Supplementary materials

Chemicals and Reagents

Des-fluoro-sitagliptin (an analog of sitagliptin) was provided by Merck Company (1). Bezafibrate, niacin, carboxymethylcellulose (CMC), sodium palmitate, bovine serum albumin (BSA), mercaptoethanol, exendin-4, native GLP-1 and GIP were purchased from sigma. [D-Ala^2]GIP$_{(1\text{-}30)}$ (D-GIP) was synthesized by GenScript. Cyclic AMP EIA kit was from Cayman. Rat/mouse Insulin ELISA kit was from Lincon. Triglyceride kit, NEFA kit and cholesterol kit were obtained from Wako Company. RPMI1640 medium, DMEM medium, fetal bovine serum (FBS), sodium pyruvate and antibiotics were from HyClone. Rabbit phosphor-CREB (Ser133) antibody, rabbit CREB antibody and mouse β-actin antibody were from Cell Signaling. Guinea-pig anti-insulin was from DAKO. Mouse anti-BrdU antibody was from BD Biosciences. Mouse anti-glucagon, cy2-goat anti-guinea pig and cy3-donkey anti mouse were from Jackson. An 8 mM stock solution of sodium palmitate with 10.5% BSA (the molar ratio of palmitate to BSA was about 5:1) was prepared by dissolving sodium palmitate in RPMI1640 medium as published (2).

Islet isolation and culture, construction of Ad-GLP1R, RT-PCR, Western blotting, and measurement of cAMP Production and insulin secretion

Islet isolation and culture. Pancreatic islets were isolated from 8 to 9 week-old male C57BL/6J mice as previously described (3). Islets were cultured at 37°C in RPMI 1640 medium supplemented with 0.2 mmol/l glutamine, 10% (vol./vol.) heat-inactivated FBS, 100 U/ml penicillin and 100 g/ml streptomycin. After overnight culture, the islets were washed with PBS and incubated in the medium containing 0.4 mmol/l palmitate or BSA only for 48 hours.

Construction of Ad-GLP1R. In brief, full length mouse GLP-1R was amplified from mouse pancreas tissue cDNA by polymerase chain reaction. The PCR product was then cloned into pCDNA3.1$^+$ vector with BamH1/Xho1 restriction site. The used primers were forward (F):5- CTTggatccATGGCCAGCACCCCAAGCCT-3, reverse(R): 5-TAGctcgag GCCACCC AGCCAGAAGCAA-3. Then, mouse GLP-1R

was amplified from pCDNA-mGLP-1R and inserted into directional pENTR Topo vector to produce ENTR-GLP-1R shuttle plasmids. Followed by sequencing conformation, ENTR-GLP-1R was recombined into pAD/CMV/V5–DEST Gateway-based vector. At last, recombinant Ad-GLP-1R was linearized by PacI digestion and purified with ethanol precipitation, and 3 g linearized vectors were transfected into a 6-well 293A cells for viruses production. The viruses were amplified and condensed, and titer of viruses was determined before transducing into cells.

RNA Extraction and Quantitative RT-PCR. Total RNA was extracted using the TRIZOL (Invitrogen) according to the manufacturer's instructions. For real-time quantification, the first strand cDNA was prepared using the Reverse Transcription Kit (Invitrogen). Real-time PCR was performed using the SYBR-Green Kit (ABI) on an ABI Prism 7500 TH (Applied Biosystems). Data was normalized to the expression levels of β-actin in each sample.

Analysis of Phosphorylation of CREB. After being treated with BSA or palmitate for 24 hours, cells were starved in fresh medium without FBS for 1 hour, and then stimulated by PBS, GLP-1, or GIP for the indicated time. Total proteins were extracted with cold RIPA lysis buffer containing 10 mM Tri-HCl (pH7.6), 5 mM EDTA, 50 mM NaCL, 50 mM NaF, 1% Triton X-100, and protein inhibitors. Protein levels were assayed by BCA, and then 20-40 μg proteins were loaded for the following 10% SDS-PAGE and Western blot analysis.

Measurement of cAMP Production. For the cAMP production assay, 7.5×10^5 INS-1E cells or 5×10^5 min 6 cells were seeded in each well of a 12-well plate and cultured for 48 hours, then drugs treated or virus infected as indicated. Before the experiment, medium was refreshed to KRBH (129 mM NaCl, 4.8 mM KCl, 1.2 mM $KH2PO_4$, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 20 mM HEPES, and 24 mM $NaHCO_3$) with 0.2% BSA and 3 mM glucose for 1 hour. Then cells were stimulated by medium containing 500 μM IBMX in the presence of 100 nM GLP-1 or 100 nM GIP for 10 min. Medium was aspirated and cells were lysed in 300 μL of 0.1 M HCl for the following assay. Intracellular cAMP content was determined using Cyclic AMP EIA

KIT (Cayman), and protein level was assayed by BCA for the correction.

Measurement of Insulin Secretion. INS1-E cell line was sending in 12-well plates for 48 hours, and then palmitate or BSA treated for 24 hours. The plate was washed with PBS and preincubated in KRBH (129 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2mM $CaCl_2$, 20mMHEPES, and 24mM$NaHCO_3$) with 0.2% BSA and 3 mM glucose for 1 hour. Medium was then changed to KRBH with 16.7 mM glucose and additions as specified and incubated for 1 hour, the medium was collected for insulin assay according to the manufacturer's instructions. Protein level was assay by BCA for the correction of insulin secretion. Mouse islets were treated with palmitate or BSA for 48 hours, and then 50 islets were used to measure insulin secretion.

RT-QPCR analysis of mouse *Glp1r* expression. INS-1E cell was infected with Ad-GFP or Ad-GLP-1R for 48 hours, total RNA was extracted using the TRIZOL (Invitrogen) according to the manufacturer's instructions. cDNAs were synthesized using extracted RNA with the Reverse Transcription Kit (Invitrogen). PCR was performed using the SYBR-Green Kit (ABI) on an ABI Prism 7500 TH (Applied Biosystems) for 30 cycles. β-actin was used to as internal control.

Oral glucose tolerance test (OGTT), insulin tolerance test (ITT), and serum lipid profile measurement

For OGTT, mice were fasted overnight (~17 hours). Glucose levels from tail vein blood were determined immediately using a glucometer at 0, 30, 60, and 120 min after an oral administration of 0.3 g/kg glucose for *db/db* mice model. For ITT, 6 hours after fasting, mice were intraperitoneally injected with 2 IU/kg human insulin for db/db mice. Glucose levels were measured the same as OGTT at 0, 30, 60, and 120 min after injection. For insulin measured during OGTT, blood was obtained from tail vein by a heparinzed tube 30 min after the oral glucose administration and centrifuged at 7500 rpm at 4 ℃ for 10 minutes, and the supernatant plasma was used for insulin analysis by the Rat/mouse insulin ELISA kit (Millipore) according to the manufacturer's instructions.

Mice were sacrificed and serum was collected and stored at -80 ℃ before further analysis. Triglycerides (TG), free fatty acid (FFA) and total cholesterol (TC) concentrations were measured using related kits (Wako Labassay™, Japan) according to the manufacturer's instructions. High density lipoprotein (HDL) was determined by enzymatic assays using an Olympus automated analyzer.

1. Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, Feng Y, Zhu L, Li C, Howard AD, Moller DE, Thornberry NA, Zhang BB. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes 2006;55:1695-1704.
2. Mott DM, Stone K, Gessel MC, Bunt JC, Bogardus C. Palmitate action to inhibit glycogen synthase and stimulate protein phosphatase 2A increases with risk factors for type 2 diabetes. Am J Physiol Endocrinol Metab 2008;294:E444-450.
3. Chan JY, Cooney GJ, Biden TJ, Laybutt DR. Differential regulation of adaptive and apoptotic unfolded protein response signalling by cytokine-induced nitric oxide production in mouse pancreatic beta cells. Diabetologia 2011;54:1766-1776.