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Suppression of FAT/CD36 mRNA by human growth hormone in pancreatic β-cells

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ABSTRACT

Fatty acid-induced damage in pancreatic β-cells is assumed to play an important role in the development of type 2 diabetes. Lactogens (prolactin, placental lactogen and growth hormone) improve β-cell survival via STAT5 activation but the molecular targets are incompletely characterized. The aim of this study was to examine the effect of human growth hormone (hGH) on mRNAs of fatty acid transport and binding proteins expressed in pancreatic β-cells, and to examine this in relation to β-cell survival after exposure to fatty acids. hGH decreased mRNA levels of FAT/CD36, whereas mRNAs of GPR40, FASN, FABP2, FATP1 and FATP4 were unchanged. RNAi against FAT/CD36 decreased fatty acid-induced apoptosis. Overexpression of constitutively active STAT5 was able to mimic hGH's suppression of FAT/CD36 expression, whereas dominant negative STAT5 was unable to block the effect of hGH indicating that STAT5 did not bind directly to the FAT/CD36 promoter. The hGH-mediated suppression of FAT/CD36 mRNA was associated with a decrease in palmitate uptake and fatty acid-induced basal hyper-secretion of insulin resulting in improved glucose-stimulated insulin secretion. This study suggests that hGH can protect β-cells against fatty acid-induced damages.

1. Introduction

The obesity epidemic of the westernized world causes dyslipidemia and increased circulating fatty acids (FAs). Fatty acid uptake and metabolism in non-adipose tissues may play important roles in the development of type 2 diabetes. Furthermore, chronic exposure of pancreatic β-cells to elevated glucose and FA concentrations lead to increased rates of apoptosis and a blunted glucose-stimulated insulin secretion with hypersecretion of insulin at basal glucose concentrations [1–3]. This increased apoptosis is an important intermediate step in the β-cell decompensation phase when obesity-associated type 2 diabetes is developing, because persistent hyperlipidemia together with post-prandial hyperglycemia may prone β-cells to apoptosis and impaired function [3–5]. Since β-cell dysfunction is a prerequisite for development of type 2 diabetes, it is important to identify molecular mechanisms and pharmacological agents to prevent or correct β-cell impairment.

The hormones of the growth hormone (GH) family; GH, placental lactogen (PL) and prolactin (PRL) are potent stimulators of proliferation and insulin production in pancreatic β-cells [6]. In rodent β-cells human GH (hGH) activates both the PRL receptor and the GH receptor, resulting in JAK2 activation, dimerization and nuclear translocation of STAT 5A and 5B. The trophic effects of hGH on rodent β-cells are well described and is a combination of GH and PRL sensitive effects, and include increased proliferation and insulin biosynthesis [7–10]. Anti-apoptotic effects of GH have been described in various cell types incl. β-cells and is mediated by STAT5 [11–13]. Other signaling pathways activated by GH include activation of PI3 kinase, the MAP-kinase cascade and suppression of FoxO1 [14–16].

The effects of hGH on human skeletal muscle and adipose tissue FA metabolism are well documented: hGH increases β-oxidation in skeletal muscle and has acute anti-lipolytic effects in adipose tissue, whereas the long-term effect of hGH is to increase lipolysis and inhibit lipogenesis [17,18] concordant with the dual role of hGH as an anabolic and catabolic hormone. However, the effects of hGH on β-cell FA uptake and signalling have not been investigated.

The aim of the current study was to examine the effects of hGH on mRNAs encoding proteins involved in FA uptake, transport or signalling in pancreatic β-cells, and to characterize the impact of this regulation in the β-cell.

Abbreviations: CA, constitutively active; DN, dominant negative; FA, fatty acid; GSIS, glucose-stimulated insulin secretion; FATP, fatty acid transport protein; FABP, fatty acid binding protein; FASN, fatty acid synthase; FAT, fatty acid translocase; GFP, green fluorescent protein; hGH, human growth hormone; STAT, signalling transducer and activator of transcription; O/P, oleate/palmitate; PRL, prolactin; PRLR, prolactin receptor.

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Our results indicate that FA-induced apoptosis is mediated via FAT/CD36 and may be counteracted either by RNAi against FAT/CD36 or via STAT5-mediated suppression of FAT/CD36 by hGH.

2. Materials and methods

2.1. Materials

The insulin-producing cell line INS-1E was provided by Dr. Claes Wollheim, University of Geneva, Switzerland [19]. Recombinant hGH was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark) and used at a concentration of 500 ng/mL unless otherwise noted. Recombinant adenovirus encoding GFP, dominant negative (DN)-STAT5 and constitutively active (CA)-STAT5B have been described previously [7,20,21]. Chemicals were from Sigma–Aldrich (Broendby, Denmark) and media from Invitrogen (Taastrup, Denmark) unless otherwise stated. Radiochemicals were from GE Healthcare (Hilleroed, Denmark).

2.2. Cell culture

Cells were cultured at 37 °C in a 5% CO_2 incubator in RPMI 1640 supplemented with glutamax, 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 50 μmol/L 2-mercaptoethanol. All experiments were performed in media with 0.5% serum. Rat neonatal islets were isolated and cultured as described [13].

2.3. Preparation of FA-conjugated BSA

Palmitic acid was dissolved in heated 100 mmol/L NaOH to a concentration of 10 mmol/L. Dissolved palmitate was added to RPMI 1640 containing fatty-acid free BSA (Calbiochem, Roedovre, Denmark) (molar ratio of palmitate to BSA: 6:1) and pH adjusted to 7.2. Oleic acid (12 mmol/L) was conjugated to 12.5% fatty-acid free BSA, sterile-filtered and stored under liquid nitrogen. In some experiments palmitate and oleate were used together at a molar ratio of 1:2 as described [22]. A palmitate:albumin ratio of 6:1 was used, since albumin has up to 7 binding sites for FAs [23].

2.4. Isolation of RNA, reverse transcription and quantitative real-time PCR

Total RNA from fatty-acid-treated cells was isolated using Trireagent (Sigma–Aldrich) and reverse transcribed using Superscript III (Invitrogen). Subsequently, mRNA levels of FATP1 and -4, FABP2, PRLR, FASN and FAT/CD36 were determined using real-time PCR with SYBR-green detection. All biological replicates were assayed in duplicate. Transcription Factor IIB (TFIIB) was used as a reference gene [24]. Primer sequences are listed in the Supplementary Table 1.

2.5. Transfection of INS-1E cells using siRNA

INS-1E cells grown in 12-well plates were transfected with either a pool of specific siRNAs targeting FAT/CD36 (ON-TARGET-plus rat CD36 SMARTPool) or a negative control siRNA (both from Dharmacon, ThermoScientific, Lafayette, Co., USA) using the transfection agent Dharmacon 1 following manufacturers' instructions. FACS-analysis of transfected fluorophore-labelled siRNA (SiGLO Lamin A/C control, Dharmacon) showed that up to 70% of cells had taken up the siRNA. The overall knock-down efficiency of FAT/CD36 mRNA was determined by QPCR to be 52 ± 17% of FAT/CD36 mRNA levels in control cells transfected with the negative control siRNA.

2.6. Apoptosis assays

AnnexinV binding was quantitated using FACS analysis (MBL 4700, Nordic Biosite, Täby, Sweden). For TUNEL assay cells were seeded in 3 cm^2 culture plates, cultured 24 h with FAs, BSA, hGH or combination thereof before they were harvested, applied to poly-lysine-treated slides and fixed in 10% formalin and stained using the Dead-End Colorimetric TUNEL kit (Promega, Mannheim, Germany).

2.7. Palmitate oxidation and cellular palmitate uptake

Cells were trypsinized, washed in 1 × PBS and aliquoted into 3.5×10^6 cells per replicate. Palmitate oxidation was measured as previously described [25]. Subsequently, cells were extracted using a Folch extraction procedure [26] to establish the amount of palmitic acid taken up by cells, and quantified by scintillation counting. The total FA uptake was calculated by adding counts from oxidized and extracted FAs.

2.8. Glucose-stimulated insulin secretion (GSIS)

Cells were seeded in 12-well plates and treated with FAs as described. Prior to the GSIS assay cells were incubated for 3 h in RPMI 1640 containing 3 mmol/L glucose for basal secretion. Cells were then washed in 1 × PBS and incubated in Krebs–Ringer Bicarbonate buffer with HEPES (KRBH) (123.5 mmol/L NaCl, 4.75 mmol/L KCl, 1.19 mmol/L $MgSO_4$, 1.19 mmol/L KH_2PO_4, 5 mmol/L $NaHCO_3$, 10 mmol/L HEPES, 2.54 mmol/L $CaCl_2$, 0.2% FA-free BSA, and glucose as indicated) for 2 h. The KRBH was then aspirated, centrifuged and insulin content of the supernatant was determined using RIA with human insulin as standard [27]. Total insulin was extracted from the cells using acid–ethanol as described previously [27].

2.9. Statistical analysis

Results are expressed as mean value ± SE. Multiple groups were compared using one-way ANOVA, and post hoc comparison of individual groups with Tukey's post-test using Graphpad Prism software. Comparisons between two groups were made with *t*-test. Differences between treatments were considered significant at a P-value < 0.05 (two-tailed).

3. Results and discussion

3.1. Database searches for GH-regulated FA binding and transport proteins

Gene-expression profiles in the NCBI database (http://www.ncbi.nlm.nih.gov/gds) [28] was searched to identify transcripts involved in FA transport, binding or signalling regulated by lactogens. We identified two publicly available datasets: GDS1489 assaying 3T3-F442 adipocytes treated with GH [29] and GDS2019 assaying livers from Snell dwarf mice [30], which lack pituitary somatotropic cells and secrete no GH or PRL [31]. In Snell dwarf mice, FA translocase (FAT/CD36) mRNA levels were increased about 4-fold in older mice (22 months) compared with age-matched control. FA binding protein (FABP)-2 and FA synthase (FASN) mRNA were decreased and FA transport protein (FATP)-1 and FATP4 mRNA were unchanged (data from Boylston et al. (2004) [30] are shown in Fig. S1 A–E). FAT/CD36 and FASN mRNA were suppressed 50% by GH in adipocytes (dataset GDS1489 of Huo et al. (2006) [29] presented in Fig. S1 F–J). Suppression of FASN by GH is due to binding of STAT5 to an atypical STAT responsive element in the FASN promoter [32].