Supporting Information

Potter et al. 10.1073/pnas.0909024107

SI Materials and Methods

Porcine IAPP Sequencing. The primer sequences used were: Forward, CTGAATTTCCAAAGGATTGTACTGGGAA, ACCAAAACACTGGGTTACTGATAGGAAGA; reverse, GTGCGTTGACCTCTAAAGGGGTAAGTA, CTGGCAGCAAACATGGGACACA.

Peptide Synthesis and Preparation. The synthesis was conducted on a 0.25-mmol scale using an Applied Biosystems 433A Peptide Synthesizer, using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry as previously described (46). Use of a 5-(4′-Fmoc-aminomethyl-3′, 5-dimethoxyphenol) valeric acid (PAL-PEG) resin afforded an amidated C terminus as in the naturally occurring peptide. The Bachem peptide was used without further purification. The peptides synthesized at the State University of New York at Stony Brook were purified using HPLC. The peptide prepared in house was oxidized using the DMSO-based method previously described (45). The purified peptide was analyzed by electrospray mass spectrometry using a Micromass Platform LCZ single quadrupole instrument to confirm its identity. Mass spectra were acquired by averaging scans over the *m*/*z* range of 500–4,000. Purity was checked by analytical HPLC before each experiment. Biophysical studies used samples derived from the same peptide solution to ensure comparable conditions in all experiments. Peptide stock solutions for these studies were prepared by adding HFIP to dry oxidized peptide, sonicating at room temperature, and lyophilizing. Stock solutions (1.58 mM) of both porcine and human IAPP were prepared using 100% HFIP. The solutions were filtered through a 0.4 μm GHP Acrodisc syringe filter before use. All biophysical measurements were initiated by diluting the stocks solutions into buffered aqueous solutions to yield a final HFIP concentration of 2% by volume. These are standard protocols for solution biophysical studies of IAPP. For toxicity assays, peptide was dissolved in 100% HFIP, lyophilized, and dissolved in cell media immediately before assays.

IAPP Cytoxicity Assays. INS-1 cells were grown in RPMI 1640 (Gibco-BRL) supplemented with 10% FBS, 11 mM glucose, 10 mM Hepes, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, 100 U/mL penicillin, and 100 U/mL streptomycin (Gibco-BRL). Cells were maintained at 37 °C in a humidified environment supplemented with 5% CO_2. Cells were grown for two passages before use and used in assays between passages 59 and 65. For Alamar blue experiments, cells were seeded (60,000 cells/well) in 96-well plates and cultured for 24 h before addition of peptide. For TUNEL assays, INS-1 cells were seeded at a density of 100,000 cells per well in 8-well chamber slides and cultured for 48 h before addition of peptide.

Neonatal Porcine Islet Isolation and Transplantation. The pancreas was removed from anesthetized animals following laparotomy and exsanguination and placed into Hanks' balanced salt solution. The pancreas was cut into small pieces, digested with 2.5 mg/mL collagenase (Sigma-Aldrich), and filtered through a 500 μm nylon mesh. The filtrate containing NPI was cultured for 7–10 days at 37 °C, 20% CO_2 in Ham's F-10 medium supplemented with 10 mM glucose, 50 μM isolbutalmethylxanthine (IBMX; ICN Biomedicals), 0.5% BSA (fraction V, RIA grade, Sigma-Aldrich), 2 mM L-glutamine, 3 mM $CaCl_2$, 10 mM nicotinamide, 100 U/mL penicillin, and 100 μg/mL streptomycin (all from Invitrogen).

Adult Porcine Islet Isolation and Transplantation. Pig pancreata were harvested under aseptic conditions and transported in UW solution. Upon receipt, the organ was infused with either Liberase-PI or Collagenase-P (Roche). The islets were isolated as previously described (47) using a large Ricordi digestion chamber with gentle shaking. Separation of islet fractions was performed using discontinuous Euro-Ficoll gradients (at densities of 1.108, 1.096, and 1.037) in a COBE 2991 Cell Separator (48). Purity was determined with dithizone staining of islet samples and expressed as percentage of islets/whole tissue. The islets were then cultured in CMRL 1066 media supplemented with 10% porcine serum until tested or transplanted. Additionally, immediately before testing or transplantation, porcine islets were hand-picked for greater purity. The recipient animal was anesthetized with a 2.5% solution of avertin, injected i.p. at a dose of 0.015–0.017 mL/g body weight. The kidney was externalized and a small tear was made in the capsule at one pole of the kidney. The islets were aspirated into a piece of PE50 tubing (Harvard Apparatus) and slowly expelled into the renal subcapsular space (49).

Table S1. Blood glucose (mM) in murine recipients of human islet transplants

Experiment ID	Pretransplant	Following streptozotocin	4 weeks posttransplant	8 weeks posttransplant
SB1	N/A	16.4	8.8	
SB2	N/A	18.9	5.1	3.8
SB3	N/A	21.1	30	23
SB4	N/A	26.1	30.2	
SB5	N/A	17.5	17.2	25.4
SB6	N/A	23	7.6	6.7
SB7	N/A	19.2	18.8	
CRA1	5.7	26.3	9.9	14.1
CRC3	6.1	31.6	9.2	6.4
HTC1	6.5	24.4	5.9	3.5
KP17C1M1	6.3	20.3	4.9	4.7
KP17C1M3	5.9	25.3	4.9	4.9
KP17C1M5	6.5	29	4.7	4.8
KP19 1–3K	6.4	28.2	5.2	3.9
KP19 2–1	6.3	15.8	7	4.0
KP19 2–3	7.3	15.5	5.4	4.2
DD35 c2m3	7.2	21.4	8.8	18.2
AK18 2–1	5.7	24.7	5.5	
AK18 2–2	6.2	24.9	5	
AK18 3–1	7.4	25.7	5.3	
AK19 1–3	6.8	23.2	10.4	
AK19 1–4	8.3	23.1	13	
AK21 1–3	6.6	26.9	23.1	
AK21 3–3	7.7	30	15.8	
AK21 3–4	6.9	24.5	12.1	
AK36 1–3	7.9	22.1	9	
AK36 1–4	7.1	28.4	11.3	
AK36 4–2	7.6	24	19.2	
KPTCC2	7.9	19	4.5	
KPTCC3	7.9	19	4.5	
KPSC 2–1	8.8	30.1	15.3	
KPSC 2–2	12.7	24.4	12.3	
KPSC 2–3	10.8	24.7	4.8	
AK18 1–1	6.3	23.7	7.9	
AK18 2–3	7.8	25.3	5.7	
AK18 3–2	7.5	23.4	4.6	
AK19 1–1	7.8	24.6	5	
AK19 1–2	7.8	23.7	8.8	
AK19 2–4	6.3	23.6	4.2	
AK21 1–2	5.9	21.4	10.1	
AK21 3–2	6.4	24.5	13.3	
AK36 1–1	5	23.9	7	
AK36 4–1	3.1	23.3	5.9	

Table S2. Blood glucose (mM) in murine recipients of adult porcine islet transplants

Experiment ID	Following streptozotocin	2 weeks posttransplantation	4 weeks posttransplantation	Final
Mouse 1	25.6	13.7	14.2	12.2 (34 days)
Mouse 2	22.7	9.8	7.2	5.3 (37 days)
Mouse 3	19.4	5.7	5.6	4.8 (87 days)
Mouse 4	21.5	6.6	4.5	7.7 (195 days)
Mouse 5	21.4	6.2	3.7	4.0 (113 days)
Mouse 6	24.0	4.2	4.2	4.2 (25 days)

Table S3. Blood glucose (mM) in murine recipients of neonatal porcine islet transplants

Experiment ID	Following streptozotocin	2 weeks posttransplantation	4 weeks posttransplantation	8 weeks posttransplantation	10 weeks posttransplantation
Mouse 1	28.6	25.1	17.1	5.8	5.6
Mouse 2	29.2	26.2	14.2	5.2	5.2
Mouse 3	27.4	24.1	13.1	5.8	5.6
Mouse 4	29.8	26.1	12.0	6.2	6.2
Mouse 5	26.1	25.1	11.1	5.7	6.1
Mouse 6	27.8	24.2	12.6	6.2	5.9
Mouse 7	26.1	22.1	9.9	6.0	5.6
Mouse 8	29.8	19.9	10.1	5.5	5.7
Mouse 9	29.2	23.1	9.8	6.1	5.8
Mouse 10	27.6	24.2	11.1	6.3	5.6