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Activation of SIRT1 protects pancreatic β-cells against palmitate-induced dysfunction☆

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ABSTRACT

Sirtuin 1 (SIRT1), a nicotinamide adenosine dinucleotide-dependent histone deacetylase, is an important regulator of energy homeostasis in response to nutrient availability. In pancreatic β-cells, SIRT1 has been shown to up-regulate insulin secretion in response to glucose stimulation. However, the potential roles of SIRT1 in islet β-cells against lipotoxicity remain poorly understood. Here, we demonstrated that SIRT1 mRNA and protein expressions were markedly reduced in the islets isolated from rats infused with 20% Intralipid for 24 h. Long-term exposure to 0.4 mmol/L palmitate also decreased SIRT1 expression in cultured INS-1 cells and isolated rat islets, which was prevented by 10 μmol/L resveratrol, a SIRT1 agonist. In addition, resveratrol improved glucose-stimulated insulin secretion decreased by palmitate, which was abrogated by EX527, a specific SIRT1 inhibitor. Furthermore, inhibition of SIRT1 activity by EX527 or a knockdown of SIRT1 suppressed insulin promoter activity, along with decreased insulin, v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), and NK6 homeodomain 1 (NKX6.1) mRNA expressions. Activation of SIRT1 by resveratrol or overexpression of SIRT1 antagonized palmitate-inhibited insulin transcriptional activity. SIRT1 overexpression exerted an additive effect on pancreatic and duodenal homeobox 1 (PDX1)-stimulated insulin promoter activity and abolished forkhead box O1 protein (FOXO1)-decreased insulin transcriptional activity. Resveratrol reversed FOXO1 nuclear translocation induced by palmitate. Our findings indicate that SIRT1 protects against palmitate-induced β-cell dysfunction.

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1. Introduction

Type 2 diabetes mellitus is characterized by insufficient insulin secretion in response to elevations of plasma glucose, and excessive circulating lipid levels have been suggested to contribute, in conjunction with chronic hyperglycemia, to the progressive deterioration of β cell function in this disease [1,2]. Acute exposure of the β cell to free fatty acid (FFA) results in an increase of insulin release, whereas chronic exposure leads to desensitization and suppression of secretion [3,4]. Evidence also indicates that elevated FFA inhibits insulin biosynthesis and decreases cell viability [5–7]. However, the mechanisms underlying the detrimental impact of FFAs on the β cell are still incompletely understood.

The NAD^+-dependent deacetylase Sir2 extends the life span of a lower eukaryote [8]. Its mammalian ortholog sirtuin 1 (SIRT1) plays pivotal roles in various physiological processes including cell cycle regulation, gene silencing, metabolism, and inflammation [9–11]. In skeletal muscle, SIRT1 is suggested to interact with and deacetylate peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) to enhance mitochondrial biogenesis [12]. It has been shown that SIRT1 regulates glucose and lipid homeostasis through deacetylating signal transducer and activator of transcription 3 (STAT3) and liver X receptor (LXR) in liver [13,14]. Moreover, glucose-stimulated insulin secretion (GSIS) in islets of SIRT1-knockout mice is blunted [15], whereas GSIS is enhanced in β-cell-specific SIRT1-overexpressing mice [16], suggesting that SIRT1 functions as a positive regulator of insulin secretion and maintenance of β cell function. Sun et al. [17] found that palmitate decreased SIRT1 protein expression in C2C12 myotubes and overexpression of SIRT1

Abbreviations: FFA, free fatty acid; PDX1, pancreatic and duodenal homeobox 1; GSIS, glucose-stimulated insulin secretion; JNK, Jun N-terminal kinase; SIRT1, sirtuin 1; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; NeuroD, neurogenic differentiation; NKX6.1, NK6 homeodomain 1; MafA, v-maf musculoaponeurotic fibrosarcoma oncogene homolog A; AMPK, AMP-activated protein kinase

☆ Disclosure summary: the authors have nothing to declare. There are no other conflicts of interest in regard to this study.

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improved palmitate-induced insulin resistance. But whether SIRT1 could protect against lipotoxicity in β cells remains elusive.

In the present study, we showed that fatty acids decreased SIRT1 expression in INS-1 insulinoma cells and isolated rat islets *in vivo* and *in vitro*. Resveratrol, a SIRT1 agonist, ameliorated palmitate-suppressed insulin secretion. Therefore, we further investigated the effect of SIRT1 on insulin transcriptional activity under lipotoxic conditions, demonstrating that SIRT1 is a positive regulator of insulin gene expression.

2. Materials and methods

2.1. Reagents

Palmitate, resveratrol, and fatty acid-free bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 medium, fetal bovine serum (FBS), and other culture reagents were obtained from Gibco Life Technologies (Grand Island, NY). EX527 was from Tocris Bioscience (Bristol, UK). Compound C was purchased from Calbiochem (San Diego, CA). Rat insulin RIA kit and anti-SIRT1 antibody were purchased from Millipore Technologies (Billerica, MA, USA). Anti-GAPDH, anti-acetyl-p65, anti-p65, anti-acetyl-p53, anti-p53, and anti-mouse or rabbit IgG conjugated with horseradish peroxidase were from Cell Signaling Technology (Beverly, MA, USA).

2.2. Cell culture

INS-1 cells (passage 22–35) were cultured in RPMI 1640 medium with 11.1 mmol/L glucose, 10% FBS, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, and β-mercaptoethanol (5 μl/L). At 24 h before the experiment (24 h after seeding), the medium was replaced with RPMI 1640 containing 5.6 mmol/L glucose or 16.7 mmol/L glucose supplemented with either BSA alone or BSA coupled to palmitate. The fatty acid coupling procedure was performed as described previously [18]. This procedure generated BSA-coupled palmitate in a molar ratio of 5:1 (generally, 0.4 mmol/L to 0.52% BSA, final).

2.3. Rat infusions

The animal protocol was reviewed and approved by the Animal Care Committee of Shanghai Jiaotong University School of Medicine. Male Sprague Dawley rats (Shanghai Laboratory Animal Company, Shanghai, China) weighing 250–300 g were housed under controlled temperature (21 °C) and a 12-h light–dark cycle with unrestricted access to water and standard laboratory diet. The animals were randomized into two groups, receiving 0.9% saline or 20% Intralipid with heparin (40 units/mL). The infusion technique was similar to that described by Hagman et al. [19]. Under general anesthesia, indwelling catheters were inserted into the right jugular vein. The catheters were tunneled subcutaneously and exteriorized at the base of the neck. The animals were recovered for 5 days after surgery. Catheter patency was maintained with 50 units/mL heparin in 0.9% saline. Intralipid and saline were infused at a constant rate of 1 mL/h. After 24 h infusion, the animals were killed for islet isolation.

2.4. Islet isolation and treatment

Islets of Langerhans were isolated by *in situ* pancreas collagenase infusion and separated by density gradient centrifugation [20] from male Sprague Dawley rats. The concentration of collagenase type XI was 0.5 mg/mL. Freshly isolated rat islets were transferred to 24-well plates (10 islets per well) or 6-well (150 islets per well) plates and cultured overnight in RPMI 1640 containing 5.6 mmol/L glucose, 10 mmol/L HEPES, 100 U/mL penicillin G sodium, and 100 μg/mL streptomycin sulfate supplemented with either BSA alone or BSA coupled to palmitate at 37 °C and 5% CO_2.

2.5. Insulin secretion

Islets were washed once in Krebs-Ringer bicarbonate (KRB) buffer [128.8 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, 2.5 mmol/L $CaCl_2$, 5 mmol/L $NaHCO_3$, and 10 mmol/L HEPES (pH 7.4)] with 0.1% BSA containing 3.3 mmol/L glucose, and then were preincubated for 30 min in 1 mL of the same medium at 37 °C. This buffer was then replaced with 1 mL of prewarmed KRB containing other additions as indicated for a further 60 min at 37 °C. An aliquot was then removed for analysis of insulin secretion by RIA. Islets were extracted with 0.18 N HCl in 70% ethanol for determination of insulin content.

2.6. Western blotting

INS-1 cells or isolated islets in 6-well plates were washed twice with ice-cold PBS and placed immediately in lysis buffer containing 25 mmol/L HEPES (pH 7.4), 1% Nonidet P-40, 100 mmol/L NaCl, 2% glycerol, 5 mmol/L NaF, 1 mmol/L EDTA, 1 mmol/L Na_3VO_4, 1 mmol/L NaPPi, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 5 μg/mL leupeptin, and 5 μg/mL pepstatin. Lysates were gently mixed for 10 min at 4 °C and then centrifuged at 13,000 *g* for 15 min at 4 °C. The protein concentration of the extracts was determined according to the method of Bradford, using BSA as the standard. Samples were separated by SDS-PAGE on 8% polyacrylamide gels and transferred to PVDF-Plus membranes (Bio-Rad, Hercules, CA). Primary antibodies were detected with donkey anti-rabbit at 1:2000 for 1 h at room temperature. Blotted membrane was developed with ECL Advance (Cell Signaling Technology, Boston, MA) and imaged with a LAS-4000 Super CCD Remote Control Science Imaging System (Fuji, JAP).

2.7. RNA isolation and real-time PCR

Total RNA was extracted from isolated islets or INS-1 cells using Trizol (Invitrogen) according to the manufacturer's instructions. In order to quantify the transcript abundance of genes of interest, real-time PCR was performed using a SYBR Green Premix Ex Taq (Takara, Shiga, Japan) with an Applied Biosystems 7300 Real-Time PCR machine (Applied Biosystems, Foster City, CA). The results of relative expression were normalized to β-actin mRNA levels in each sample. The specific primers were as follows: SIRT1, 5′-AGG GAACCTCTGCCTCATCTAC-3′ (forward), 5′-GGCATACTCGCCACCTAA CCT-3′ (reverse); Insulin 1 (Ins1), 5′-CCTGCTCGTCCTCTGGGAGCCC AAG-3′ (forward), 5′-CTCCAGTGCCAAGGTCTGAAGATCC-3′ (reverse); Insulin 2 (Ins2), 5′-CCTGCTCATCCTCTGGGAGCCCCGC-3′ (forward), 5′-CTCCAGTGCCAAGGTCTGAAGGTCA-3′ (reverse); neurogenic differentiation (NeuroD), 5′-GGCTCCAGGGTTATGAGATC-3′ (forward), 5′-GCATT CATGGCTTCAAGC-3′ (reverse); pancreatic and duodenal homeobox 1 (PDX1), 5′-CTTTCCCGAATGGAACCGAG-3′ (forward), 5′-GAATTCCTTCT CCAGCTCC-3′ (reverse); NK6 homeodomain 1 (NKX6.1), 5′-CTATTCTCT GGGGATGACGG-3′ (forward), 5′-TCTCGTCGTCAGAGTTCGGGTC-3′ (reverse); v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), 5′-AGCAAGGAGGAGGTCATC-3′ (forward), 5′-CGTATTTCTCCTT GTACAGG-3′ (reverse); and β-actin, 5′-AGGCCCCTCTGAACCCTAAG-3′ (forward), 5′-GGAGCGCGTAACCCTCATAG-3′ (reverse).

2.8. Plasmid transfection and siRNA interference

Transfection was conducted using Lipofectamine 2000 (Invitrogen) according to the manufacturer's manual. Briefly, INS-1 cells were plated in 24-well plates for 1 day before transfection. At 70–80% confluency, each well of cells was transfected with pECE-SIRT1, pcDNA3.1-PDX1, pcDNA3.1-forkhead box O1 protein (FOXO1) or a control empty vector. After 24 h of transfection, the medium was changed for a further 24 h at 37 °C. SIRT1-specific small interfering RNA (siRNA) and negative control siRNA were designed and synthesized by Shanghai GenePharma

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