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Malin Hult, Henrik Ortsäter, Gertrud Schuster, Florian Graedler, Johannes Beckers, et al.. Short-term glucocorticoid treatment increases insulin secretion in islets derived from lean mice through multiple pathways, mechanisms. *Molecular and Cellular Endocrinology*, 2009, 301 (1-2), pp.109. 10.1016/j.mce.2008.09.038 . hal-00532090

HAL Id: hal-00532090

<https://hal.science/hal-00532090>

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Accepted Manuscript

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PII: S0303-7207(08)00452-8
DOI: doi:10.1016/j.mce.2008.09.038
Reference: MCE 7011

To appear in: *Molecular and Cellular Endocrinology*

Received date: 31-8-2008
Revised date: 30-9-2008
Accepted date: 30-9-2008

Please cite this article as: Hult, M., Orstäter, H., Schuster, G., Graedler, F., Beckers, J., Adamski, J., Ploner, A., Jörnvall, H., Bergsten, P., Oppermann, U., Short-term glucocorticoid treatment increases insulin secretion in islets derived from lean mice through multiple pathways, mechanisms, *Molecular and Cellular Endocrinology* (2008), doi:10.1016/j.mce.2008.09.038

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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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SUMMARY

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 hrs) 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 hrs 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.

Running Title: Glucocorticoid mediated changes in pancreatic islet function

Key words: insulin resistance, glucose stimulated insulin secretion, glucocorticoid, inflammation, 11 β -hydroxysteroid dehydrogenase

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

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 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, Kawai and Kuzuya, 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 analyse by microarray analysis the underlying transcriptional changes in pancreatic islets.

EXPERIMENTAL

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 [20]. IgG-certified 96-well microtiter plates were purchased from Nunc (Roskilde, Denmark).

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 hrs 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 hrs, culture medium was changed every 48 hrs. A group of corticosterone-treated islets (18 hrs treatment) were treated with 1 μ M 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.

Measurement of insulin release: After cultivation, individual islets were perfused 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- μ l chamber, made of Teflon-tubing and perfused at a rate of 150 to 160 μ L per min. The perfusion medium was supplemented with 1 mg/mL albumin and contained (in mM): NaCl 125, KCl 5.9, MgCl₂ 1.2, CaCl₂ 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 perfusion 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 perfusate samples were stored at - 20 °C until assayed for insulin. Insulin in the perfusate 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 posthoc test. P-values lower than 0.05 were considered significant. Values were expressed as means $\pm$ standard error of the mean (SEM).

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 6-7 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).

Quantitative real time PCR (RTQ-PCR): cDNA was prepared as previously described using 1 µg total RNA (Stulnig et al., 2002, Stulnig et al., 2002). 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.

11 β -HSD1 dehydrocorticosterone reductase activity in islets of Langerhans. The dehydrocorticosterone reductase activity of the isolated islets was investigated with islets treated overnight at 37°C in 50 nM dehydrocorticosterone, 200 CPM ³H-dehydrocorticosterone +/- 5 µM carbenoxolone. Additional samples without islets were used as negative controls. Each point was run as triplicate samples, and each sample contained approximately 40 islets. Islets were grown in 6-well plates at 37 °C overnight in 2 ml culture medium. Steroids were extracted from the medium (OASIS HLB extraction columns, Waters), eluted in 2 ml MeOH, evaporated under a nitrogen stream and reconstituted in 100 µl MeOH. The conversion of steroid was evaluated by reverse-phase HPLC separation, UV and radioactivity detection on a C₁₈ column in 30% acetonitrile, 0.1% ammonium acetate, pH 7.0, at a flow rate of 1 ml/min.

RESULTS

Insulin secretion. Pancreatic islets from lean C57BL/6J mice were isolated and cultured for various time periods ranging from 2-120 hrs. Islets were left untreated or treated with 200 nM corticosterone, with or without the glucocorticoid receptor antagonist RU486, to investigate the effects of glucocorticoid on glucose-stimulated insulin secretion (GSIS) (Fig 1A and 1B). To dissect the insulin secretion response phases upon glucose stimulation, perfusate aliquots were taken at different time points and analyzed for insulin content. Figure 1A shows a representative pattern from islets cultured for 18 hrs. The average insulin secretory rate in untreated islets was $10 \pm 2 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ at 3 mM glucose. Increasing the glucose concentration to 11 mM caused a pronounced early phase of insulin secretion. During the time period 2-6 min post glucose elevation average insulin secretory rate was $195 \pm 42 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ ($p < 0.05$ compared to 3 mM glucose). During the time period 10-25 min post glucose elevation, the late phase of insulin secretion, the average secretory rate in untreated islets was $72 \pm 17 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ ($p < 0.05$ compared to 3 mM glucose). Treating islets with 200 nM corticosterone did not affect the basal insulin secretory rate but enhanced both the early and the late phases of GSIS 2- and 3-fold, respectively ($p < 0.05$). Including RU486 together with corticosterone during culture reduced the corticosterone-induced enhancement of GSIS ($p < 0.05$) for the early phase and blocked completely the late phase effects. Indeed, such islets had secretory rates comparable with those from untreated islets (Fig. 1A). Basal and GSIS were further investigated as a function of duration of glucocorticoid exposure (Fig. 1B). Whereas no significant changes in either basal or any of the two GSIS phases were observed after 2 hrs incubation with the steroid, distinct differences were observed after longer treatment. Basal insulin secretion was not affected by corticosterone in any of the time points investigated. However, corticosterone induced a significant rise in early phase response after 18 hrs, which remained elevated even after 120 hrs of treatment. The late phase was elevated in islets treated for 18 and 24 hrs but declined towards basal levels after prolonged treatment.

11 β -HSD1 dehydrocorticosterone reductase activity and GSIS. Islets were treated with 50 nM of the glucocorticoid precursor dehydrocorticosterone to investigate its effects on insulin release. The effects resulted in, similar to corticosterone treatment, a

3.5-fold increase of GSIS in comparison to the untreated islets (Fig. 2A), demonstrating glucocorticoid activation by 11 β -HSD1 in islets derived from lean mice. Pancreatic islets were isolated and cultured overnight with 50 nM dehydrocorticosterone, with or without 5 μ M carbenoxolone to investigate the 11 β -HSD1 reductase activity. The dehydrocorticosterone (DHC) reductase activity in the islets was estimated to 1.4 ± 0.8 pmol/30 min/islet (Fig. 2B). A complete inhibition of the activity was obtained upon co-culturing with the 11 β -HSD antagonist carbenoxolone.

Microarray data analysis. Mouse microarray chips containing nearly 21,000 non-redundant cDNA clones were used for the study of pancreatic islets in response to glucocorticoid treatment of perfused islets derived from lean mice. The 100 most differentially expressed genes upon corticosterone treatment were grouped according to function, as shown in Table 2. The majority of the 100 genes revealed functions in signal transduction (15 genes), stress response (25 genes), metabolism (13 genes), or were unassigned (21 genes). Within the 100-top-result list, genes were selected from the various functional classes for RTQ-PCR (cf Table 1). A scatter plot (Fig. 3) of p-value vs. estimated fold change displays all genes on the chip, discriminating the ones of $p < 0.05$ and > 2 -fold change, including the genes that were quantified by RT-PCR. The four original RNA samples were investigated by RTQ-PCR (Fig 4) using target primer concentrations of 100 nM. Of 11 genes selected for RTQ-PCR, 2 were chosen on biological basis of glucocorticoid regulations and responses, although they did not appear among the 100-top list (Pdx1, 11 β -HSD1). Differential expression of 8 out of the 9 remaining genes selected from the 100-top list could be qualitatively confirmed by RTQ-PCR. 11 β -HSD1 was found to be upregulated by the glucocorticoid treatment, whereas Pdx1 was downregulated. In general, the RTQ-PCR results strengthen the results of the microarray data, showing a clear response to the glucocorticoid treatment with particular genes including Itmap1, Fabp4, Rgs2, Lcn2 and RNase4, being differentially expressed.

DISCUSSION

This study highlights two important effects of glucocorticoid hormones (GCs) on β -cell function. The first regards their function as a modulator of GSIS and the second

their ability to provoke a transcriptional response. GCs are stress hormones regulating a multitude of important effects in metabolism and inflammation. GCs provoke hyperglycemia and hyperinsulinemia, effects thought to arise from increased hepatic gluconeogenesis and decreased peripheral insulin sensitivity. In addition, direct effects on β -cells are reported, with partially contradictory results on glucose-stimulated insulin secretion. Whereas long-term increased GC sensitivity leads to decreased ability of the β -cell to respond to glucose by insulin secretion, probably through upregulation of α_2 -adenergetic receptors and G-protein coupled receptor (GPCR) signaling (Davani et al., 2004), short- to medium term GC effects on the β -cells are not that clear-cut. Most reports show decreased insulin responsiveness upon GC treatment in various animal models (Davani et al., 2004, Delaunay et al., 1997, Jeong et al., 2001, Koizumi and Yada, 2008, Swali et al., 2008) including in islets derived from the hyperglucocorticoidemic *ob/ob* mouse model (Davani et al., 2000). In contrast to our results with *ob/ob* islets (Davani et al., 2000, Ortsater et al., 2005), we now show that islets treated over different time intervals and derived from lean C57BL/6J mice, respond with a significant increase in insulin secretion upon GC treatment. This is in concordance with other reports demonstrating increase in insulin secretion upon GC treatment (Karlsson et al., 2001, Kawai and Kuzuya, 1974, Kawai and Kuzuya, 1977), the latter study showing similar responses (3-4 fold increase in GSIS) to GC treatment as in our experiments. These effects are mediated through the glucocorticoid receptor (GR), as demonstrated by decrease in insulin secretion to control levels using the receptor antagonist RU486 (Fig 1A). Furthermore, we demonstrate that different phases in GSIS responses are differentially affected by glucocorticoids, indicating distinct glucocorticoid regulated components in glucose response pathways.

An important determinant of GC signaling is amplification of the steroid hormone signal by intracellular activation of precursor 11-oxoglucocorticoids (cortisone in humans, dehydrocorticosterone, DHC, in rodents), activated through 11 β -HSD1. We here show (Fig 2) by substrate conversion and carbenoxolone inhibition experiments that 11 β -HSD1 is present in islets from lean mice. Importantly, islets receiving the DHC precursor respond in a manner equivalent to that of corticosterone-treated cells, by an increased response in GSIS (Fig 2). Differential expression of 11 β -HSD1 in

islets appears to be a distinguishing feature, as observed earlier in experiments using lean and diabetic ZDF rats (Duplomb et al., 2004). In this study, PPAR γ agonist treatment reduced expression levels of 11 β -HSD1 in islets, as noted earlier in adipocytes or liver (Berger et al., 2001, Stulnig et al., 2002). Assuming an essential role of 11 β -HSD1 in GC signal transmission in islets, we note that the effects of PPAR γ treatment of control and non-steroid induced, diabetic patients (Willi et al., 2002, Willi et al., 2002), resulting in increased insulin secretion, are in agreement with insulin secretion data obtained in the present and our previous studies (Davani et al., 2000, Ortsater et al., 2005). A recent study (Swali et al., 2008) shows that 11 β -HSD1 is colocalized to cells expressing glucagon and not found in β -cells, thus a paracrine effect of glucocorticoid activation on insulin secretion is apparent.

We conducted microarray analysis to investigate glucocorticoid regulated genes potentially involved in insulin secretion. We selected a time point of 18 hrs steroid incubation, showing a response to the treatment, with several signal transduction pathways affected. We demonstrate that several genes are upregulated. In this manner, increased expression of Fabp4 is a hallmark of metabolic dysregulation. Fkbp5 regulates GC response (Davies et al., 2002) by binding to the GC receptor and is likely to cause GC resistance in response to high doses of GCs (Denny et al., 2000), and upregulation of Rgs2 will lead to a decreased response in glucose-dependent insulinotropic polypeptide (GIP) mediated insulin secretion (Tseng and Zhang, 2000). However, several distinct components are upregulated and lead to enhanced insulin secretion. These genes comprise ion channels (amiloride sensitive cation channel, leading through depolarization to Ca²⁺ release), elements of GPCR signaling (Grb10, interaction with PI3 kinase) (Deng et al., 2003), protein and inositol phosphate kinases (inositol trisphosphate 3 kinase), metabolic enzymes (lipoprotein lipase, allowing uptake of free fatty acids) and cytoskeleton or vesicle components (chromogranin B). Taken together, this part of the study shows that GCs regulate a distinct set of genes involved in enhanced insulin secretion.

Surprisingly we found a large amount of differentially regulated genes mediating stress responses or inflammatory signals. Besides acute phase proteins (e.g. lipocalin, trefoil factor 2, serum amyloid 3), we found a considerable number of extracellular proteases, inhibitors, heat shock proteins and chaperones involved in tissue

remodelling, or stress response enzymes such as glutamyl cysteine ligase. It will be of future interest to compare the expression pattern changes observed in this work with a recent study conducted on islets derived from spontaneously diabetic GK rats (Ghanaat-Pour et al., 2007).

Furthermore, the detection of the proinflammatory cytokine IL-1 β in primary islet culture confirms an earlier report on expression of this mediator (Johansson et al., 2003). Downregulation of IL1 β by glucocorticoids explains the decreased levels of Serpina12 observed in the microarray experiments, since this and other protease components are regulated through inflammatory signals and further highlights the anti-inflammatory properties of glucocorticoids.

Taken together, this study presents clear evidence that in the early phase of GC exposure, β -cells of primary islet cultures from lean mice act by increased insulin secretion, displaying a distinct profile of stress regulation. This situation possibly primes the cells for a switch in GC response, leading to glucose insensitivity and decrease in insulin secretion.

ACKNOWLEDGEMENTS

This study was supported by grants from the NovoNordisk Foundation, the Swedish Medical Research Council (72x-14019), the European Foundation for the Study of Diabetes, the Swedish Foundation for Strategic Research, the Swedish Diabetes Association, the Swedish Medical Association, the Swedish Foundation for International Cooperation in Research and Higher Education the Swedish Society for Medical Research, Göran Gustafsson Foundation, Magnus Bergvall Foundation, Filip Lundbergs Foundation, Family Ernfors Fund and Syskonen Svenssons Fund and Karolinska Institutet. Expert technical assistance by Monica Lindh is gratefully acknowledged.

REFERENCES

- Ashcroft, F.M., Proks, P., Smith, P.A., Ammala, C., Bokvist, K. and Rorsman, P. (1994) Stimulus-secretion coupling in pancreatic beta cells. *J Cell Biochem* 55 Suppl, 54-65.
- Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N. and Golani, I. (2001) Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 125, 279-84.

- Berger, J., Tanen, M., Elbrecht, A., Hermanowski-Vosatka, A., Moller, D.E., Wright, S.D. and Thieringer, R. (2001) Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11beta -hydroxysteroid dehydrogenase type 1 expression and activity. *J Biol Chem* 276, 12629-35.
- Berggren, P.O. and Larsson, O. (1994) Ca²⁺ and pancreatic B-cell function. *Biochem Soc Trans* 22, 12-8.
- Bergsten, P. and Hellman, B. (1993) Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. *Diabetes* 42, 670-4.
- Brunstedt, J. and Nielsen, J.H. (1981) Direct long-term effect of hydrocortisone on insulin and glucagon release from mouse pancreatic islets in tissue culture. *Acta Endocrinol (Copenh)* 96, 498-504.
- Davani, B., Khan, A., Hult, M., Martensson, E., Okret, S., Efendic, S., Jornvall, H. and Oppermann, U.C. (2000) Type 1 11beta -hydroxysteroid dehydrogenase mediates glucocorticoid activation and insulin release in pancreatic islets. *J Biol Chem* 275, 34841-4.
- Davani, B., Portwood, N., Bryzgalova, G., Reimer, M.K., Heiden, T., Ostenson, C.G., Okret, S., Ahren, B., Efendic, S. and Khan, A. (2004) Aged Transgenic Mice With Increased Glucocorticoid Sensitivity in Pancreatic beta-Cells Develop Diabetes. *Diabetes* 53 Suppl 1, S51-S59.
- Davies, T.H., Ning, Y.M. and Sanchez, E.R. (2002) A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. *J Biol Chem* 277, 4597-600.
- Delaunay, F., Khan, A., Cintra, A., Davani, B., Ling, Z.C., Andersson, A., Ostenson, C.G., Gustafsson, J., Efendic, S. and Okret, S. (1997) Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest* 100, 2094-8.
- Deng, Y., Bhattacharya, S., Swamy, O.R., Tandon, R., Wang, Y., Janda, R. and Riedel, H. (2003) Growth factor receptor-binding protein 10 (Grb10) as a partner of phosphatidylinositol 3-kinase in metabolic insulin action. *J Biol Chem* 278, 39311-22.
- Denny, W.B., Valentine, D.L., Reynolds, P.D., Smith, D.F. and Scammell, J.G. (2000) Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. *Endocrinology* 141, 4107-13.
- Drobyshev, A.L., Machka, C., Horsch, M., Seltmann, M., Liebscher, V., Hrabec de Angelis, M. and Beckers, J. (2003) Specificity assessment from fractionation experiments (SAFE): a novel method to evaluate microarray probe specificity based on hybridisation stringencies. *Nucleic Acids Res* 31, E1-1.
- Dudoit, S., Shaffer, J.P. and Boldrick, J.C. (2003) Multiple Testing in Microarray Experiments. *Statistical Science* 18, 71-103.
- Duplomb, L., Lee, Y., Wang, M.Y., Park, B.H., Takaishi, K., Agarwal, A.K. and Unger, R.H. (2004) Increased expression and activity of 11beta-HSD-1 in diabetic islets and prevention with troglitazone. *Biochem Biophys Res Commun* 313, 594-9.
- Ghanaat-Pour, H., Huang, Z., Lehtihet, M. and Sjöholm, A. (2007) Global expression profiling of glucose-regulated genes in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats. *J Mol Endocrinol* 39, 135-50.

- Hedge, P., Qi, R., Abernathy, K., Gay, C., Dharap, S., Gaspard, R., Hughes, J., Snesrud, E., Lee, E. and Quackenbush, J. (2000) A concise guide to cDNA microarray analysis. *Biotechniques* 29, 548-56.
- Itoh, N. and Okamoto, H. (1980) Translational control of proinsulin synthesis by glucose. *Nature* 283, 100-2.
- Jeong, I.K., Oh, S.H., Kim, B.J., Chung, J.H., Min, Y.K., Lee, M.S., Lee, M.K. and Kim, K.W. (2001) The effects of dexamethasone on insulin release and biosynthesis are dependent on the dose and duration of treatment. *Diabetes Res Clin Pract* 51, 163-71.
- Johansson, U., Olsson, A., Gabrielsson, S., Nilsson, B. and Korsgren, O. (2003) Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. *Biochem Biophys Res Commun* 308, 474-9.
- Karlsson, S., Ostlund, B., Myrsen-Axcrona, U., Sundler, F. and Ahren, B. (2001) Beta cell adaptation to dexamethasone-induced insulin resistance in rats involves increased glucose responsiveness but not glucose effectiveness. *Pancreas* 22, 148-56.
- Kawai, A. and Kuzuya, N. (1974) [Proceedings: Stimulatory action of glucocorticoids on insulin secretion]. *Nippon Naibunpi Gakkai Zasshi* 50, 226.
- Kawai, A. and Kuzuya, N. (1977) On the role of glucocorticoid in glucose-induced insulin secretion. *Horm Metab Res* 9, 361-5.
- Khan, A., Ostenson, C.G., Berggren, P.O. and Efendic, S. (1992) Glucocorticoid increases glucose cycling and inhibits insulin release in pancreatic islets of ob/ob mice. *Am J Physiol* 263, E663-6.
- Koizumi, M. and Yada, T. (2008) Sub-chronic stimulation of glucocorticoid receptor impairs and mineralocorticoid receptor protects cytosolic Ca²⁺ responses to glucose in pancreatic beta-cells. *J Endocrinol* 197, 221-9.
- Lambillotte, C., Gilon, P. and Henquin, J.C. (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 99, 414-23.
- Lang, J. (1999) Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *Eur J Biochem* 259, 3-17.
- Leibiger, B., Moede, T., Schwarz, T., Brown, G.R., Kohler, M., Leibiger, I.B. and Berggren, P.O. (1998) Short-term regulation of insulin gene transcription by glucose. *Proc Natl Acad Sci U S A* 95, 9307-12.
- Lenzen, S. and Bailey, C.J. (1984) Thyroid hormones, gonadal and adrenocortical steroids and the function of the islets of Langerhans. *Endocr Rev* 5, 411-34.
- Oppermann, U. (2006) Type 1 11beta-hydroxysteroid dehydrogenase as universal drug target in metabolic diseases? *Endocr Metab Immune Disord Drug Targets* 6, 259-69.
- Oppermann, U.C., Persson, B. and Jornvall, H. (1997) Function, gene organization and protein structures of 11beta-hydroxysteroid dehydrogenase isoforms. *Eur J Biochem* 249, 355-60.
- Ortsater, H., Alberts, P., Warpman, U., Engblom, L.O., Abrahmsen, L. and Bergsten, P. (2005) Regulation of 11beta-hydroxysteroid dehydrogenase type 1 and glucose-stimulated insulin secretion in pancreatic islets of Langerhans. *Diabetes Metab Res Rev* 21, 359-66.
- Stulnig, T.M., Oppermann, U., Steffensen, K.R., Schuster, G.U. and Gustafsson, J.A. (2002) Liver X receptors downregulate 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. *Diabetes* 51, 2426-33.

- Stulnig, T.M., Steffensen, K.R., Gao, H., Reimers, M., Dahlman-Wright, K., Schuster, G.U. and Gustafsson, J.A. (2002) Novel roles of liver X receptors exposed by gene expression profiling in liver and adipose tissue. *Mol Pharmacol* 62, 1299-305.
- Swali, A., Walker, E.A., Lavery, G.G., Tomlinson, J.W. and Stewart, P.M. (2008) 11beta-Hydroxysteroid dehydrogenase type 1 regulates insulin and glucagon secretion in pancreatic islets. *Diabetologia*.
- Tomlinson, J.W. and Stewart, P.M. (2007) Modulation of glucocorticoid action and the treatment of type-2 diabetes. *Best Pract Res Clin Endocrinol Metab* 21, 607-19.
- Tseng, C.C. and Zhang, X.Y. (2000) Role of G protein-coupled receptor kinases in glucose-dependent insulintropic polypeptide receptor signaling. *Endocrinology* 141, 947-52.
- Walker, B.R. and Seckl, J.R. (2003) 11beta-Hydroxysteroid dehydrogenase Type 1 as a novel therapeutic target in metabolic and neurodegenerative disease. *Expert Opin Ther Targets* 7, 771-83.
- Willi, S.M., Kennedy, A., Brant, B.P., Wallace, P., Rogers, N.L. and Garvey, W.T. (2002) Effective use of thiazolidinediones for the treatment of glucocorticoid-induced diabetes. *Diabetes Res Clin Pract* 58, 87-96.
- Willi, S.M., Kennedy, A., Wallace, P., Ganaway, E., Rogers, N.L. and Garvey, W.T. (2002) Troglitazone antagonizes metabolic effects of glucocorticoids in humans: effects on glucose tolerance, insulin sensitivity, suppression of free fatty acids, and leptin. *Diabetes* 51, 2895-902.
- Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J. and Speed, T.P. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* 30, e15.

Table 1: Primer pairs used for real-time quantitative PCR using SYBRgreen labeling.

Gene	Sequences of forward and reverse primers (5' to 3')	Source of sequence data
Rgs2	CGG CCC CAA GGT CGA CAA ACG GGT CTT CCA ATC CTT	af_43291
Lcn2	CAA AAT TAC CCT GTA TGG AAG AAC C ACA CTC ACC ACC CAT TCA GTT GT	nm_008491
Fkbp5	CAG GCT GCC ATC GTG AAA TGC GTG TAC TTG GGT GGG TT	nm_010220
RNase4	ATA TCG GGG TCG TGT TTC CA ATC CAT CAT TAC CTA GAA AGT GCC T	nm_041472
Hspb1	CAA GGA AGG CGT GGT GGA AGC CAT GTT CGT CCT GCC	nm_013560
Meth	CAT ACA AAC GCT GAC GGA TGA TCT GCC TTC TGT GCT TTC AGT TC	bc_052040
Itmap1	ACT TAA TCA GCA GCG GAT GTT GTT CGA GAT GTT TGG TCA TTG TTA TCA C	nm_008411
Fabp4	GAA TTC GAT GAA ATC ACC GCA CTC TTT ATT GTG GTC GAC TTT CC	nm_024406
Pdx1	CCT TTC CCG TGG ATG AAA TC TCG GGT TCC GCT GTG TAA	nm_008814
11 β -HSD1	AAGCAGAGCAATGGCAGCAT GAGCAATCATAGGCTGGGTCAT	X83202
Serpina12	GCA GGA GAG CTG GCT GTC A GCA GGA GAG CTG GCT GTC A	nm_026535

Table 2: List of the 100 most differentially expressed genes obtained by short-term corticosterone treatment of islets derived from lean C57BL/6J mice. n = 5 experiments. The table contains the following information 1) function, divided into main groups and subclasses, 2) Genbank ID, 3) gene name (underlined genes were further processed by RT Q-PCR, 4) log2-fold change, 5) p-values, adjusted using false discovery rate (p.fdr) (See (Dudoit et al., 2003) for an overview).

Function	Genbank ID	name	log2 fold change	p.fdr
<u>Transport</u>				
Vesicle	BC016584	glycolipid transfer protein	-1.66	0.0921
	NM_008411	<u>integral membrane-associated protein 1 (Itmap1)</u>	2.35	0.0026
	NM_007694	chromogranin B	-0.77	0.0921
	NM_026168	PTX1 protein	1.49	0.0921
<u>Signal Transduction</u>				
	BB191620	Ras-related GTP binding D	-1.46	0.0812
	AF432916	<u>Regulator of G-protein signaling 2 (Rgs2)</u>	2.01	0.0089
	NM_009061	K0617F02-3 N1A	1.61	0.0198
	NM_016693	Mitogen-activated protein kinase kinase kinase 6	1.40	0.0448
	NM_181593	inositol 1,4,5-trisphosphate 3-kinase C	1.31	0.0862
	BB017040	RIKEN BB017040	-2.32	0.0921
	NM_023456	Neuropeptide Y precursor	1.26	0.0921
	BF136563	Similar to KIAA1741 protein	0.90	0.0921
	NM_010345	growth factor receptor bound protein 1	0.91	0.1027
		transcription factor		
	BG965788	transcription factor ETV6	1.11	0.0762
	NM_010638	basic transcription element binding protein 1	1.00	0.0921
	NM_010330	Embigin precursor	-0.96	0.0974
ion channel	NM_009206	adaptor protein kanadaplin	1.09	0.1048
	NM_019664	potassium inwardly-rectifying channel	1.96	0.0069
	NM_007384	amiloride-sensitive cation channel 1	0.85	0.0974
<u>Stress response</u>				
	NM_013560	<u>heat shock protein 1 (hspb1)</u>	2.02	0.0089
	NM_012032	membrane protein TMS-1	1.52	0.0974
	NM_031165	heat shock protein 8	1.39	0.0762
		inflammation		
	BC011437	interleukin 1 beta	-2.47	0.0448
	NM_010492	Islet cell autoantigen 1	2.73	0.0064
	NM_008013	fibrinogen-like protein 2	0.97	0.0577
	NM_008491	<u>lipocalin precursor (Lcn2)</u>	2.00	0.0089
	NM_011315	Serum amyloid A 3	2.05	0.0806
	NM_009363	Trefoil factor 2 precursor	-0.82	0.0974
	NM_010220	<u>FK506 binding protein 5 (Fkbp5)</u>	1.02	0.0398
	NM_010501	Interferon-induced protein tetraatricopeptide repeats 3	-1.44	0.0188
		extracellular matrix		
	NM_146248	a-helical protein	1.17	0.0640
	NM_009930	procollagen, type III, alpha 1	0.84	0.0809
	NM_008877	Plasminogen precursor	-2.70	0.0843
	NM_007743	Collagen alpha 2(I) chain precursor	1.11	0.0921
	NM_028072	sulfatase 2 (Sulf2-pending)	0.67	0.0921
		Proteolytic processing		
	NM_026535	<u>serine proteinase inhibitor (Serpina 12)</u>	2.89	0.0056
	M64085.1	Contrapsin-like protease inhibitor (CPI-26)	2.26	0.0064
	BI694738	spi2 proteinase inhibitor	2.19	0.0193
	BE851987	Kallikrein-binding protein precursor (Serpina3g)	1.42	0.0198
		protein turnover		
	NM_011458	Contrapsin precursor (Serpina3k).	2.44	0.0269
	NM_011595	Metalloproteinase inhibitor 3 precursor	2.96	0.0921
	NM_175340	NHL repeat containing 1	-1.75	0.0921
	NM_021301	solute carrier family 15 (H+/peptide transporter)	-0.98	0.1027
	NM_010295	Glutamate-cysteine ligase	1.06	0.0577

Metabolism

	NM_008898	NADPH-cytochrome P450 reductase	1.30	0.0089
	BC011202	Selenium-binding protein 1	1.08	0.0357
protein metabolism				
	BC016492	transglutaminase 2	0.67	0.1027
nucleotide metabolism				
	BI713856	alkaline phosphodiesterase	-2.29	0.0974
	NM_021472	<u>Ribonuclease 4 precursor (RNase4)</u>	-1.27	0.0089
	BM196407	<u>Methionyl-tRNA synthetase (Meth, BM196407)</u>	-1.11	0.0554
	NM_021335	U2 small nuclear ribonucleoprotein B	-1.45	0.0921
	NM_011690	valyl-tRNA synthetase 2	0.86	0.1027
transport				
	NM_009155	Selenoprotein P 1	1.41	0.0762
	NM_019414	Selenium-binding protein 2	1.41	0.0772
	BC011202	Mus musculus selenium binding protein 1	1.68	0.0974
lipid metabolism				
	NM_024406	<u>Fatty acid-binding protein (Fabp4)</u>	1.21	0.0308
	NM_008509	Lipoprotein lipase precursor	-1.31	0.0921
<u>miscellaneous</u>				
	NM_011359	Pulmonary surfactant-associated protein C precursor	1.81	0.0997
growth factor				
	NM_013655	chemokine (C-X-C motif) ligand 12	-1.71	0.0058
	NM_009264	Small proline-rich protein 1A	-2.37	0.0089
	NM_015776	microfibrillar-associated protein 5	1.81	0.0762
	NM_008733	nebulin-related anchoring protein	1.98	0.0921
	NM_134471	kinesin family member 2C	-1.94	0.1027
calcium binding				
	NM_025994	swiprosin 1.	1.33	0.0261
	NM_022315	SPARC related modular calcium binding 2	1.66	0.0772
<u>unassigned</u>				
	AK013920	RIKEN 3100002J23	1.13	0.0188
	NM_027727	RIKEN cDNA 4933427L07	-1.37	0.0193
	AA987153	Sugano mouse kidney mkia	1.92	0.0309
	AC125091	BAC clone RP24-165P5 from chromosome 1	1.79	0.0357
	AV223084	RIKEN 3830408C12	2.16	0.0398
	AC122337	BAC clone RP23-351M10 from chromosome 12	2.08	0.0501
	NM_175329	16.7Kd protein	-1.02	0.0577
	NM_153546	O-acyltransferase (membrane bound) domain containing 1	0.98	0.0577
	BQ175097	NIH_BMAP_DJ2	2.37	0.0586
	AI196943	cryptochrome 2	0.85	0.0613
	BQ177102	UI-M-DJ2-bwa-j-15-0-UIs1 NIH_BMAP_DJ2 Mus musculus cDNA clone	1.50	0.0709
	BC010768	crystallin, alpha B	0.82	0.0759
	AJ000262	huntingtin associated protein 1	-0.69	0.0762
	AL591544	clone RP23-386D6 on chromosome 11	2.53	0.0812
	AF197878	AF197878 Mus musculus C57BL/6J	0.88	0.0812
	AW123290	NIH_BMAP_M_S3.1	-1.91	0.0836
	BC028925	Immunoglobulin kappa chain variable 1 (V1)	-1.76	0.0836
	NM_026293	RIKEN 4930540L03	-2.18	0.0843
	BI738207	NIH_MGC_94	1.10	0.0872
	NM_027829	RIKEN 9030607L17	-2.27	0.0921
	BB199157	RIKEN BB199157	-1.81	0.0921
	AL837516	clone RP23-234L8 on chromosome 2	-1.65	0.0921
	NM_175533	RIKEN 5830411N06	-1.13	0.0921
	AA174873	Soares mouse 3NbMS cDNA clone	1.12	0.0921
	AI414870	Soares mouse p3NMF19.5 cDNA clone	1.25	0.0947
	AF291467	HASH1 gene	-1.13	0.0974
	AV359767	RIKEN AV359767	-2.13	0.0990
	NM_009749	brain expressed X-linked 2	-0.77	0.1027
	AK019807.1	RIKEN 4930572D21	2.34	0.1027
	BM219859	C0931B05-3 NIA	1.96	0.1027
	BC049975	hypothetical protein LOC238393	1.47	0.1027

LEGENDS TO FIGURES

Figure 1: Corticosterone mediated amplification of glucose-stimulated insulin secretion. **(A)** Islets from lean mice were cultured for 18 hours in the absence or presence of 200 nM corticosterone together or not with 1 μ M RU486. After culture, the islets secretory capacity was analyzed in perfusion experiments. Results show average insulin secretion (mean $\pm$ SEM) for 17-25 separate experiments at 3 and 11 mM glucose. **(B)** Corticosterone effect on basal and the early and late phase of glucose-stimulated insulin secretion was examined over 2-120 hrs of glucocorticoid treatment. Data are presented as fold over control at the different time points.

Figure 2: 11 β -HSD1 activity and GSIS in islet cultures derived from lean mice. Islets were cultivated for 18 hrs with and without dehydrocorticosterone and analyzed as follows: **(A)** GSIS was 3.5-fold increased upon treatment with the inactive glucocorticoid dehydrocorticosterone, indicating glucocorticoid conversion by 11 β -HSD1 in these islets. Insulin release in the presence of 11 mM glucose in the absence (left bar) or presence of DHC (right bar) is presented as mean $\pm$ SEM for 6 experiments. **(B)** Dehydrocorticosterone reductase activity in pancreatic islets is completely inhibited by the 11 β -HSD inhibitor carbenoxolone. Data presented as mean $\pm$ SEM for n=3 experiments for each treatment with 40 islets in each sample.

Figure 3: Scatter plot of p-value vs. estimated fold change for all features on the chip (volcano plot). The horizontal axis shows log₂ of the observed mean of fold change; positive values correspond to overexpression in treated mice. The vertical axis shows -log₁₀ of the p-value, comparing treated and untreated animals, adjusted for false discovery rate. The horizontal line is drawn at the traditional value of 0.05, the vertical lines at -1 and 1, corresponding to a two-fold change in mRNA. The labeled spots were chosen for QRT-PCR (see also Fig. 4).

Figure 4: Quantitative real-time PCR. Genes selected from functional classes showed high correlation (8/9) with the microarray results in differential expression patterns. Gene names as in Table 2.

Figure 1

Figure 2

Figure 3

Figure 4.