Supporting Information

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SI Experimental Procedures

Islet Isolation. Human islets were isolated as described (1). Monkey islets were isolated from cynomolgus monkeys (*Macaccafascicularis*) >4 years of age at the time of pancreas procurement, as previously described (2). Pig pancreata were procured from the local slaughterhouse. Mice (C57BL/6) and rat (Lewis rat; Harlan) islets were isolated using a rodent-islet isolation technique (3). All animal protocols were approved by the University of Miami Care and Use Committee.

Human islets were dissociated into single cells using enzyme-free cell dissociation buffer (Invitrogen). Islets and islets cells from all species were cultured identically (37 °C and 5% CO_2) in Connaught Medical Research Laboratories (CMRL) medium-1066 (Invitrogen), niacinamide (10 mM; Sigma), insulin, human transferrin, and selenous acid (ITS) universal culture supplement (BD Biosciences), Zn_2SO_4 (15 μM, Sigma), GlutaMAX (2 mM; Invitrogen), Hepes (25 mM; Sigma), FBS (10%; Invitrogen), and penicillin–streptomycin (100 IU/mL–100 μg/mL; Invitrogen).

$[Ca^{2+}]_i$ Imaging. $[Ca^{2+}]_i$ imaging was performed as previously described (4, 5). Dispersed islet cells were immersed in Hepes-buffered solution (125 mM NaCl, 5.9 mM KCl, 2.56 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM Hepes, and 0.1% BSA, pH 7.4). Glucose was added to give a final concentration of 3 mM. Islets or dispersed islet cells were incubated in Fura-2 AM (2 μM; 1 h) and placed in a closed small-volume imaging chamber (Warner Instruments). Stimuli were applied with the bathing solution. Islets loaded with Fura-2 were excited alternatively at 340 and 380 nm with a monochromator light source (Cairn ReseachOptoscanMonochromator; Cairn Research Ltd). Images were acquired with a Hamamatsu camera (Hamamatsu) attached to a ZeissAxiovert 200 microscope (Carl Zeiss). Changes in the 340/380 fluorescence emission ratio over time were analyzed in individual islets and dispersed cells using Kinetic Imaging AQM Advance software (Kinetic Imaging). Peak changes in the fluorescence ratio constituted the response amplitude.

Beta cells were distinguished from other endocrine cells by their $[Ca^{2+}]_i$ responses to high glucose concentrations, and alpha cells were identified by their $[Ca^{2+}]_i$ responses to kainate (glutamate receptor agonist) (4, 5).

Insulin and Glucagon Secretion. Insulin and glucagon secretion were measured as previously described (4, 5). A high-capacity automated perifusion system was developed to dynamically measure hormone secretion from pancreatic islets. A low pulsatility peristaltic pump pushed Hepes-buffered solution (125 mM NaCl, 5.9 mM KCl, 2.56 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM Hepes, and 0.1% BSA, pH 7.4 at a perifusion rate of 100 μL/min) through a column containing 100 pancreatic islets immobilized in Bio-Gel P-4 Gel (BioRad). Except when otherwise stated, glucose concentration was adjusted to 3 mM for all experiments. Stimuli were applied with the perifusion buffer. The perifusate was collected in an automatic fraction collector designed for a 96-well plate format. The columns containing the islets and the perifusion solutions were kept at 37 °C, and the perifusate in the collecting plate was kept at <4 °C. Perifusates were collected every 1 min. Hormone release in the perifusate was determined with the human or mouse Endocrine LINCOplex Kit following manufacturer's instructions (Lincoresearch).

Human islet preparations varied considerably in their quality. Thus, the magnitudes of the responses to different stimuli were compared within the same recording or using recordings from the same preparation.

Immunohistochemistry. Sections (14 μm) were incubated overnight with anti-P2X receptor antibodies (Alomone Labs), antiinsulin antibodies (1:500; Accurate Chemical & Scientific), antiglucagon antibodies (1:4,000; Sigma), and/or antisomatostatin antibodies (1:1,000; Accurate Chemical & Scientific). As a negative control, purified peptide (50 μg) was preincubated with purinergic receptor primary antibodies (1 μg) for 1 h (room temperature). Pancreatic sections containing islets were examined using a Zeiss LSM 510 scanning confocal microscope (viewed at magnifications 20× and 40×).

In Situ Hybridization. In situ hybridization using digoxigenin (DIG)-labeled RNA probes for mRNA detection of human P2XRs was performed as described (6). A total of 30 ng of DIG-labeled probe was diluted in 150 μl of hybridization buffer, applied to the slides, and allowed to hybridize at 70 °C overnight. Slides were then washed for 1 h at 70 °C in 0.2 sodium, sodium citrate (SSC) solution (Ambion–Applied Biosystems) and incubated with alkaline phosphatase-conjugated sheep anti-DIG antibody (Roche) overnight at 4 °C. Alkaline phosphatase reaction was carried out in polyvinyl alcohol (PVA) with 200 μL of $MgCl_2$ 1 M and 140 μL of nitro-blue tetrazolium chloride/5-bromo-4-chloro-3′-indolyphosphate p-toluidine salt (NBT/BCIP) stock (Roche). Sense strand probes were used as a negative control for each P2XR. Immunofluorescence localization of antigens, double-labeled immunofluorescence, and confocal microscopy were carried out as described (6). Antibodies used were mouse antiinsulin (1/1,000; Sigma), guinea pig antiglucagon (1/50; Dako), Alexa Fluor 488-conjugated goat anti-mouse (1/400; Molecular Probes), and Alexa Fluor 568-conjugated goat anti-guinea pig (1/400; Molecular Probes). DAPI was used as nuclear counterstaining. Hybridization and immunofluorescence signals were merged by digitally converting the chromogen signal into a color signal in red, green, blue (RGB) scale. The hybridization signal was pseudocolored in red. This signal was then merged with the insulin signal (green). Both transformations were done using Photoshop.

Western Blotting. Immunoblot analysis was carried out by standard methods using the antibodies used for P2X immunohistochemistry (1:1,000). In control experiments, primary antibodies were incubated with corresponding control peptide (Alomone Labs) at a ratio of 50 μg antigenic peptide/1 μg antibody at room temperature for 5 h.

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2. Kenyon NS, et al. (1999) Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci USA* 96:8132–8137.
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5. Cabrera O, et al. (2008) Automated, high-throughput assays for evaluation of human pancreatic islet function. *Cell Transplant* 16:1039–1048.
6. Apelqvist A, Ahlgren U, Edlund H (1997) Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. *Curr Biol* 7:801–804.

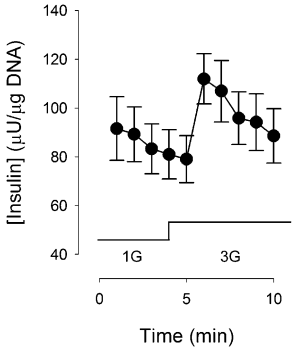

Fig. S1. Slight increases in insulin secretion occurring at low glucose concentrations. Insulin secretion was stimulated in human islets by raising the glucose concentration from 1 mM to 3 mM. Average traces of insulin secretion are shown (n = 8 perifusions).

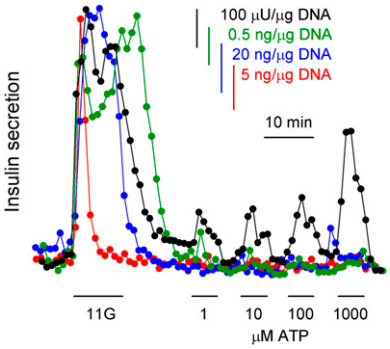

Fig. S2. Species differences in ATP-induced insulin secretion. Monkey islets (black symbols) responded to increasing concentrations of ATP like human islets. No responses to ATP were observed in mouse (red symbols), rat (blue symbols), or pig (green symbols) islets in the range of concentrations tested (1–1,000 μM). Shown are representative experiments ($n \geq 3$ islet preparations per species).

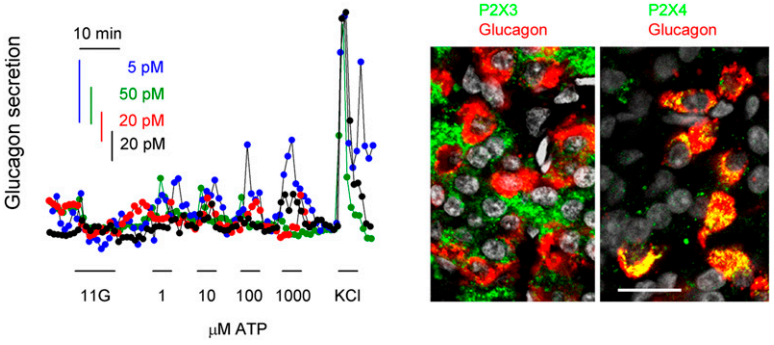

Fig. S3. ATP elicits small increases in glucagon secretion. (*Left*) Glucagon responses to ATP were small in monkey islets (blue symbols) and human islets (black symbols) and were difficult to discern in mouse (red symbols) or pig islets (green symbols). Shown are representative experiments from two or more islet preparations per species. (*Right*) $P2X_3$ immunoreactivity, shown on the left, was not present in alpha cells (glucagon immunostaining; red). Confocal images of human pancreatic sections showing immunoreactivity for $P2X_4$ (green) in alpha cells are on the right. Merge appears in yellow. (Scale bar, 20 μm.)