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Research Article

Specific and redundant roles of PKBα/AKT1 and PKBβ/AKT2 in human pancreatic islets

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ABSTRACT

Protein kinase Bα (PKBα)/AKT1 and PKBβ/AKT2 are required for normal peripheral insulin action but their role in pancreatic β cells remains enigmatic as indicated by the relatively mild islet phenotype of mice with deficiency for either one of these two isoforms. In this study we have analysed proliferation, apoptosis, β cell size and glucose-stimulated insulin secretion in human islets overexpressing either PKBα or PKBβ. Our results reveal redundant and specific functions. Overexpression of either isoform resulted in increased β cell size, but insulin production and secretion remained unchanged. Proliferation and apoptosis of β cells were only significantly stimulated and inhibited, respectively, by PKBα/AKT1. Importantly, overexpression of PKBα/AKT1 in dissociated islets increased the ratio of β cells to non-β cells. These results confirm our previous findings obtained with rodent islets and strongly indicate that PKBα/AKT1 can regulate β cell mass also in human islets.

1. Introduction

Mammals can adapt their capacity for insulin production to changes in demand. Type 2 diabetes (T2D) manifests as this ability is lost [1]. How much insulin can be produced depends on total mass of β cells located within the pancreatic islets of Langerhans and on how well they function (functional β cell mass) [2]. Pancreatic β cells compensate for increased demand via proliferation, cellular growth and reduced cell death [3]. However, the capacity for compensation is often limited by chronically elevated concentrations of D-glucose, high concentrations of saturated free fatty acids (FFA), and low-grade inflammation that can induce apoptosis in β cells. As a consequence, β cell mass progressively decreases in later stages of the disease [4].

The proper regulation of maintenance and expansion of functional islet mass depends on insulin receptor substrate (IRS) 2 [5]. IRS proteins are components of canonical insulin/IGF signalling and IRS1 and IRS2 are required for metabolic insulin action in muscle, liver, and adipose tissue, via activation of phosphoinositide 3-kinase (PI3K). It was shown that IRS2, but not IRS1, is required in β cells for proper regulation of survival, proliferation and function of islets [6,7]. Indeed, IRS2 deficient mice are unable to compensate for peripheral insulin resistance and develop severe diabetes. Furthermore, overexpression of IRS2 in islets can autonomously increase proliferation in β cells and reduces high-glucose-induced β cell apoptosis [8,9]. These observations indicated that IRS2 is necessary and sufficient for the maintenance and compensatory increase of pancreatic β cell mass.

Protein kinase B (PKB)/AKT is required for peripheral insulin action and also functions as a global regulator of differentiation, growth, proliferation, and survival of cells in mammals. PKB is evolutionary highly conserved and an important component of IRS/PI3K signalling [10,11]. Three isoforms of PKB exist in mammals: PKBα/AKT1, PKBβ/AKT2 and PKBγ/AKT3. The isoforms are encoded by separate genes, but share 80–85% sequence identity and the same structural organisation. They show distinct but overlapping expression patterns and differ in their function, despite their structural similarities [10]. Functions of PKB isoforms have been studied in mice deficient for individual isoforms [12–21]. Mice with whole body knockout (KO) for *pkbα* or *pkbβ* show complementary phenotypes: $pkb\beta^{-/-}$ mice are glucose intolerant and insulin resistant while $pkb\alpha^{-/-}$ mice show improved glucose tolerance and are more insulin sensitive. $Pkb\gamma^{-/-}$ mice show impaired brain development but normal metabolic control.

Abbreviations: (PKB), Protein kinase B; (T2D), type 2 diabetes; (FFA), free fatty acids; (IRS), insulin receptor substrate; (PI3K), phosphoinositide 3-kinase; (mTORC2), mTOR complex 2; (KO), knockout; (INS), rat insulinoma cells; (GOF), gain of function; (GSIS), glucose-stimulated insulin secretion; (ECM), extracellular matrix; (HA), hemagglutinin; (MOI), multiplicities of infection; (IL-1β), interleukin-1β; (BrdU), 5-Bromo-2′-deoxyuridine; (LOF), loss of function

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Although all three isoforms are expressed in human pancreatic β cells [12], it is not clear if the regulation of functional β cell mass is isoform-specific. Transgenic mice expressing constitutively active PKBα showed a two-fold increase in β cell proliferation [22] and overexpression of PKBα in rat insulinoma cells (INS1) protects from FFA-induced apoptosis [23]. Furthermore, insulin secretion was found to be impaired after downregulation of total PKB activity in pancreatic β cells, suggesting PKB is required for normal β cell function [24]. When overexpressed in isolated islets *in vitro*, only overexpression of PKBα but not PKBβ increased β cell proliferation. In contrast to gain of function (GOF) experiments, mice specific deficient for *pkb* in pancreatic β cells, show no or only mild islet phenotypes. Whole body deficiency for *pkbβ* even results in β cell compensation and hyperinsulinaemia. The activation/phosphorylation of PKB isoforms was studied in β cells overexpressing IRS2 [12]. Only PKBα but not β or γ was activated by IRS2 and IRS2-dependant enhancement of β cell proliferation was impaired in islets lacking *pkbα* but normal in the case of deficiency for *pkbβ* or γ indicating that PKBα is regulated and required downstream of IRS2 in islets.

The aim of this present study was to clarify if the regulation of pancreatic β cell mass is evolutionary conserved in islets between rodents and humans. To this end, we assessed in human islets if and how GOF for the metabolically relevant isoforms (PKBα and PKBβ) affects β cell function, proliferation, apoptosis, and β cell size, respectively.

2. Material and methods

2.1. Human islets

Human islets were obtained from the Juvenile Diabetes Research Fund (JDRF) and the European Consortium for Islet Transplantation's (ECIT) “Islets for Research Distribution Programme”.

The islets were cultured in CMRL medium containing 5 mM D-glucose (Invitrogen, Carlsbad, USA), 10% FCS (HyClone Laboratories Inc., Logan, USA), 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen, Carlsbad, USA).

Approximately 50 islets were plated on dishes coated with extracellular matrix (ECM) derived from bovine corneal cells (Novamed, Jerusalem, Israel) and allowed to attach and flatten out for three days prior to the start of experiments [25]. Each condition was carried out in triplicate. β cells cannot be unambiguously distinguished from non-β cells in intact human islets cultured on ECM-coated dishes. Therefore islets were dispersed by collagenase digestion as described by Linetsky et al. [26]. Approximately 40,000 cells were plated on ECM-coated dishes in triplicate and allowed to attach for 24 h.

2.2. Adenoviral transfection

Transfections and adenoviral constructs were described previously [12]. In brief, adenoviral constructs for expression of Hemagglutinin (HA)-tagged PKBα, PKBβ, or GFP were supplied by Vector BioLabs (Philadelphia, USA). Intact or dispersed islets on ECM plates were exposed for two days to viral particles at multiplicities of infection (MOI) of 500–1000 or 200–400, respectively. Afterwards islets or cells were washed twice with PBS to remove residual viral particels. For untransfected control, islets or cells were treated equally.

2.3. Western blotting

Cells were lysed in a buffer containing 50 mM HEPES, 140 mM NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 3 μg/mL aprotinin, 3 μg/mL 10 mM sodium fluoride, 1 mM disodium pyrophosphate, and 1 mM sodium orthovanadate. Protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, USA). SDS-PAGE and Western blotting was performed with the NuPAGE system from Invitrogen (Carlsbad, USA) according to the manufacturers instructions. Equal amounts of protein were loaded on NuPAGE Novex 4–12% gradient Bis–Tris Gels and afterwards transferred onto nitrocellulose membranes (GE Healthcare, Buckinghamshire, England). The membranes were incubated with 1° antibodies overnight at 4 °C and incubated with 2° antibody for 2 h at RT. Lumi-Light Western blotting substrate (Roche, Basel, Switzerland) was used for visualisation of signals in a Fuji LAS-3000 ChemImager (Fujifilm, Tokio, Japan). Bands were quantified using AIDA software (Raytest, Germany). Antibody against HA was derived from a hybridoma cell line (12CA5). An antibody against PKBβ was purchased from Cell Signalling Technology (Beverly, MA, cat no. 2964). PKBα was detected using an antibody from BD Bioscience (Franklin Lakes, USA cat no. 610860). Phosphorylated-PKB was detected using antibodies against p-Ser473 and p-Thr308 (both from Cell Signalling Technology, Beverly, USA, cat 9271 and 5106, respectively). An antibody against actin was purchased from Millipore Chemicon International (Billerica, USA). Secondary HRP-linked antibodies were used (anti-mouse from Santa Cruz Biotechnology, Santa Cruz, USA cat no. 2005, and anti-rabbit from Bio-Rad, Hercules, USA, cat no. 172-1019).

2.4. Glucose-stimulated insulin secretion and determination of total insulin content

Some 50 islets per dish in triplicate were incubated in Krebs–Ringer bicarbonate (KRB) buffer with 0.5% BSA and 2.8 mM D-glucose for 1 h at 37 °C than stimulated with 16.7 mM D-glucose for 1 h at 37 °C. To determine total insulin content insulin was extracted from islets by incubating overnight in acid–alcohol (0.18 M HCl in 70% ethanol). Insulin in supernatants and acid–alcohol extracts was measured by radioimmunoassay (RIA, CIS Bio international, Gif-Sur-Yvette, France) and normalised to protein content as measured by the Pierce Protein BCA assay kit (Thermo Scientific, Rockford, USA).

2.5. Analysis of apoptosis and proliferation

Apoptosis was assessed using the In Situ cell death detection kit from Roche (Basel, Switzerland). TUNEL-positive cells were detected with an antibody coupled to FITC followed by amplification of the signal by anti-FITC antibody linked to alkaline phosphatase (AP) [27]. Intact or dispersed islets were cultured for four days in the presence or absence of 2 ng/mL interleukin-1β (IL-1β) (ProSpec, Ness Ziona, Israel). Cells were fixed in 4% formalin for 30 min. Islets or β cells were visualised by co-staining for insulin (mouse anti-insulin; Sigma-Aldrich, Saint Louis, USA, cat. I2018, clone K36AC10).

To assess proliferation the BrdU Labelling and Detection Kit II (Roche, Basel, Switzerland) was used. BrdU-positive cells were detected by staining with alkaline phosphatase (AP). Intact or dispersed islets on ECM plates were cultured for 4 days in the presence of 5-Bromo-2′-deoxyuridine (BrdU) (10 μM) and were fixed in 4% formalin for 30 min at room temperature, co-staining for insulin (mouse anti-insulin; Sigma-Aldrich, Saint Louis, USA, cat. I2018, clone K36AC10) and DAPI allowed detection of islets or β cells.

Intact islets: the number of islets as well as mean islet- and β cell-size was determined and used to calculate the total number of islet cells per dish. Apoptotic/proliferating islet cells were counted, expressed in percent of total number of cells and normalised to GFP control. For assessment of β cell proliferation and apoptosis in dispersed islets at least 2000 cells were analysed.