immunoelectron Microscopy**—Islets were fixed in 2% paraformaldehyde with 0.25% glutaraldehyde and processed for immunoelectron microscopy. Ultrathin sections were immunolabeled as described previously (25), with rabbit antiserum A110 raised against IAPP1–37 and compared with 5% uranyl acetate in water and Reynold’s lead solution. For morphological analysis of aggregates formed in vitro, samples were diluted 1:30 in double-distilled H2O2, adhered on formvar-coated copper grids and negatively contrasted with 2.5% uranyl acetate in 50% ethanol. Samples were studied at 75 kV in a Hitachi H-7100 transmission electron microscope (Hitachi, Tokyo, Japan), and images were obtained with Gatan 832 Orius SC1000 (Gatan).

**Isolation and Characterization of HS from Pancreas**—One heparanase overexpressing mouse and one C57BL mouse were injected intraperitoneally with 100 μCi of Na35SO4 (catalog no. NEX041H, PerkinElmer LifeSciences). After 1 h, the mice were sacrificed, and the whole pancreata were dissected. Following homogenization, the lysates were incubated with 0.5 mg of protease at 55 °C overnight to digest proteins, followed by incubation with benzoxonase (12.5 units) to degrade DNA. After 10-min centrifugation at 8000 × g, the supernatants were collected and applied to a DEAE-Sepharose to purify 35S-labeled glycosaminoglycans (heparan sulfate and chondroitin sulfate). The yielded products were treated with chondroitinase ABC (#100330; Serkagaku, Tokyo, Japan) and re-applied to DEAE-Sepharose column to remove degraded chondroitin sulfate. The purified 35S-labeled heparan sulfate was analyzed by Superose-12 column (GE Healthcare) connected to a HPLC system. The purity of 35S-labeled heparan sulfate was confirmed by complete heparanase/heparitinase digestion.

**Heparin Labeling**—The 3H-labeled heparin was prepared by N-deacetylation followed by re-N-acetylation with N-[14C]acetic anhydride (26). The heparin fragments were generated by partial deaminative cleavage of the 3H-labeled heparin, followed by reduction with NaBH4. The fragmented heparin samples were separated on a Bio-gel P-10 column (Bio-Rad) as described previously (27).

**Binding Studies**—Heparin binding with a panel of peptides was analyzed with a nitrocellulose filter binding assay. A peptide corresponding to human proIAPP6–15 (Ulla Engström, Ludwig Institute, Uppsala, Sweden), hIAPP1–37 (Keck Biotechnologies, Yale University), hIAPP8–37 and rIAPP1–37 (Peninsula Laboratories), human α-CGRP1–37 (Sigma-Aldrich), insulin (Humulin-R, Eli Lilly), and C-peptide (Chiron Mimotopes Peptide Systems, Clayton, Victoria, Australia) dissolved in DMSO were diluted in PBS (pH 7.4) with 0.1% BSA to yield a final concentration of 6 μM peptide (2.5–5% final DMSO concentration). Peptides were incubated with 3H-labeled heparin (1400 cpm) or 3H-labeled heparin fragments of different sizes (4-, 6-, 8-, 12-, 16-, or 18-mers; 10,000 cpm) for 1 h, and then protein-bound 3H-heparin/heparin fragments were determined using a Tri-Carb 2910 TR scintillation analyzer (Perkin Elmer Life Sciences).

**Thioflavin T Assay**—A stock solution of hIAPP1–37 dissolved in DMSO was diluted to a final concentration of 1.4 μM (0.5% DMSO) in PBS (pH 7.4) with 10 μM thioflavin T (ThT, Sigma). Fibrillation of hIAPP was measured by monitoring emission at 480 nm with 440-nm excitation on a FLUOstar Omega microplate reader (BMG Labtech, Stockholm, Sweden) at room temperature. All reactions were carried out in Sigma-cote-treated (Sigma-Aldrich) black 96-well plates (Nunc). Fibrillation studies were performed in the absence or presence of equimolar full-length heparin or 12-mer heparin fragments. Also, an aliquot of the samples used for the FRET cell assay was incubated with 10 μM ThT in PBS, and aggregation was monitored as described above but at 37 °C.

**Cell Transfection**—Mutant CHO cells with defective HS biosynthesis (pgsD-677) (28) and wild-type CHO cells (CHOWT) (ATCC) were maintained in RPMI 1640 medium supplemented with 10% (v/v) FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5 mM sodium pyruvate. Both cell types were transfected either with the pRcCMV.FRET-DEVD12 vector or the pRcCMV.FRET-KEAF vector (29) using the TurboFect transfection reagent kit (Thermo Scientific, Gothenburg, Sweden). Twenty-four hours post-transfection, medium with G418 (400 mg/ml) (Gibco) was added for selection of stable expressing clones of CHOWT-DEVD and pgsD-677-DEVD or CHOWT-KEAF and pgsD-677-KEAF cells.

**FRET Assay**—Cells were seeded into black 96-well optical bottom plates (Nunc) and cultured in the presence of 4% DMSO for 48 h to increase vector expression because long-term culture is known to inactivate the CMV promotor (30). The assay was performed in Krebs-Ringer solution (120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 12 mM MgSO4, and 0.5 mM KH2PO4 (pH 7.4)) supplemented with 20 mM HEPES and 2 mM glucose (KRHG), shown previously to give low autofluo-
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resonance at 480 nm and 540 nm (31). Cell culture medium was replaced with KRHG, and the experiment was initiated by addition of a mixture of 50 μM hIAPP1–37 and sonicated preformed fibrils (seeds) corresponding to 125 nm monomeric IAPP. Monomeric IAPP and seeds were diluted in KRHG, and final DMSO concentration was 1.5%. IAPP fibril seeds were added to ensure the consistency of IAPP fibrillation kinetics between experiments. To analyze the effect of heparin and 12-mer heparin fragments on IAPP-induced cytotoxicity, an equimolar concentration (50 μM) was mixed with the seeded IAPP solution just before addition to the cells. For caspase inhibitor analysis, cells were preincubated for 30 min with 200 μM caspase-8 inhibitor (Z-IETD-fmk) or caspase-9 inhibitor (Z-LEHD-fmk) (R&D Systems) and present at 100 μM during the analysis. As positive control for induction of apoptosis, the cells were incubated with 2 μM staurosporine (Sigma-Aldrich) or 4 μg/ml mouse Fas Ligand His6, with 10 μg/ml polyhistidine antibody (R&D Systems). Cells incubated with only seeds or polyhistidine antibody served as negative controls. FRET was measured by monitoring emission at 480 and 540 nm with 440-nm excitation once per hour in a FLUOstar Omega microplate reader at 37 °C.

**Immunolabeling of Cell-associated Aggregates—**CHO<sup>WT</sup>-DEVD and pgd-D677-DEVD cells incubated for 48 h in 4% DMSO were lysed in Qiazol lysis reagent (Qiagen), and total RNA was purified using RNeasy MiniElute cleanup columns (Qiagen). 5 μg of RNA, quantified using Nanodrop 2000c (Thermo Scientific), was used for first-strand cDNA synthesis with oligo(dT) primer according to the instructions of the manufacturer (catalog no. K1632, Thermo Scientific). Reactions (10 μl) containing 10 ng of cellular cDNA, 400 nM primer, and FastStart Universal SYBR Green Master (ROX) (Roche Diagnostics) were used for real-time quantitative PCR on a 7900HT real-time PCR system (Applied Biosystems) at 95 °C for 5 min, followed by 50 cycles of 95 °C for 30 s and 54 °C for 30 s. The following primer sequences were used: Fas receptor (XM 003505961), 5’ CACGGGAAGGAAATACA and 3’ CAGTTTGCAGACA-GAAGC; housekeeping gene GAPDH, 5’ AACTTTGGCATGGAAAG and 3’ CACATTTGGGGTAGAACAC. All primers were designed with Primer3 and produced at TAG (Copenhagen, Denmark). PCR products were confirmed by agarose gel electrophoresis and melting curve analysis.

**Statistical Analysis—**All statistics were carried out using GraphPad Prism version 6.01 (GraphPad Software, La Jolla, CA). p < 0.05 was considered statistically significant.

**Results**

**Heparanase Overexpression in hpa-hIAPP Islets—**Heparanase degrades cell surface-associated and extracellular heparan sulfate glycosaminoglycan chains, and, to study the effect of heparanase overexpression in IAPP amyloidogenesis, a double-transgenic mouse expressing both human heparanase and human IAPP (hpa-hIAPP) was generated. Littermates expressing hIAPP were used as controls (hIAPP). Animals with double transgene expression did not develop any apparent phenotypic alterations, and the pancreas showed a normal histological picture, in line with observations from the single-transgenic mice (21, 22). Overexpression of heparanase in islets from hpa-hIAPP mice was verified using an anti-heparanase antibody (733) that detected the cleaved 50-kDa form (Fig. 1A). In pancreas sections, most heparanase reactivity was found in the isocrine pancreas tissue (Fig. 1B). The lack of reactivity in hIAPP mice indicates low levels of endogenous mouse heparanase in islets.

Heparanase overexpression resulted in reduced length of HS chains in the pancreas, as demonstrated by gel chromatography of HS extracted from whole pancreata (Fig. 1C). Because HS and heparanase expression is pronounced in islets and low in exocrine tissue (33), the shift in HS size observed in whole pancreata from hpa mice should reflect islet-associated HS.
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Heparanase Overexpression Reduced Islet Amyloid Load—Culture of isolated hpa-hIAPP and hIAPP islets in 20 mM glucose resulted in the deposition of amyloid, detected by thioflavin S staining (Fig. 2, A and B). After 19 days of culture in 20 mM glucose, islets from hpa-hIAPP mice contained significantly less amyloid compared with islets from hIAPP mice (0.92% ± 0.17% versus 0.35% ± 0.06% for hIAPP versus hpa-hIAPP; p = 0.007, Student’s t test) (Fig. 2C). Culture of islets in 11 mM glucose for 19 days (n = 33 islets/group) resulted in few deposits of amyloid (0.13% ± 0.03% versus 0.07% ± 0.005% for hIAPP versus hpa-hIAPP; p = 0.006, Mann-Whitney test) (Fig. 2C). Islets cultured for 19 days were incubated with Mayer’s nuclear stain, and the number of pyknotic nuclei/islet area was determined to be 0.055 ± 0.015 versus 0.034 ± 0.003 for hIAPP (n = 3) versus hpa-hIAPP (n = 4) (p = 0.18, Student’s t test). This 40% reduction of apoptosis in islets from hpa-hIAPP islets points to a trend for increased cell death in the islets with amyloid. Electron microscopy analysis of the amyloid deposits revealed a fibrillar appearance and reactivity with antisera against IAPP (Fig. 2, D and E). Islet amyloid did not spontaneously develop in 21-month-old mice fed standard chow.

Heparanase Overexpression Does Not Affect β Cell Hormone Secretion or Content—Potential effects of heparanase overexpression on islet function were examined by determining the fraction of β cells and α cells per islet in pancreas sections. No difference was observed between hIAPP and hpa-hIAPP mice (β cells, 72.5% ± 0.3% versus 74.3% ± 2.5%; α cells, 23.7% ± 0.9% versus 27.3% ± 1.9% for hIAPP versus hpa-hIAPP) (Fig. 3A). Next, isolated islets were analyzed for glucose-stimulated insulin and IAPP release. Released insulin or IAPP during basal and stimulated conditions was similar in hIAPP and hpa-hIAPP islets (insulin, basal, 0.71 ± 0.13 versus 0.98 ± 0.31 ng/10 islets/h; insulin, stimulated, 5.40 ± 1.40 versus 5.40 ± 0.62 ng/10 islets/h for hIAPP versus hpa-hIAPP; IAPP, basal, 0.08 ± 0.005 versus 0.07 ± 0.002 ng/10 islets/h; IAPP, stimulated, 0.40 ± 0.03 versus 0.43 ± 0.02 ng/10 islets/h for hIAPP versus hpa-hIAPP) (Fig. 3, B and D). No difference was observed in total hormone content (insulin content, 1030 ± 116 versus 934 ± 80 ng/30 islets; IAPP content, 38 ± 8 versus 32 ± 7 ng/30 islets for hIAPP versus hpa-hIAPP) (Fig. 3, C and E).

Heparin Binds to the N-terminal Region of IAPP in a Size-Dependent Manner—The interaction between heparin and IAPP peptides was analyzed with a nitrocellulose filter binding assay using 3H-labeled heparin. Heparin is used as a model for HS and only differs in its higher sulfate content and more uniform sulfation pattern. Heparin bound hIAPP–37 as well as rat IAPP1–37 and the IAPP related peptide hCGRP1–37, whereas no binding was seen with a peptide spanning the N-terminal cleavage site of hproIAPP (hproIAPP6–15) or with N-terminally truncated hIAPP (hIAPP8–37) (Fig. 4A). Rat IAPP differs from the human variant at six positions and has an N-terminal region identical to hIAPP. Human CGRP shares almost 50% overall sequence identity with hIAPP, with the greatest homology found in the N-terminal region, where identity increases up to more than 70% (residues 1–12). No interaction of heparin with the other components of the secretory granule, insulin or...
C-peptide, was observed. The binding between heparin and hIAPP1–37 was affected by the size of heparin, and heparin fragments ≤8-mers displayed little or no binding with hIAPP, whereas the longer chains of 12-, 16-, and 18-mers showed progressively increased binding to hIAPP (Fig. 4B).

The 12-mer Heparin Fragment Attenuated Amyloid Load in Human Islets—Next we determined the potential for extracellular heparin and heparin fragments to influence amyloid load in cultured islets. Isolated islets from hIAPP mice (hIAPP FVB/N, the same strain used to generate hpa-hIAPP mice) were cultured in 20 mM glucose in the absence or presence of heparin fragments; 4-, 8-, 12-, or 18-mer heparin fragments; or full-length heparin. Of the five fragments tested, only 12-mer had a tendency to lower islet amyloid load (Fig. 5A). Therefore, the culture of transgenic mouse islets in 20 mM glucose with or without addition of 12-mer heparin fragments was repeated (in total three times). The amyloid load in percent was determined, and, for comparison, the value was first normalized against the HG group for analysis (HG, 100% ± 0% versus 12-mer, 71.63% ± 2.8%; p < 0.05, ANOVA, Bonferroni correction) (Fig. 5A). The failure of 4- and 8-mer heparin fragments to interfere with IAPP amyloid formation is in accordance with the result from the binding assay (Fig. 4B), and the reason for the lack of effect by the longer heparin chains (>12-mer) is unclear. It was not due to their inability to diffuse into the islets because biotinylated full-length heparin was detected throughout the islets, including the islet core, already after 3 h of incubation. In a single experiment, hIAPP transgenic mouse islets isolated from three mice were cultured in 20 mM glucose and 20 mM glucose supplemented with 150 mM or 300 mM of 12-mer heparin fragments for 19 days. Irrespective of the 12-mer heparin fragment concentration used for incubation, there was a tendency for lowering of islet amyloid load (HG, 1.85% ± 0.9%; 12-mer, 150 mM, 1.28% ± 0.8%; 12-mer, 300 mM, 1.14% ± 0.9%). To test whether the effect of heparin fragments also applies to human tissues, isolated human islets from three different donors were cultured in 20 mM glucose with or without addition of 12-mer heparin fragments. Culture in the presence of 150 mM 12-mer compared with islets cultured in HG only (p < 0.05 versus HG). A 2-fold increase of the 12-mer heparin fragment concentration to 300 mM resulted in reduction of the amyloid load; however, it did not reach statistical significance (12-mer, 300 mM, 0.16% ± 0.04%).

The Heparin Fragment Promotes in Vitro IAPP Fibril Growth—To determine how heparin chain size affects IAPP fibril formation, aggregation kinetics in the presence of full-length heparin or 12-mer heparin fragments were analyzed. We used a ThT assay where fibril formation is monitored as an increase in fluorescence intensity at 480 nm with excitation at 440 nm. The lag phase is the period before any rise in fluores-
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Heparan sulfate (HS) proteoglycans (PGs) are critical to islet amyloid formation (IAP) (1). HS-deficient pancreatic β cells (∼20% prevalence) are a feature of type 2 diabetes (T2D) (3). The formation of amyloid IAPP fibrils is critical to IAPP-induced cell death and β cell failure (4). However, the role of HS in IAPP-induced apoptosis remains unclear. Here, we investigate the role of HS in IAPP-induced apoptosis. This was achieved by using human islet amyloid precursor protein (hIAPP)-positive (CHO WT) cells, which lack HS, and human islet amyloid precursor protein (hIAPP)-negative (CHO Δ677) cells, which express HS. CHO WT cells were stably transfected with a vector encoding protein pairs for caspase-3 activation and subsequent induction of apoptosis in real time, cells were stably transfected with a vector encoding protein pairs for caspase-3 activation and subsequent induction of apoptosis in real time.

Incubation of CHO WT-DEV cells with hIAPP and sonicated IAPP fibrils (seeds) resulted in a progressive loss of FRET over time (p < 0.05, two-way ANOVA with Bonferroni correction) (Fig. 7A). In contrast, exposure of CHO WT-DEV to seeded hIAPP did not reduce FRET at any time point studied (≤11 h) (Fig. 7B). Staurosporine was used as a positive control and reduced FRET in both CHO WT-DEV and CHO WT-DEV and pgsD-677-DEV after 4–5 h (Fig. 7, A and B) but had no effect in CHO WT-KEAF cells (Fig. 7D). Incubation of CHO WT-DEV with solution containing seeds only did not result in activation of caspase-3. At the end point of the FRET assay, after 12 h of incubation with seeded hIAPP, large aggregates of hIAPP were found mainly extracellularly and associated with the plasma membrane of both cell types (Fig. 7E). However, more extensive hIAPP reactivity was found associated with CHO WT-DEV cells compared with pgsD-677-DEV cells.

Fibril Formation Neutralizes Toxic Species—In the ThT assay, hIAPP and 12-mer heparin fragments shortened the lag phase for IAPP aggregation (Fig. 6A), and the 12-mer fragment attenuated the amyloid load in cultured islets (Fig. 5). When hIAPP and 12-mer fragments were added to seeded IAPP solution in the caspase-3 activation assay, it lead to a brief acceleration of apoptosis at 4 and 6 h, but, after 12 h, toxicity was attenuated significantly compared with seeded IAPP alone (p < 0.05, two-way ANOVA with Bonferroni correction) (Fig. 6B). The early caspase-3 activation at 4 and 6 h is in line with the ThT results and reinforces the assumption that rapid fibril formation neutralizes toxic species.

Human IAPP Induces Apoptosis in CHO WT via a Caspase-8-Dependent Pathway—The signaling pathway involved in hIAPP-induced apoptosis of CHO WT cells was determined using caspase-8 and caspase-9 inhibitors. Treatment of CHO WT-DEV cells with caspase-8 inhibitor, but not caspase-9 inhibitor, effectively prevented the observed hIAPP-induced reduction of FRET (p < 0.05, one- and two-way ANOVA with Bonferroni correction) (Fig. 7, F and G). Caspase inhibitors had no effect on cells incubated with seeds alone, demonstrating that the effect of caspase-8 inhibition was specific to hIAPP-induced apoptosis. On the basis of these results, we analyzed whether a defect in the extrinsic pathway could explain the lack of IAPP-induced apoptosis in pgsD-677-DEV cells. However, this seems unlikely because no difference in Fas receptor mRNA levels (Fig. 8A) or FasL-induced loss of FRET was

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observed in CHOWT-DEV and pgsD-677-DEV cells (Fig. 8, B and C).

Discussion

The codeposition of heparan sulfate proteoglycan with islet amyloid in patients with type 2 diabetes, Aβ-amyloid in Alzheimer disease, and amyloid in all other amyloid-related diseases suggests that this ubiquitously expressed macromolecule plays an active role in amyloidogenesis (35, 36). Overexpression of heparanase has been reported to reduce the amyloid load in animal models of other amyloid-related diseases, including AA amyloidosis (20) and Alzheimer disease (37). In this study, we show, for the first time, that overexpression of the HS-degrading enzyme heparanase in pancreatic islets reduces islet amyloid formation.

For the implementation of this work, we generated a double transgene hpa-hIAPP mouse and found active heparanase in islet β cells. HS isolated from whole pancreata demonstrated decreased chain size, consistent with an overproduction of active heparanase, which must originate from islets because...
Several in vitro studies have demonstrated an accelerating effect of HS on fibril formation of amyloid peptides, including hIAPP and hproIAPP (14, 17). When binding of heparin to IAPP was analyzed by nitrocellulose filter assay, we detected the same degree of binding to hIAPP1–37 as to rIAPP1–37, a result that differs from previous studies in which binding between proteoglycans and IAPP was missing (14, 15). The amino acid sequences of hIAPP and rIAPP are identical in the N-terminal region spanning residues 1–17, and this, together with the absence of heparin binding to hIAPP 8–37, suggest that residues 1–7 comprise part of the binding site. We also found that heparin binds to hCGRP, an IAPP-related peptide that shares five of seven N-terminal residues with IAPP.

Amyloid fibrils consist of a β sheet structure where strands are arranged perpendicularly to the fibril axis. There are several models describing IAPP aggregates (42–45). Commonly, the seven to ten most N-terminal residues are not included in the fibril core. Biological activity of IAPP and CGRP requires a disulfide bond between cysteines at positions 2 and 7, which causes a restriction in mobility in this region. We used oxidized hIAPP, rIAPP, and CGRP in our study, and the observed binding between the N-terminal region and HS may lead to an orientation of bound peptides allowing intermolecular interactions and, in the case of hIAPP, aggregation. Such binding at the N terminus of the peptide may also prevent other interactions of IAPP regions important for β sheet formation.

In addition to HS effects on IAPP amyloid formation, we investigated its role in IAPP-induced cytotoxicity. It is still unknown what drives the conversion of native protein to amyloid fibrils, but it is clear that the process involves the formation of smaller aggregates, oligomers (46, 47). Although the species responsible for toxicity is debated (48), ex vivo studies show that oligomers or seeded IAPP amyloid propagation induce apoptosis in various cell lines, whereas the addition of mature fibrils does not (49–51). Molecular dynamic studies on IAPP aggregation show that hIAPP trimers form a stable core that can seed IAPP fibrillization, whereas rIAPP aggregates never adopt the stability required for fibril propagation (52). It is possible that HS creates a microenvironment on the cell surface that allows extracellular hIAPP seeds to form.

In CHOWT-DEV cells, IAPP-induced toxicity corresponded to aggregated species early in the elongation phase of the ThT curve, although already with fibrillar morphology. It is likely that IAPP fibril formation is initiated much earlier in the presence of cells compared with what was seen in the ThT assay because cellular components such as GAGs and negatively charged lipids can promote the aggregation of IAPP (5).

Previously, B-TC6 cells transfected with the FRET-DEV vector were used in a study in which we showed that there was no difference between the ability of hproIAPP and hIAPP to activate caspase-3, whereas incubation with monomeric hIAPP, mature hIAPP fibrils, or rIAPP failed to do so (31). B-TC6-DEV and CHOWT-DEV cells with HS on the cell surface are expected to bind both hIAPP and rIAPP, but only hIAPP activates caspase-3. Therefore, it seems unlikely that binding of hIAPP or rIAPP to HS is sufficient for induction of toxicity. Instead, further aggregation or fibril propagation in close association with the cell membrane is required. This is very low levels of active heparanase were found in the exocrine pancreas.

Hull et al. (38) demonstrated that blocking GAG chain elongation in islets with an N-acetylgalactosamine analogue significantly reduced both islet amyloid prevalence and severity, with an associated increase in β cell area. However, the authors raised a concern regarding impaired islet viability and islet hormone secretion as a consequence of loss of GAGs. HS is ubiquitously expressed in virtually all tissues, including pancreatic islets (33) and β cells (38), and HS has been shown to affect both β cell survival (33) and insulin release (39). However, the decreased islet amyloid deposition in hpa-hIAPP mice was not due to reduced IAPP release because glucose-induced IAPP secretion remained unaltered. The preserved hormone secretion from β cells in hpa-hIAPP mice may be explained by the specific degradation of HS, leaving the other GAGs intact, which may act as substitutes for HS. In addition, small HS fragments have been shown to retain the ability to perform some of the functions of full-length HS.

We hypothesize that the observed reduction in islet amyloid load by overexpression of heparanase results from the digestion of HS present on cell surface-associated syndecans and glypicans or in the extracellular matrix, reducing the availability of HS, which, otherwise, would act as a promoter of amyloid formation. A further effect of heparanase overexpression is the generation of soluble heparin fragments that compete with cellular and extracellular HS for binding of the released IAPP, thereby preventing IAPP from forming aggregates directly adjacent to the cell surface. However, the contribution of other effects of heparanase overexpression, in addition to increased enzymatic digestion of HS, such as increased sulfation degree of HS (40), altered gene expression (41), or increased release of extracellular matrix-bound factors (21), cannot be ruled out.

**FIGURE 8.** HS-deficient pgsD-677 cells have intact activation of apoptosis via the extrinsic pathway. A, no difference in Fas receptor gene expression was found between CHOWT-DEV and pgsD-677-DEV cells at initiation of the FRET assay. Data are presented as the fold change in Fas receptor gene expression normalized to an endogenous reference (GADPH) and relative to CHOWT cells (n = 2). B and C, both CHOWT-DEV (B) and pgsD-677-DEV (C) cells demonstrated a significant reduction in FRET signal after incubation with mouse Fas Ligand His6 and polyhistidine antibody for 1 h. Data are representative of n = 2. * p < 0.05 versus control; two-way ANOVA; Bonferroni correction; mean ± S.E.

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supported by the finding that immunolabeling of cells incubated with hIAPP and IAPP seeds showed an enrichment of IAPP-reactive material in close proximity to the CHO<sup>WT</sup>-DEVD cell membrane, different from the picture in pgsD-677-DEVD cells. Also, pgsD-677-DEVD cells lacking HS on their surface remain unaffected by exogenously added hIAPP.

In CHO<sup>WT</sup>-DEVD cells, incubation with aggregating hIAPP resulted in caspase-3 activation after 6 h, and this is in good agreement with the results from the ThT assay, where IAPP aggregates could be demonstrated after 6 h. When CHO<sup>WT</sup>-DEVD cells were incubated with heparin and IAPP or a 12-mer heparin fragment and IAPP, caspase-3 activation started at 4 and 6 h, respectively, but never reached the same level of cell toxicity as after incubation with IAPP alone. In the ThT assay, both heparin and 12-mer fragments triggered fibrillation. This supports the hypothesis that fibrils are less toxic and that rapid fibrillation of IAPP prevents cytotoxicity. A recent study showed that copper ions interfere with IAPP aggregation and can suppress fibril formation but potentiate the toxicity of formed aggregates (53). The presence of heparin and 12-mer heparin fragments in the extracellular medium also competes with IAPP binding to cell surface HS and redirects the fibrillation of IAPP away from the vicinity of the cell surface, discussed above as an important aspect in the induction of cytotoxicity. Culture of CHO<sup>WT</sup> cells in the presence of IAPP and IAPP seeds induced apoptosis via the caspase-8-dependent extrinsic pathway, in agreement with earlier work with β cells demonstrating the extrinsic pathway, involving Fas receptor signaling, as the primary pathway in hIAPP-induced apoptosis (8, 54, 55).

In a recent paper, Carufel et al. (56) demonstrated that the removal of cell surface GAGs had no effect on IAPP-induced cytotoxicity, measured 24 h after exposure to ≥25 μM hIAPP. This is not unexpected because formation of fibrillar species and, therefore, cytotoxicity should occur at such supraphysiological concentrations of hIAPP well before 24 h, independent of the presence or absence of GAGs. In addition to type 2 diabetes, IAPP amyloid develops rapidly in human islet implanted under the kidney capsule of nude mice (57) and in allogenic islets transplanted to the liver as a treatment strategy for improvement of blood glucose regulation in type 1 diabetes (58). In the human situation, islets from more than one donor are required to achieve insulin independence. A vast mass of the implant is lost in connection with the transplantation and is ascribed to the process of instant blood-mediated inflammatory reaction. The addition of heparin has been shown to improve immediate islet survival (59). However, heparin increases the risk of bleeding and is therefore given as a bolus dose for peritransplantation treatment. In an attempt to improve long-term islet survival, functional heparin has been immobilized on the islet surface to create a shield that, after implantation, prevents coagulation and activation of the complement cascade (60). This heparin shield can create foci with high binding for IAPP released continuously from β cells in the entrapped islet. Therefore, it is possible that IAPP aggregation is activated in islets pretreated with heparin or implanted into a heparin-rich environment.

In conclusion, we show, for the first time, that overexpression of the HS-degrading enzyme heparanase reduces islet amyloid formation and that the presence of small heparin fragments (12-mers) decreases the islet amyloid load in cultured human islets. Moreover, we show that cell-associated HS is important for IAPP-induced caspase-3 activation. This study provides proof of HS playing a causative role in islet amyloid formation and IAPP-induced toxicity in β cells. HS may be a suitable target for the development of new approaches to preserve β cell viability and function in type 2 diabetes and after islet transplantation.

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References

1. Westermark, P., Andersson, A., and Westermark, G. T. (2011) Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol. Rev. 91, 826–862

2. Glabe, C. G. (2006) Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. Neurobiol. Aging 27, 570–575

3. Shin, J. T., Ward, J. E., Collins, P. A., Dai, M., Semigran, H. L., Semigran, M. J., and Seldin, D. C. (2012) Overexpression of human amyloidogenic light chains causes heart failure in embryonic zebrafish: a preliminary report. Amyloid 19, 191–196

4. Mirzabekov, T. A., Lin, M. C., and Kagan, B. L. (1996) Pore formation by the cytotoxic islet amyloid peptide amylin. J. Biol. Chem. 271, 1988–1992

5. Knight, J. D., and Miranker, A. D. (2004) Phospholipid catalysis of diabetic amyloid assembly. J. Mol. Biol. 341, 1175–1187

6. Trika, S., and Jeremic, A. M. (2011) Clustering and internalization of toxic amylin oligomers in pancreatic cells require plasma membrane cholesterol. J. Biol. Chem. 286, 36086–36097

7. Bag, N., Ah, A., Chauhan, V. S., Wohland, T., and Mishra, A. (2013) Membrane destabilization by monomeric hIAPP observed by imaging fluorescence correlation spectroscopy. Chem. Commun. 49, 9155–9157

8. Park, Y. J., Lee, S., Kieffer, T. J., Warnock, G. L., Safikhani, N., Speck, M., Hao, Z., Woo, M., and Marzban, L. (2012) Deletion of Fas protects islet β cells from cytotoxic effects of human islet amyloid polypeptide. Diabetologia 55, 1035–1047

9. Sipe, J. D., Benson, M. D., Buxbaum, J. N., Ikeda, S., Merlini, G., Saraiva, M. J., and Westermark, P. (2014) Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. Amyloid 21, 224–224

10. Hass, G. (1942) Studies of amyloid: II: the isolation of a polysaccharide from the amyloid fibril. Acta Pathol. Microbiol. Scand. 19, 105–107

11. Snow, A. D., Bramson, R., Mar, H., Wight, T. N., and Kisilevsky, R. (1991) A temporal and ultrastructural relationship between heparan sulfate proteoglycans and AA amyloid in experimental amyloidosis. J. Histochem. Cytochem. 39, 1321–1330

12. Norling, B., Westermark, G. T., and Westermark, P. (1988) Immunohistochemical identification of heparan sulfate proteoglycan in secondary systemic amyloidosis. Clin. Exp. Immunol. 73, 333–337

13. Sarrazin, S., Lamanna, W. C., and Esko, J. D. (2011) Heparan sulfate proteoglycans. Cold Spring Harb. Perspect. Biol. 3, 1–33

14. Castillo, G. M., Cummings, J. A., Yang, W., Judge, M. E., Sheardown, M. J., Rimvall, K., Hansen, J. B., and Snow, A. D. (1998) Sulfate content and specific glycosaminoglycan backbone of perlecan are critical for perlecan’s enhancement of islet amyloid polypeptide (amylin) fibril formation. Diabetes 47, 612–620

15. Potter-Perigo, S., Hull, R. L., Tsoi, C., Braun, K. R., Andrikopoulos, S., Teague, J., Bruce Verchere, C., Kahn, S. E., and Wight, T. N. (2003) Proteoglycans synthesized and secreted by pancreatic islet β-cells bind amylin. Arch. Biochem. Biophys. 413, 182–190

16. Park, K., and Verchere, C. B. (2001) Identification of a heparin binding domain in the N-terminal cleavage site of pro-islet amyloid polypeptide: implications for islet amyloid formation. J. Biol. Chem. 276, 16611–16616

17. Abedini, A., Tracz, S. M., Cho, J. H., and Raleigh, D. P. (2006) Characterization of the heparin binding site in the N-terminus of human pro-islet amyloid polypeptide: implications for amyloid formation.
HS Proteoglycans Are Important for Islet Amyloid Formation

Alzheimer’s disease and other amyloidoses. *Neurobiol. Aging* 10, 481–497

36. Young, J. D., Ailles, L., Narinderosasaraks, S., Tan, R., and Kisilevsky, R. (1992) Localization of the basement membrane heparan sulfate proteoglycan in islet amyloid deposits in type II diabetes mellitus. *Arch. Pathol. Lab. Med.* 116, 951–954

37. Jendresen, C. B., Cui, H., Zhang, X., Vlodavsky, I., Nilsson, L. N., and Li, J. P. (2015) Overexpression of heparanase lowers the amyloid burden in amyloid-β precursor protein transgenic mice. *J. Biol. Chem.* 290, 5053–5064

38. Hull, R. L., Zraika, S., Udayasankar, J., Kisilevsky, R., Szarek, W. A., Wight, T. N., and Kahn, S. E. (2007) Inhibition of glycosaminoglycan synthesis and protein glycosylation with WAS-406 and asazerenine result in reduced islet amyloid formation in vitro. *Am. J. Physiol. Cell Physiol.* 293, C1586–C1593

39. Takahashi, I., Noguchi, N., Kata, K., Yamada, T., Kawiwa, T., Mizumoto, S., Ikeda, T., Sugihara, K., Asano, M., Yoshikawa, T., Yamauchi, A., Sernani, N. J., Urano, A., Kato, U., Uno, M., Sugahara, K., Takasawa, S., Okamoto, H., and Sugawara, A. (2009) Important role of heparan sulfate in postnatal islet growth and insulin secretion. *Biochem. Biophys. Res. Commun.* 383, 113–118

40. Escobar Galvis, M. L., Jia, J., Zhang, X., Jastrebova, N., Spilmann, D., Gottfridsson, E., van Kuppevelt, T. H., Zcharia, E., Vlodavsky, I., Lindahl, U., and Li, J. P. (2007) Transgenic or tumor-induced expression of heparanase upregulates sulfation of heparan sulfate. *Nat. Chem. Biol.* 3, 773–778

41. Nobuhisa, T., Naoi, Y., Takaoka, M., Tabuchi, Y., Ookawa, K., Kitamoto, D., Gunduz, E., Gunduz, M., Nagatsu, H., Haisa, M., Matsuoka, J., Nakajima, M., and Tanaka, N. (2005) Emergence of nuclear heparanase induces differentiation of human mammary cancer cells. *Biochem. Biophys. Res. Commun.* 331, 175–180

42. Luca, S., Yao, W. M., Leaman, R., and Tycko, R. (2007) Peptide conformation and supramolecular organization in amylin fibrils: constraints from solid-state NMR. *Biochemistry* 46, 13505–13522

43. Wiltzius, J. J., Sievers, S. A., Sawaya, M. B., Cascio, D., Popov, D., Riekel, C., and Eisenberg, D. (2008) Atomic structure of the cross-β spine of islet amyloid polypeptide (amylin). *Protein Sci.* 17, 1467–1474

44. Buchanan, L. E., Dunkelberger, E. B., Tran, H. Q., Cheng, P. N., Chiu, C. C., Cao, P., Raleigh, D. P., de Pablo, J. J., Nowick, J. S., and Zanni, M. T. (2013) Mechanism of IAPP amyloid fibril formation involves an intermediate with a transient β-sheet. *Proc. Natl. Acad. Sci. U.S.A.* 110, 19285–19290

45. Kajava, A. V., Aebi, U., and Steven, A. C. (2005) The parallel superpleated β-structure as a model for amyloid fibrils of human amylin. *J. Mol. Biol.* 348, 247–252

46. Anguiano, M., Nowak, R. J., and Lansbury, P. T., Jr. (2002) Protobifibrill islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. *Biochemistry* 41, 11338–11343

47. Butler, A. E., Jang, J., Gurlo, T., Carty, M. D., Soeller, W. C., and Butler, P. C. (2004) Diabetes due to a progressive defect in β-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. *Diabetes* 53, 1509–1516

48. Abedini, A., and Raleigh, D. P. (2005) The role of His-18 in amyloid formation by human islet amyloid polypeptide. *Biochemistry* 44, 16284–16291

49. Lorenzo, A., Razzaboni, B., Weir, G. C., and Yankner, B. A. (1994) Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature* 368, 756–760

50. Zhang, S., Liu, J., Saati, E. L., and Cooper, G. J. (1999) Induction of apoptosis by human amylin in RINm5F islet β-cells is associated with enhanced expression of p53 and p21WAF1/CIP1. *FEBS Lett.* 455, 315–320

51. Janson, J., Ashley, R. H., Harrison, D., McIntyre, S., and Butler, P. C. (1999) The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. *Diabetes* 48, 491–498

52. Liang, G., Zhao, J., Yu, X., and Zheng, J. (2013) Comparative molecular dynamics study of human islet amyloid polypeptide (IAPP) and rat IAPP oligomers. *Biochemistry* 52, 1089–1100

53. Ma, L., Li, X., Wang, Y., Zheng, W., and Chen, T. (2014) Cu(II) inhibits hIAPP fibrillation and promotes hIAPP-induced β cell apoptosis through
induction of ROS-mediated mitochondrial dysfunction. J. Inorg. Biochem. 140, 143–152
54. Zhang, S., Liu, J., Dragunow, M., and Cooper, G. J. (2003) Fibrillogenic amylin evokes islet β-cell apoptosis through linked activation of a caspase cascade and INK1. J. Biol. Chem. 278, 52810–52819
55. Park, Y. J., Woo, M., Kieffer, T. J., Hakem, R., Safikhan, N., Yang, F., Ao, Z., Warnock, G. L., and Marzban, L. (2014) The role of caspase-8 in amyloid-induced β cell death in human and mouse islets. Diabetologia 57, 765–775
56. De Carufel, C. A., Nguyen, P. T., Sahnouni, S., and Bourgault, S. (2013) New insights into the roles of sulfated glycosaminoglycans in islet amyloid polypeptide amyloidogenesis and cytotoxicity. Biopolymers 100, 645–655
57. Westermark, P., Eizirik, D. L., Pipeleers, D. G., Hellerström, C., and Andersson, A. (1995) Rapid deposition of amyloid in human islets transplanted into nude mice. Diabetologia 38, 543–549
58. Westermark, G. T., Westermark, P., Berne, C., Korsgren, O., and Nordic Network for Clinical Islet Transplantation (2008) Widespread amyloid deposition in transplanted human pancreatic islets. New Eng J. Med. 359, 977–979
59. Bennet, W., Sundberg, B., Groth, C. G., Brendel, M. D., Brandhorst, D., Brandhorst, H., Bretzel, R. G., Elgue, G., Larsson, R., Nilsson, B., and Korsgren, O. (1999) Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 48, 1907–1914
60. Cabric, S., Sanchez, J., Lundgren, T., Foss, A., Felldin, M., Källen, R., Salmela, K., Tibell, A., Tufveson, G., Larsson, R., Korsgren, O., and Nilsson, B. (2007) Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. Diabetes 56, 2008–2015