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Research Paper

BDE-47 and BDE-85 stimulate insulin secretion in INS-1 832/13 pancreatic β-cells through the thyroid receptor and Akt

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ABSTRACT

PBDEs (polybrominated diphenyl ethers) are environmental pollutants that have been linked to the development of type 2 diabetes, however, the precise mechanisms are not clear. Particularly, their direct effect on insulin secretion is unknown. In this study, we show that two PBDE congeners, BDE-47 and BDE-85, potentiate glucose-stimulated insulin secretion (GSIS) in INS-1 832/13 cells. This effect of BDE-47 and BDE-85 on GSIS was dependent on thyroid receptor (TR). Both BDE-47 and BDE-85 (10 μM) activated Akt during an acute exposure. The activation of Akt by BDE-47 and BDE-85 plays a role in their potentiation of GSIS, as pharmacological inhibition of PI3K, an upstream activator of Akt, significantly lowers GSIS compared to compounds alone. This study shows that BDE-47 and BDE-85 directly act on pancreatic β-cells to stimulate GSIS, and that this effect is mediated by the thyroid receptor (TR) and Akt activation.

1. Introduction

Type 2 diabetes is a metabolic disorder characterized by chronic hyperglycemia, which develops as a consequence of peripheral insulin resistance and defective insulin secretion from pancreatic β-cells (Sargis, 2014). Diabetes prevalence has been on the rise and it can lead to major health complications, which increase the impacts of the disease in our society (Guariguata et al., 2014). A high calorie diet coupled with physical inactivity are known risk factors for the development of type 2 diabetes; however, these alone fail to account for the rapid rise of the disease (Sargis, 2014).

Recent attention has turned to the role of environmental pollutants in the development of metabolic diseases. Persistent organic pollutants (POPs), as their name suggests, are compounds that do not degrade easily and can bioaccumulate in the environment (Manzetti et al., 2014). They are man-made chemicals that are byproducts of various industrial processes (Manzetti et al., 2014). Polybrominated diphenyl ethers (PBDEs) are a class of POPs, and have been extensively used as flame retardants (Airaksinen et al., 2011; Darnerud et al., 2001). BDE-47 (2,2′,4,4′- tetrabromodiphenyl ether) and BDE-85 (2,2′,3,4,4′-pentabromodiphenyl ether) are two of the congeners in this class (Darnerud et al., 2001; Vagula et al., 2011). Data from epidemiological studies have suggested that PBDEs may be involved in the development of type 2 diabetes (Airaksinen et al., 2011; Lim et al., 2008; Zhang et al., 2016). PBDEs have been positively associated with diabetes and metabolic syndrome (Lim et al., 2008), although in this study BDE-47 did not reach statistical significance. Data from animal studies show that BDE-47 exposure increases fasting blood glucose in mice (Zhang et al., 2016), whether commercial mixture penta-BDE exposure (containing BDE-47 and BDE-85 among others) increased lipolysis and decreased glucose oxidation in rat adipocytes (Hoppe and Carey, 2007). These findings suggest that exposure to these compounds may lead to changes in glucose and lipid homeostasis and thus contribute to diabetes development.

Limited studies have been done to address the diabetogenic potential of BDE-47 and BDE-85. Although some PBDE studies have focused on BDE-47, as it is one of the most abundant PBDE congeners (Darnerud et al., 2001), there are no studies done on BDE-85. Particularly, the direct effect of these compounds on pancreatic β-cell function remains underassessed. β-cells secrete an appropriate amount of insulin in response to elevated blood glucose levels (such as after a meal), which helps re-establish normoglycemia by promoting glucose uptake and utilization by insulin-sensitive peripheral tissues (Kahn et al., 2014). Altering this normal β-cell function can disrupt glucose homeostasis; inadequate insulin secretion can cause severe hyperglycemia whether oversecretion can possibly lead to hyperinsulinemia, resulting in peripheral insulin resistance and β-cell defects. In order to minimize the impacts of the disease it is important to identify potential risk agents

Abbreviations: GSIS, glucose-stimulated insulin secretion; POP, Persistent organic pollutant; PBDE, poly-brominated diphenyl ether; BDE-47, 2,2′,4,4′- tetrabromodiphenyl ether; BDE-85, 2,2′,3,4,4′-pentabromodiphenyl ether; TR, thyroid receptor

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that can cause β-cell dysfunction. In the present study, we examined whether BDE-47 and BDE-85 exposure alters GSIS in insulin-producing INS-1 832/13 cells, and the potential underlying molecular mechanisms involved.

2. Materials and Methods

2.1. Chemicals

BDE-47 and BDE-85 were purchased from AccuStandard (New Haven, CT). Thyroid hormone T3 (3,3′,5-Triiodo-L-thyronine) was purchased from Alfa Aesar (Lancashire, United Kingdom). Thyroid hormone receptor antagonist 1–850 was purchased from EMD Millipore (Darmstadt, Germany). Wortmannin was purchased from Acros Organics (Geel, Belgium). Stock solutions of BDE-47, BDE-85, T3, 1–850, and wortmannin were prepared in dimethyl sulfoxide (DMSO) and were added to the culture medium and/or KRB buffer to achieve the indicated concentrations. Final concentration of DMSO did not exceed 0.1%. All other chemicals were purchased from Sigma (St Louis, MO) unless otherwise specified.

2.2. Cell culture

INS-1 832/13 cells were a kind gift by Dr. Christopher Newgard (Duke University School of Medicine) and were cultured in RMPI-1640 glucose-free medium supplemented with 11 mmol/l glucose, 10% fetal bovine serum, 1 mmol/l sodium pyruvate, 5 mmol/l HEPES, 2 g/l sodium bicarbonate, 2 mmol/l L-glutamine, 50 μmol/l 2-mercaptoethanol, 10000 U/ml penicillin, and 10 mg/ml streptomycin. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2 and used at passages 51–56.

2.3. Cell viability

Cell viability was measured by the reduction of CellTiter-Blue® (Promega, Madison, WI) according to the manufacturer's protocol. In brief, cells were plated in 96-well plates and treated with indicated concentrations of compounds for 48 h in culture medium, after which CellTiter-Blue® was added to wells and the increase in fluorescence (560 nm excitation, 590 nm emission) was measured using a SpectraMax M5 multi-mode microplate reader (Molecular Devices, Sunnyvale, CA). IC_{50} was calculated using a least squares fit with variable slope using GraphPad Prism (version 6.07).

2.4. Glucose-stimulated insulin secretion (GSIS)

INS-1 832/13 cells grown to confluency in 24-well plates, were washed 3 times with and pre-incubated in Krebs Ringer Buffer (KRB, 120 mM NaCl, 25 mM HEPES, 4.6 mM KCl, 1 mM $MgSO_4$, 0.15 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5 mM $NaHCO_3$, 2 mM $CaCl_2$) buffer containing 3 mmol/l glucose at 37 °C for 2 h; followed by a static 1 h incubation at 37 °C in KRB containing 3 (basal) or 16 (stimulating) mmol/l glucose. For acute GSIS, compounds were present only during the 1hr static incubation phase. KRB buffer was collected and centrifuged at 5000 x g for 3 min at 4 °C to pellet out any cells. Insulin released in buffer was measured by an ELISA kit (Alpco Diagnostics, Salem, NH) and data were normalized to the protein content, measured by the Micro-BCA Protein Assay kit (Pierce, Rockford, IL). For antagonist experiments, after a 2 h preincubation with 3 mmol/l glucose KRB, cells were preincubated with antagonists or vehicle control (DMSO) at indicated concentrations for 30 min in 3 mmol/l glucose KRB, washed once with 3 mmol/l glucose KRB, followed by static 1 h incubation at 37 °C in KRB buffer containing 16 mmol/l glucose. For chronic pre-treatment, cells were exposed to indicated concentrations of BDE-47 and BDE-85 for 48 h, after which cells were washed and preincubated in KRB buffer containing 3 mmol/l glucose and static incubation was performed as described above (compounds not present during the 2 h pre-incubation or static 1 h glucose stimulation). For all insulin secretion experiments, controls cells were treated with vehicle (DMSO) at 0.1% concentration.

2.5. Western blot analysis

INS-1 832/13 cells were grown to confluence in 6-well plates, washed two times in serum-free growth media, and incubated for 30 min at 37 °C in serum-free growth media containing BDE-47, BDE-85, or T3. For inhibitor experiments, cells were preincubated with inhibitor or vehicle control (0.1% DMSO) at indicated concentrations for 30 min in serum-free growth media, washed once, and incubated for 30 min at 37 °C in serum-free media containing BDE-47 or BDE-85. After exposure, cells were solubilized in RIPA lysis buffer (Pierce, Rockford, IL). Protein content was determined using a BCA Protein Assay Kit (Pierce, Rockford, IL) and SDS samples were prepared. Equal amounts of protein were electrophoretically separated on SDS-polyacrylamide gel, followed by blotting onto PVDF membrane. Following the transfer, membranes were blocked with TBST (10 mmol/l Tris-HCl pH 7.4, 150 mmol/l NaCl, and 0.1% Tween 20) containing 5% nonfat dry milk (blocking buffer) and incubated with the primary antibodies diluted in blocking buffer overnight at 4 °C, followed by application of appropriate secondary antibodies for 1 h at room temperature. Proteins were detected by using enhanced chemiluminescence (ECL).

2.6. Reverse transcription and quantitative real-time RT-PCR (qRT-PCR)

INS-1 832/13 cells were grown to confluence in 6-well plates and total RNA was prepared using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA) and single-strand cDNA was synthesized from the RNA using a Maxime RT PreMix kit (iNtRON Biotechnology, Seongnam, South Korea). qRT-PCR amplifications were performed using rEVAlution 2x qPCR Master Mix (Empirical Bioscience, Grand Rapids, MI) in an MyIQ2 Real-Time PCR Detection System (Bio-Rad, Richmond, CA) following manufacturer's protocol. To determine the specificity of amplification, melting curve analysis was applied to all final PCR products. The relative amount of target mRNA was calculated by the comparative threshold cycle method by normalizing target mRNA threshold cycle to those for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers were purchased from Integrated DNA Technologies (Coralville, IA) and were as follows: rat TRα (NM_031134) forward 5′-CCTGGATGATACGGAAGTG-3′, reverse 5′-AGTGCGGAATGTTGTGTT-3′; rat TRβ (NM_012672) forward 5′-ATCATCACACCAGCAATCA-3′, reverse 5′-GTCCGTCACCTTCATCAG-3′; rat GAPDH (NM_017008) forward 5′-GACATGCCGCCTGGAGAAAC-3′, reverse 5′-AGCCCAGGATGCCCTTTAGT-3′

2.7. Statistical analysis

Data are expressed as means ± SEM and are results from at least three independent experiments performed in quadruplicate measurements. Significance was determined for multiple comparisons using two-way analysis of variance (ANOVA) followed by Sidak post-hoc analysis (Abdi, 2007). A p-value of ≤ 0.05 was considered significant. All analyses were conducted using the GraphPad Prism (version 6.07) statistical program software.

3. Results

To evaluate the role of chronic BDE-47 and BDE-85 exposure on INS-1 832/13 cell function, cells were exposed to different concentrations of compounds for 48 h, and GSIS was measured after the removal of these compounds. Chronic pre-treatment with 10 μM BDE-47 or BDE-85 didn't affect insulin secretion (Fig. 1A and B). The concentrations used for chronic pre-treatment GSIS did not affect cell viability during