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Short-term glucocorticoid treatment increases insulin secretion in islets derived from lean mice through multiple pathways and mechanisms

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ABSTRACT

Chronic exposure to elevated levels of glucocorticoids leads to metabolic dysfunctions with hyperglycemia and insulin resistance. Long-term treatment with glucocorticoids induces severe impairment of glucose-stimulated insulin secretion. We analyzed the effects of short-, and medium-term (2–120 h) treatment with 50–200 nM glucocorticoids on primary pancreatic islet cultures derived from lean C57BL/6J mice. In contrast to animal models of insulin resistance, β-cells from lean mice respond with an increased glucose-stimulated insulin secretion, with a peak effect around 18–24 h of treatment. Analyses of the insulin secretion response reveal that early and late phase responses are dissociated upon glucocorticoid treatment. Whereas late phase responses return to basal levels after long treatment, early phase responses remain increased over several days. Increased insulin secretion is also obtained by incubation with the inactive glucocorticoid dehydrocorticosterone, pointing to an important role of the enzyme 11β-hydroxysteroid dehydrogenase type 1 in mediating glucocorticoid effects in β-cells.

Transcript profiling revealed differential regulation of genes involved in mediation of signal transduction, insulin secretion, stress and inflammatory responses. The results show that short- to medium-term glucocorticoid treatment of pancreatic islets derived from lean mice leads to an increased insulin release and may constitute an important parameter in changing towards a pro-diabetic phenotype.

1. Introduction

The β-cells of the pancreatic islets of Langerhans are an essential component in vertebrate physiology by their unique ability to synthesize and release insulin, the vital hormone regulating maintenance of glucose homeostasis. Insulin synthesis, intracellular storage and secretion are tightly controlled through multiple mechanisms including complex sets of stimuli such as nutrients, neural and humoral factors (Lang, 1999). Glucose-stimulated insulin secretion is mediated through glucose metabolism within the β-cell, providing ATP which in turn triggers regulation of ATP-dependent potassium channels, membrane depolarization and opening of voltage-dependent Ca^{2+} channels (reviewed in (Ashcroft et al., 1994; Berggren and Larsson, 1994). This cascade leads to release of insulin from intracellular storage vesicles, produced in the secretory pathway. Whereas this stimulus-secretion coupling takes seconds or minutes, longer term adaptation of the β-cell to nutrient states includes transcriptional responses (Itoh and Okamoto, 1980; Leibiger et al., 1998). Derangement of any of these processes is linked to reduced insulin production and secretion, and constitutes an important factor in the development of diabetes mellitus (Itoh and Okamoto, 1980; Lang, 1999; Leibiger et al., 1998).

Glucocorticoid hormones regulate essential physiological functions in mammals by binding to nuclear receptors controlling gene transcription. An important determinant in glucocorticoid physiology is a system of enzymes involved in pre-receptor control of

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; GR, glucocorticoid receptor; GPCR, G-protein coupled receptor; GC, glucocorticoid; GSIS, glucose-stimulated insulin secretion.

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active hormone ligand (Tomlinson and Stewart, 2007; Walker and Seckl, 2003). The tissue-specific metabolic activation of glucocorticoid precursors (cortisone in humans, dehydrocorticosterone in rodents) to the active ligand (cortisol, corticosterone) is carried out by type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1), which acts as an NADPH-dependent reductase (Oppermann et al., 1997; Oppermann, 2006). The reverse reaction is carried out by a NAD^+-dependent type 2 11β-HSD (11β-HSD2), predominantly localized in mineralocorticoid receptor (MR) positive cells. Hyperglycemia, insulin resistance and hyperinsulinemia result from excess of glucocorticoids (Lenzen and Bailey, 1984). These conditions are believed to be the result of interactions between the different organs involved in glucose homeostasis, i.e., liver, skeletal muscle, adipose tissue and the islets of Langerhans.

Direct effects of glucocorticoids on β-cells have been reported and show different results. Whereas increased insulin release was found in long-term islet culture (3 weeks) with hydrocortisone or dexamethasone (Brunstedt and Nielsen, 1981; Karlsson et al., 2001; Kawai and Kuzuya, 1974, 1977) glucocorticoid mediated suppression of insulin secretion is reported from islets derived from normal animals (Koizumi and Yada, 2008; Lambillotte et al., 1997) or animal models of type 2 diabetes mellitus, e.g., *ob/ob* mice (Delaunay et al., 1997; Khan et al., 1992). We have described a decrease in insulin secretion in islets from *ob/ob* mice by glucocorticoid activation through 11β-HSD1, and reversal of this effect by use of 11β-HSD1 inhibitors (Davani et al., 2000; Ortsater et al., 2005). Importantly, animals overexpressing the glucocorticoid receptor in β-cells ultimately develop manifest diabetes at 12–15 months of age (Davani et al., 2004). Taken together, these data point to a complex response of β-cells to glucocorticoids with regard to insulin secretion. The present study addresses the effects of glucocorticoids during short-term treatment of islets derived from lean mice. We describe how corticosterone treatment under these conditions results in increased insulin secretion, and we analyze by microarray analysis the underlying transcriptional changes in pancreatic islets.

2. Experimental

2.1. Chemicals

Analytical grade reagents and MilliQ water were used. Collagenase, HEPES and bovine serum albumin (fraction V) were from Boehringer Mannheim GmbH (Mannheim, Germany), RPMI 1640 culture medium and fetal bovine serum were from Invitrogen (Carlsbad, CA). Mifepristone (RU486) and steroids were from Sigma (St. Louis, MO), radioactive corticosterone (specific activity 70 Ci/mmol) was from NEN-Dupont, labeled dehydrocorticosterone was synthesized using recombinant 11β-HSD1, extracted, and purified by thin-layer chromatography. The rat insulin standard was from Novo Nordisk (Bagsvaerd, Denmark). Guinea pig anti-mouse insulin antibodies were produced as described Bergsten and Hellman (1993). IgG-certified 96-well microtiter plates were purchased from Nunc (Roskilde, Denmark).

2.2. Islet preparation and culture

Male 3–5 months old C57BL/6J mice (B&K, Sollentuna, Sweden) were used as islet source. Pancreatic islets were isolated by collagenase digestion, and cultured for 2–120 h in RPMI 1640 culture medium supplemented with 5.5 mM glucose, 10% fetal bovine serum and 0 or 200 nM corticosterone or 50 nM dehydrocorticosterone. For culture times longer than 48 h, culture medium was changed every 48 h. A group of corticosterone-treated islets (18 h treatment) were treated with 1 μM RU486. Corticosterone was dissolved in 95% ethanol and diluted in culture medium giving a final ethanol concentration of 0.1% (v/v), whereas RU486 was dissolved in DMSO and diluted in culture medium giving a final DMSO concentration of 0.1% (v/v). The same concentrations of ethanol and DMSO were used in the control experiments. The procedures involving animals were approved by the local animal ethical committee.

2.3. Measurement of insulin release

After cultivation, individual islets were perifused and insulin secretion was determined by ELISA as previously described (Bergsten and Hellman, 1993; Ortsater et al., 2005). In short, one individual islet per experiment was placed in a 10-μL chamber, made of Teflon-tubing and perifused at a rate of 150–160 μL per min. The perifusion medium was supplemented with 1 mg/mL albumin and contained (in mM): NaCl 125, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 1.28, HEPES 25 and glucose 3, titrated to pH 7.4 with NaOH and thermostated at 37 °C. After a 60-min equilibration period at 3 mM glucose, samples were collected every 5 min for 15 min, before elevating the glucose concentration to 11 mM. To ensure good time resolution of the initial secretory response, samples were taken every 2 min during the first 10 min at 11 mM glucose. Subsequently, samples were collected every 5 min for an additional 20 min. At the end of the experiments, islet functionality was verified by lowering the glucose concentration of the perifusion medium to 3 mM to test the reversibility of GSIS. Second, the insulin secretory capacity was confirmed by depolarization with high potassium ion concentration (30 mM). Experiments where GSIS was not reversible or potassium depolarization failed to induce insulin secretion were excluded from the calculations. The perifusate samples were stored at −20 °C until assayed for insulin. Insulin in the perifusate was assayed by a competitive enzyme-linked immunosorbant assay (ELISA) with the anti-insulin antibodies immobilized directly to the solid phase. The rate of insulin release was normalized to islet dry weight after freeze-drying and weighing the islets on a quartz fiber balance. Differences in insulin secretion were evaluated within each experiment group with an ANOVA for repeated measurements and between groups with factorial ANOVA and Fischer's post hoc test. *p*-values lower than 0.05 were considered significant. Values were expressed as means ± standard error of the mean (SEM).

2.4. Analysis of glucocorticoid-induced transcriptional changes in islets

Pancreatic islets obtained from C57BL/6J mice were isolated as described above in 2 biological replicates, each replicate consisted of islets pooled from 6 to 7 mice. Each biological replicate was divided in two halves, and either treated with 200 nM corticosterone or the appropriate volume of solvent overnight at 37 °C. Aliquots of the samples were analyzed for insulin secretion, as detailed above. Total RNA was extracted from the islets using QIAshredder Homogenizer (Qiagen) in combination with RNeasy Mini Kit (QIAgen) and on column DNAse I treatment with the RNase-Free DNAse Set (QIAgen) according to the manufacturer's instructions. An amount of 25 μg total RNA was obtained for each sample. 1 μg of each of the four samples were loaded onto 1.2% agarose gels stained with ethidium bromide, to investigate the quality of the isolated total RNA. The four pooled RNA samples described above were hybridized on microarray chips as detailed below. The experimental microarray design setup was to hybridize untreated and corresponding corticosterone-treated samples in 2 dye-swap pairs onto the slides. There were 5 slides in total, including an additional technical replicate.

Microarrays were produced using a non-redundant mouse cDNA clone library purchased from Lion Biosciences, containing nearly 21,000 sequence-verified and size-matched cDNA fragments, mainly from the 3′ untranslated region. cDNA inserts were PCR-amplified with universal amino-linked M13 primers, purified on Millipore MultiScreen membranes according to a TIGR institute procedure (Hedge et al., 2000). PCR products were spotted onto silylated glass slides (Cell Associates), using a Microgrid Pro spotting robot (Biorobotics) as described (Drobyshev et al., 2003). Amino-linked cDNA products were covalently bound to free aldehyde groups attached to the surface.

Total RNA was transcribed into cDNA and labelled with cyanine 3/5 fluorophores. Labelled cDNA products resulting from 5 μg total RNA of treated and untreated cells, were combined and hybridized overnight to the arrays at 42 °C in a buffer containing 50% formamide, 6X SSC, 0.5% SDS and 5% Denhardt's solution. Hybridized slides went through a series of washes with increasing stringency and were subsequently analyzed with an Axon 4000 scanner. Raw data were acquired and extracted using the GenePix software, version 4.0 (Axon).

Background-corrected intensity ratios were normalized using print tip-specific LOESS curves (Yang et al., 2002) and spots were also scale normalized across arrays. Analysis was performed following the least square/empirical Bayes method, using the package LIMMA 1.0.8 (LIMMA: Linear Models for Microarray Data. http://bioinf.wehi.edu.au/limma/) for the open-source statistics package R 1.7.1 (R: R development Core Team, Vienna, Austria, 2003. http://www.R-project.org). A table of the 100 top-ranked differentially expressed genes was obtained, based on the *p*-values of the *t*-statistics of the contrasts. Spots flagged by the scanning software for quality considerations were down-weighted to 10% in the print tip normalization and computation of *t*-statistics. *p*-values were adjusted for multiple comparisons by false discovery rate (FDR) (Benjamini et al., 2001).

2.5. Quantitative real-time PCR (RTQ-PCR)

cDNA was prepared as previously described using 1 μg total RNA (Stulnig et al., 2002a, 2002b). Primer sequences, designed using PRIMER EXPRESS software (PE Applied Biosystems), are listed in Table 1. The PCR reaction mixture contained, in a final volume of 25 μL, 1% of the cDNA, 12.5 μL of 2xSYBRgreen Universal Mastermix (PE Applied Biosystems, Foster City, CA), and corresponding primers. RTQ-PCR was performed in triplicates by using an ABI PRISM 7700 Sequence Detection Systems instrument and software (PE Applied Biosystems). RNA samples were normalized for comparison by determination of 18S rRNA levels.