Cellular Signalling 26 (2014) 268–278

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Activation of PPARβ/δ protects pancreatic β cells from palmitate-induced apoptosis by upregulating the expression of GLP-1 receptor☆

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ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form 27 October 2013
Accepted 11 November 2013
Available online 21 November 2013

Keywords:
PPARβ/δ
GLP-1R
Pancreatic β cells
Palmitate
Apoptosis

ABSTRACT

We previously showed that activated peroxisome proliferator-activated receptor (PPAR)β/δ can protect pancreatic β cells against lipotoxic apoptosis. However, the molecular mechanism remained unclear. Glucagon-like peptide-1 receptor (GLP-1R) has been reported to exhibit a protective effect against lipotoxic apoptosis in pancreatic β cells. In the present study, we aimed to investigate the underlying molecular mechanisms that PPARβ/δ activation suppressed apoptosis and improved β cell function impaired by fatty acids, focusing on contribution of GLP-1R. Isolated rat islets and rat insulin-secreting INS-1 cells were treated with the PPARβ/δ agonist GW501516 (GW) in the presence or absence of palmitate (PA) and transfected with siRNA for PPARβ/δ or treated with the PPARβ/δ antagonist GSK0660. Apoptosis was assessed by DNA fragmentation, Hoechst 33342 staining and flow cytometry. GLP-1R expression in INS-1 cells and islets was assayed by immunoblotting, quantitative PCR (qPCR) and immunofluorescence staining. SREBP-1c, Caveolin-1, Akt, Bcl-2, Bcl-xl and caspase-3 expression was measured using immunoblotting and qPCR. Our results showed that PPARβ/δ activation decreased apoptosis in β cells and robustly stimulated GLP-1R expression under lipotoxic conditions. GW enhanced glucose-stimulated insulin secretion (GSIS) impaired by PA through stimulation of GLP-1R expression in β cells. Moreover, SREBP-1c/Caveolin-1 signaling was involved in PPARβ/δ-regulated GLP-1R expression. Finally, GW exerted anti-apoptotic effects via interfering with GLP-1R-dependent Akt/Bcl-2 and Bcl-xl/caspase-3 signaling pathways. Our study suggested that the anti-apoptotic action of GW may involve its transcriptional regulation of GLP-1R, and PPARβ/δ activation may represent a new therapeutic method for protecting pancreatic β cells from lipotoxicity.

1. Introduction

Type 2 diabetes (T2D) is characterized by progressive failure of pancreatic β-cell function [1,2]. Evidence has indicated that lipids, especially saturated fatty acids, accumulate in β cells and induce β-cell dysfunction and exhaustion via apoptosis, which is often referred to as "lipotoxic apoptosis"; however, the molecular mechanisms underlying this phenomenon are still not clear [3,4].

Peroxisome proliferator-activated receptors (PPARs), including PPARα, PPARβ/δ, and PPARγ, are ligand-activated transcription factors that belong to the nuclear receptor superfamily. PPARs activate transcription of their target genes, which encode proteins involved in lipid metabolism and energy balance. It has been reported that PPARβ/δ can promote fatty acid oxidation, reduce triglyceride deposition, and reduce lipotoxicity in β cells [5,6]. Consequently, PPARβ/δ activation has recently been proposed as a potential treatment for insulin resistance and metabolic syndrome [7,8]. Our previous research showed that activated PPARβ/δ could protect β cells against lipotoxic apoptosis [9]. However, the protective mechanisms of PPARβ/δ on pancreatic β cells under lipotoxic conditions remain unclear.

Glucagon-like peptide-1 (GLP-1) is a major gastrointestinal hormone encoded by the proglucagon gene. GLP-1 activates a specific

Abbreviations: PPARβ/δ, peroxisome proliferator-activated receptor β/δ; GLP-1R, glucagon-like peptide-1 receptor; GW, GW501516; PA, palmitate; qPCR, quantitative polymerase chain reaction; SREBP-1c, sterol regulatory element binding protein-1c; GSIS, glucose-stimulated insulin secretion; T2D, type 2 diabetes; GPCR, G protein coupled receptor; KRBH, glucose-free Krebs–Ringer bicarbonate HEPES buffer; ELISA, enzyme-linked immunosorbent assay; FDA, fluorescein diacetate; PI, propidium iodide; Ex, exendin-4; FFA, free fatty acid; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution.

☆ No conflicts of interest were declared.

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http://dx.doi.org/10.1016/j.cellsig.2013.11.019

guanine nucleotide-binding protein (G-protein) coupled receptor (GPCR) expressed directly on pancreatic β cells, acts to promote β-cell proliferation and survival and inhibits lipotoxic apoptosis, thus leading to expansion of β-cell mass and improvement of glucose-stimulated insulin secretion (GSIS) [10–12].

Collectively, these findings suggest that a link between PPARβ/δ and GLP-1R may exist in pancreatic β cells in the presence of high palmitate (PA) levels.

In the present study, we hypothesized that PPARβ/δ activation exerted protective effects on pancreatic β cells under elevated PA conditions by modulating the GLP-1R expression. To verify this, we treated rat insulin-secreting (INS-1) cells and rat islets with PA to induce pancreatic β-cell damage at first. In addition, we aimed to investigate the relationship between PPARβ/δ and GLP-1R in the PPARβ/δ-dependent anti-apoptotic response.

2. Materials and methods

2.1. Cell culture and isolation of pancreatic islets

INS-1 cells (passages 7–20) were cultured as previously described [11,12]. Pancreatic islets were isolated from adult male Wistar rats by collagenase digestion, separated by Ficoll gradient as described previously [13,14]. All protocols were approved by the animal ethics committee of Sichuan University, China. PA (Sigma-Aldrich, St. Louis, MO) solutions were prepared by a method modified from that described by Bunn et al. [15]. The spent media were replaced by fresh media. Replacement media contained desired concentrations of PA and either 100 nM GW501516 (GW; ENZO Life Sciences, USA) or 1 μM GSK0660 (Sigma) for 24 h, whereas 0.5% BSA was used as a control.

2.2. DNA fragmentation analysis

Cells (5×10^6) from each culture condition were extracted using a DNA Isolation Kit (Beyotime, China) according to the manufacturer's instructions. DNA samples were separated on 1% agarose gel electrophoresis (60 V for 2 h) and visualized under ultraviolet light.

2.3. Flow cytometry analysis

Cell apoptosis was measured using an Annexin-FITC Apoptosis Detection Kit (Biosea, China) according to the manufacturer's protocols and then was analyzed immediately by FACSCalibur flow cytometry.

2.4. Insulin secretion assays

Insulin release from islets and INS-1 cells was measured as described previously [14,16]. Fifteen islets per sample were collected in individual tubes. INS-1 cells and islets were incubated in glucose-free Krebs–Ringer bicarbonate HEPES buffer (KRBH) containing 3 (low glucose: LG) or 20 mM glucose (high glucose: HG) for 1 h at 37 °C. Insulin levels were measured by enzyme-linked immunosorbent assay (ELISA; R&D), according to the manufacturer's instructions. Where indicated, insulin levels were normalized to total cellular protein in INS-1 cells.

2.5. Hoechst 33342 and FDA/PI staining

Cells were incubated with Hoechst 33342 (Beyotime) and the morphologies of nuclei were viewed under a fluorescence microscope. Islets were incubated with *fluorescein diacetate* (FDA; Merck Millipore)/*PI* solutions and the morphologies were then viewed under a confocal microscope.

2.6. Immunofluorescence staining

Islets and INS-1 cells were seeded on collagen-coated glass slides and subsequently fixed with 4% paraformaldehyde. Rabbit anti-GLP-1R (Abcam) or rabbit anti-sterol regulatory element-binding protein (SREBP)-1c (Santa Cruz, CA) antibodies and mouse anti-insulin antibodies (Abcam) were used. The cells were then visualized using a fluorescence microscope or confocal microscopy.

2.7. Immunocytochemical staining

INS-1 cells were fixed with 4% paraformaldehyde. Anti-GLP-1R antibodies were used. The cells were then observed under a light microscope. The positive results were visualized as claybank grains appearing in the cell membrane and cytoplasm.

2.8. Transient transfection with small-interfering RNA (siRNA)

siRNA for PPARβ/δ and SREBP-1c was purchased from Ribobio and GenePharma, China respectively, while $siRNA_{GLP\text{-}1R}$ was designed according to a previous report [17], as shown in Table 1. The final concentration of siRNA for PPARβ/δ and GLP-1R was 50 nM. $siRNA_{SREBP\text{-}1c}$ (120 nM) comprised a mixture of 3 siRNA duplexes targeting different regions of *SREBP-1c* mRNA. siRNA–Lipofectamine 2000 complexes were transfected into INS-1 cells and islets following the manufacturer's instructions. After 24 h, cells and islets were then treated with PA or BSA in the presence/absence of 100 nM GW for an additional 24 h.

2.9. Quantitative real-time PCR (qPCR) analysis

RNA isolation, cDNA synthesis, and real-time PCR analyses in INS-1 cells and islets were performed as described by Kato et al. [14]. Data were normalized to internal control β-actin mRNA. Primer sequences are as shown in Table 2.

2.10. Immunoblotting

Immunoblotting analysis was performed as described previously [7,11]. Cell extracts from INS-1 were probed with anti-GLP-1R, anti-SREBP-1c, anti-Akt (Cell Signaling Technology [CST], Danvers, MA), anti-phospho-Akt (CST), anti-Bcl-2 (CST), anti-Bcl-xl (CST), anti-Bax (CST), anti-cleaved caspase-3 (CST), and anti-β-actin (ZSGB-BIO, Beijing, China). Immunoreactive proteins were visualized by enhanced chemiluminescence (ECL; Beyotime). Levels of protein were normalized against β-actin and plotted as a histogram. Each bar represents the mean ± SD of three independent experiments.

2.11. Statistical analysis

All quantitative data were expressed as means ± SD of at least 3 independent experiments and were analyzed by one-way ANOVA or unpaired Student's t-tests, as appropriate. *P*-values of less than 0.05 were considered statistically significant.

Table 1
Oligonucleotide sequences of siRNAs.

siRNA	Sequences
Negative control	UUCUCCGAACGUGUCACGUTT; ACGUGACACGUUCGGAGAATT
siGLP-1R	AUAAUGAGCCAGUAGUUCAUGUUGG; CCAACAUGAACUACUGGCUC AUUAU
siPPARβ/δ	CCACAACGCUAUCCGCUUU dTdT; AAAGCGGAUAGCGUUGUGGTdTd
siSREBP-1c	
Primer1	CUGGAGACAUCGCAAACAATT; UUGUUUGCGAUGUCUCCAGTT
Primer2	GAGGCACAGAUGUGUCUAUTT; AUAGACACAUCUGUGCCUCTT
Primer3	GUGUGCAGGAGAUGCUAUATT; UAUAGCAUCUCCUGCACACTT