Electronic supplementary material

Animals

Three independent experiments with a total of 12 mice per group, respectively, were performed. All animals were housed in a temperature-controlled room with a 12 h light/dark cycle and were allowed free access to food and water according to the protocol approved by the UCLA Chancellor's Animal Research Committee in agreement with NIH animal care guidelines and §8 of the German animal protection law.

Average weekly food intake was stable throughout the study in both diet groups (ND and HFD) and was not changed by $P2X_7$ deficiency.

Functional analysis of $P2X_7$ in human islets

Freshly isolated human islets on dishes coated with extracellular matrix were loaded with 10 μmol/l YO-PRO-1 (0.375 kDa, Molecular Probes) in PBS. After 10 min, uptake of YO-PRO-1 by the islet cells was studied (time point 0) at 488 nm by time-lapse live imaging system under a fluorescence microscope (DMIRE2; Leica, Dearfield, IL, USA) and fluorescent nuclei in four visual fields with ×630 magnification were acquired using a CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan) and Volocity software (McBain Instruments, Chatsworth, CA, USA). After application of the treatment conditions, images were taken from each dish every 5 min over 1 h. Treatment conditions were: 5.5 mmol/l glucose (control), 11.1 or 33.3 mmol/l glucose or 0.5 mmol/l palmitate (dissolved and bound to NEFA-free albumin as described previously [1]) or 100 μmol/l benzoyl–benzoyl ATP (BzATP), a potent $P2X_7$ agonist (Sigma-Aldrich, St Louis, MO, USA). To inhibit $P2X_7$ activation, specimens were pre-treated for 30 min with 10 nmol/l KN-62 or 2 U/ml apyrase (Sigma).

IL-1Ra and IL-1β analysis

Serum obtained from retro-orbital bleedings and cell culture supernatant fractions from mouse isolated islets were evaluated using mouse IL-1Ra or IL-1β Quantikine ELISA (R&D Systems, Minneapolis, MN, USA). The minimum detectable doses were: hIL-1β: 1 pg/ml; mIL-1β: 3 pg/ml; hIL-1RA: 6.26 pg/ml; mIL-1Ra: 7 pg/ml. Serum obtained from cell culture supernatant fractions from human islets was measured by the luminex kit Fluorokine MAP Human IL-1Ra/IL-1F3 and Human IL-1β /IL-1F2 Kit and analysed by the Luminex xMAP technology. The minimum detectable doses were: hIL-1Ra: 2.06 pg/ml; hIL-1β: 0.27 pg/ml.

Beta cell mass and histochemical analyses

Ten sections (spanning the width of the pancreas) were double labelled for insulin and glucagon. An image of each slide was captured using Openlab and ImageJ software (Improvision, Lexington, MA, USA). The relative area of beta cells (green fluorescence) was determined by quantification of the cross-sectional beta cell area divided by the cross-sectional area of total tissue, respectively. The cell mass per pancreas was estimated as the product of the relative cross-sectional area of beta cells per total tissue and the weight of the pancreas. Beta cell apoptosis was analysed by the TUNEL technique according to the manufacturer's instructions (In Situ Cell Death Detection Kit, TMR red; Roche, Mannheim, Germany) and double-stained for insulin.

To obtain sections from isolated islets, islets were washed with PBS, fixed in Bouin's solution for 15 min and resuspended in 2% melted agarose in PBS, which was followed by short centrifugation and paraffin embedding. After blocking and antigen retrieval, slides were incubated with rabbit anti-$P2X_7$ (Almone Labs, Jerusalem, Israel) and guinea pig anti-insulin (Dako, Carpinteria, CA, USA). As secondary antibodies, donkey anti-rabbit Ig or anti-guinea pig Ig conjugated to FITC or cy3 were used (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and analysed as described before [2].

References

[1] Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY (2003) Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. *Diabetes* 52:726–733

[2] Sauter NS, Schulthess FT, Galasso R, Castellani LW, Maedler K (2008) The antiinflammatory cytokine interleukin-1 receptor antagonist protects from high-fat diet-induced hyperglycemia. *Endocrinology* 149:2208–2218