Chapter 3

Prednisolone-Induced Beta-Cell Dysfunction is Associated with Impaired Endoplasmic Reticulum Homeostasis in INS-1E Cells

ABSTRACT

Glucocorticoids (GCs), such as prednisolone, are widely prescribed anti-inflammatory drugs, but their use may induce glucose intolerance and diabetes. GC-induced beta-cell dysfunction contributes to these diabetogenic effects through mechanisms that remain to be elucidated. In this study, we hypothesized that activation of the unfolded protein response (UPR) following endoplasmic reticulum (ER) stress could be one of the underlying mechanisms involved in GC-induced beta-cell dysfunction. We report here that prednisolone did not affect basal insulin release, but time-dependently inhibited glucose-stimulated insulin secretion in INS-1E cells. Prednisolone treatment also decreased both PDX1 and insulin expression, leading to a marked reduction in cellular insulin content. These prednisolone-induced detrimental effects were found to be prevented by prior treatment with the glucocorticoid receptor (GR) antagonist RU486 and associated with activation of two of the three branches of the UPR. Indeed, prednisolone induced a GR-mediated activation of both ATF6 (activating transcription factor 6) and IRE1 (inositol-requiring kinase 1)/XBP1 (X-box-binding protein 1) pathways, but was found to reduce the phosphorylation of PERK (protein kinase RNA-like ER kinase) and its downstream substrate eIF2α (eukaryotic initiation factor 2α). These modulations of ER stress pathways were accompanied by upregulation of calpain 10 and increased cleaved caspase 3, indicating that long-term exposure to prednisolone ultimately promotes apoptosis. Taken together, our data suggest that the inhibition of insulin biosynthesis by prednisolone in the insulin-secreting INS-1E cells results, at least in part, from a GR-mediated impairment in ER homeostasis, which may lead to apoptotic cell death.
INTRODUCTION

Glucocorticoids (GCs), such as prednisolone, are potent and widely prescribed anti-inflammatory drugs. However, their use is frequently associated with the development of adverse metabolic effects, and may lead to the development of steroid diabetes in up to 20-50% of patients [1, 2]. GC-induced hyperglycemia has been classically attributed to progressive development of insulin resistance in peripheral tissues [1, 2], but recent studies also point toward involvement of beta-cell dysfunction [3]. A clinical study performed in healthy males recently showed that both acute and chronic treatment with prednisolone attenuated pancreatic insulin secretion during standardized meal tests by impairing multiple aspects of beta-cell function [3]. In vitro studies have also shown that GCs lower glucose-stimulated insulin secretion (GSIS) in beta-cell lines and isolated pancreatic islets by various putative mechanisms involving either downregulation of GLUT2, inhibition of phospholipase C/protein kinase C signaling, modulation of the voltage-gated K(+) channels activity, alterations in intracellular Ca(2+) homeostasis and/or decreased calcium efficacy on the insulin secretory process (see [2] for recent review). In addition, GCs may also reduce pancreatic beta-cell mass and affect insulin biosynthesis, at least in part by inducing apoptotic cell death [4-6].

Defects in endoplasmic reticulum (ER) homeostasis have been proposed to underlie beta-cell dysfunction and impairment in insulin secretion in patients with type 2 diabetes, Wolcott-Rallison and Wolfram syndrome [7-9]. Three proteins associated with the ER-membrane, activating transcription factor 6 (ATF6), inositol-requiring enzyme 1α (IRE1α), and PKR-like eukaryotic initiation factor 2α kinase (PERK) monitor the activity of ER [8]. In absence of stress, these sensors of ER homeostasis are held in an inactive state through interaction with the ER-chaperone immunoglobulin binding protein (BIP) [8]. Conditions that affect ER homeostasis in beta cells, such as increased unfolded/misfolded proteins, Ca(2+) depletion or enhanced insulin biosynthesis, result in release of BIP from the sensor proteins and activation of the unfolded protein response (UPR) [7, 8].

Activation of ATF6, the first branch of the UPR, requires its translocation from the ER to the Golgi apparatus where ATF6 is cleaved into an active nuclear transcription factor that can regulate the expression of ER genes, such as X-box binding protein-1 (XBP1) [8]. The second pathway involved in the UPR leads to activation of the endoribonuclease IRE1α and subsequent cleavage of XBP1 mRNA into XBP1s, an alternative spliced form. XBP1s alone, or in conjunction with ATF6, regulates the synthesis of ER chaperones, and proteins involved in both ER biogenesis and ER-associated protein degradation (ERAD) [7, 8]. Finally, activation of PERK, which constitutes the third branch of the UPR, occurs through autophosphorylation of
its kinase domain and results in phosphorylation of its substrate eukaryotic initiation factor 2α (eIF2α). This leads to inhibition of global protein synthesis in addition to increased synthesis of ATF4 through alternative translation [10]. ATF4 does not only regulate the expression of ER-chaperones in order to restore ER homeostasis, but can also increase the expression of genes involved in apoptosis, like C/EBP-homologous protein (CHOP) and tribbles homolog 3 (TRIB3) [8]. Mutations that functionally impair the activity of the PERK/eIF2α-pathway cause neonatal diabetes in humans (Wolcott-Rallison syndrome) and mouse models [11-13], further illustrating the importance of this pathway for beta cell function.

Given the critical role of the UPR in beta-cell homeostasis, the aim of this study was to determine whether ER stress could be one of the underlying mechanisms involved in prednisolone-induced beta-cell dysfunction.

**MATERIAL AND METHODS**

**Cell culture:** Rat insulinoma-derived insulin-secreting INS-1E cells (kind gift from Pr. P. Maechler, [14]) were cultured in a humidified atmosphere containing 5% CO₂ in RPMI 1640 medium (Invitrogen, Breda, The Netherlands) supplemented with 10 mM HEPES, pH7.4, 5% (v/v) heat-inactivated fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 50 mM 2-mercaptoethanol, 100 units/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Breda, The Netherlands) as described previously [15]. For experiments, INS-1E cells were seeded at 2x10⁵ cells/1 ml in Falcon 24-well plates (insulin secretion), or 9x10⁵ cells/2 ml in Falcon 6-well plates (western blotting, gene expression), and used 4 days later, with one medium change on day 3. When indicated, prednisolone, the glucocorticoid receptor antagonist RU486 (mifepristone) and/or their vehicle (DMSO, 0.5% v/v) were added to the medium.

**Insulin secretion:** Insulin secretion in response to glucose was performed as described earlier [15]. Briefly, cells were incubated with prednisolone or vehicle (0.5% DMSO) for various time points. Then, the cells were maintained for 2 h in glucose-free culture medium containing prednisolone or vehicle (0.5% DMSO), followed by two washes and a 30 min at 37°C incubation in glucose-free Krebs–Ringer bicarbonate HEPES buffer (KRBH: 135 mM NaCl, 3.6 mM KCl, 5 mM NaHCO₃, 0.5 mM NaH₂PO₄, 0.5 mM MgCl₂, 1.5 mM CaCl₂, 0.1% BSA and 10 mM HEPES, pH 7.4) in the presence or absence of DMSO or prednisolone. Next, cells were washed with glucose-free KRBH and incubated for 30 min in KRBH with or without prednisolone and secretagogues, as indicated. Then, plates were placed on ice and the supernatants were collected for determination of insulin secretion. Cellular insulin
content was measured in acid–ethanol extracts [15]. Insulin concentrations were measured in supernatants and acid–ethanol extracts using a rat/mouse ELISA kit (Millipore, Nuclilab, Ede, The Netherlands).

**Protein analysis:** INS-1E cells were incubated with vehicle or prednisolone as indicated. Then, cells were washed twice with phosphate buffered saline (PBS) and lysed in a buffer containing 10% (w/v) glycerol, 3% (w/v) SDS and 100 mM Tris–HCl (pH 6.8). Lysates were immediately boiled for 5 min and centrifugated (13,200 rpm; 2 min). Protein content of the supernatant was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, USA). Proteins were separated by SDS-PAGE followed by transfer to a polyvinylidene difluoride (PVDF) membrane. Membranes were blocked for 1 h at room temperature in TBST buffer (10 mM Tris–HCl (pH 7.4), 150 mM NaCl, and 0.1% (v/v) Tween 20 containing 5% (w/v) fat free milk) and incubated overnight with primary antibodies (see Supplementary Table 1). The membranes were then washed in TBST buffer and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After washing, blots were developed using enhanced chemiluminescence and quantified by densitometry analysis using ImageJ software (NIH, Bethesda, USA).

**Gene expression studies:** INS-1E cells were lysed in RLT-buffer (Qiagen, Hilden, Germany) for isolation of total RNA using the RNaseasy system including on-column DNAse I treatment (Qiagen, Hilden, Germany). For PCR analysis, total RNA (1.5 µg) was reverse transcribed using a Superscript first strand synthesis kit (Invitrogen, Breda, The Netherlands) and quantitative real-time PCR was performed with SYBR Green on a StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). All the primers sets used were designed for spanning an exon (if any) and have an efficiency of ~100+/-5% (Supplementary Table 2). Every sample was analyzed in duplicate and mRNA expression was normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) mRNA content, and expressed as arbitrary units. For whole genome expression profiling, total RNA (200 ng) was amplified, labeled and fragmented using the GeneChip 3’ IVT Express Kit (Affymetrix, Santa Clara, CA) according to the manufacturer’s instructions. Fragmented amplified RNA (10 µg) was applied to the Rat Genome 230 2.0 Array and hybridized for 16 hours at 45°C in a GeneChip Hybridization Oven 640 (Affymetrix). Following hybridization, the arrays were washed and stained with a GeneChip Fluidics Station 450 (Affymetrix) using the Affymetrix Hybridization Wash Stain (HWS) kit. The arrays were laser scanned with a GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA). Data was saved as raw image file and quantified using Affymetrix GeneChip Command Console v 1.0 (Affymetrix).
Data analysis: All data are presented as means ± SEM. Statistical analysis was performed in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Differences among groups were determined using independent t-tests or by ANOVA followed by Bonferroni correction for multiple comparisons. P<0.05 was considered as statistically significant. For whole genome expression profiling analysis, raw data were normalized using GCRMA and analyzed for identification of regulated genes with packages from the BioConductor library in R (www.r-project.org). For identifying differentially expressed genes after treatment with prednisolone, the following selection criteria were used: fold change >2 and p-value<0.05 after correction for multiple testing using the Benjamini-Hochberg correction.

RESULTS

Prednisolone reduces insulin secretion and content in INS-1E cells: Treatment of the insulin-secreting INS-1E cells with prednisolone did not affect basal insulin secretion, but led to a dose- (data not shown) and time-dependent inhibition of the glucose-stimulated insulin secretion (GSIS, Fig. 1a-b), and a 35% reduction in cellular insulin content after 20h (Fig. 1c). Treatment with the GR antagonist RU486 prevented the detrimental effects of prednisolone on both GSIS and insulin content (Fig. 1b-c).

Figure 1. Effects of prednisolone on insulin secretion and content in INS-1E cells. INS-1E cells were incubated with 700 nM prednisolone or vehicle (0.5% v/v DMSO) for the indicated time (A) or 20h in the presence or absence of 1 µM of the GR-antagonist RU486 (B-C). A-B. Glucose-stimulated insulin secretion (GSIS) was determined following incubation with either 2.5 (open circles or bars) or 20 mM glucose (black circles or bars) for 30 min. C. Insulin content was determined in cell lysates after stimulation with 2.5 mM glucose. The results are expressed as means ± SEM (n=4-5). *, p<0.05 vs 2.5 mM glucose; $, p<0.05 vs vehicle.

Prednisolone lowers PDX1 and insulin expression in INS-1E cells: The expression of PDX1, a key regulator of insulin biosynthesis in the beta cell, was lowered by prednisolone as compared to vehicle-treated INS-1E cells (Fig. 2a/b). The reduction in PDX1 expression appeared 4 h after prednisolone addition and reached a maximum at 12 h (Fig. 2b). In line
Figure 2. Effects of prednisolone on PDX1 and insulin expression in INS-1E cells. INS-1E cells were incubated with vehicle (0.5% v/v DMSO) or 700 nM prednisolone for the indicated times. A. Representative immunoblots for PDX1 and insulin protein expression are shown. Insulin receptor β-subunit (IRβ) expression was used as loading control. B-C. Densitometric quantification of immunoblots was performed and the results expressed in % of those from vehicle (DMSO)-treated cells at the same time-point. D-F. PDX1, insulin 1 and 2 mRNA expressions were determined using real time PCR, normalized for HPRT expression using the ∆Ct-method and expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs t0.

Figure 3. Effects of RU486 on prednisolone-induced changes in PDX1 and insulin expression in INS-1E cells. INS-1E cells were incubated with 700 nM prednisolone or vehicle (0.5% v/v DMSO) for 20h in the presence (black bars) or absence (open bars) of 1 µM of the GR-antagonist RU486 as indicated. A. Representative immunoblots for PDX1, insulin and IRβ protein expression are shown. B-C. Densitometric quantifications of immunoblots were performed and the results expressed in arbitrary unit. D-F. PDX1, insulin 1 and 2 mRNA expressions were determined using real time PCR, normalized for HPRT expression using the ∆Ct-method and expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs vehicle.
with the reduction in insulin content measured by ELISA, the insulin protein level was also significantly reduced 12 h following prednisolone addition when compared to vehicle-treated cells (Fig. 2a/c). The reductions in PDX1 and insulin protein expression induced by prednisolone were preceded by rapid decline in PDX1, insulin 1 and insulin 2 mRNA levels, which already became significant 1 h after addition of the drug (Fig. 2d-f). All these prednisolone-induced decrease in PDX1 and insulin mRNA and protein levels were prevented by prior treatment with RU486 (Fig. 3).

Figure 4. Effects of prednisolone and RU486 on ATF6-mediated XBP1 mRNA expression in INS-1E cells. INS-1E cells were incubated with vehicle (0.5% v/v DMSO) or 700 nM prednisolone for the indicated times (A) or for 20h in the presence (black bars) or absence (open bars) of 1 µM of RU486 as indicated (B). A-B. XBP1 mRNA expression was determined using real time PCR, normalized for HPRT expression using the ∆Ct-method and expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs t0 or vehicle.

Prednisolone differently affects endoplasmic reticulum stress pathways in INS-1E cells:
To investigate whether the detrimental effects of prednisolone on insulin biosynthesis could be ascribed to the induction of ER-stress in INS-1E cells, markers of the three main branches of this pathway were determined. Activation of the ATF6 branch of the ER-stress pathway involves cleavage of p90-ATF6 into the active transcription factor p50-ATF6 [16]. Because we failed to detect p50-ATF6 by western blot, we determined the mRNA expression of XBP1, one of the major ATF6-regulated genes. As shown in Fig. 4, mRNA levels of XBP1 were significantly enhanced 12h after exposure to prednisolone, an effect blunted by pre-treatment with RU486. The second well-characterized arm of the ER-stress pathway involved IRE1α activation and subsequent XBP1 mRNA splicing [16]. Interestingly, IRE1α protein expression was significantly increased by prednisolone as compared to vehicle-treated cells (Fig. 5a) and associated with enhanced XBP1s mRNA and protein levels (Fig. 5b-c). Pre-treatment with RU486 prevented the induction of IRE1α and XBP1s by prednisolone in INS-1E cells (Fig. 5d-g). Surprisingly, prednisolone was found to lower the activity of the third branch of the ER-stress pathway. Indeed, phosphorylation of PERK and its substrate eIF2α were significantly
Figure 5. Effects of prednisolone and RU486 on IRE1α/XBP1s pathway in INS-1E cells. INS-1E cells were incubated with vehicle (0.5% v/v DMSO) or 700 nM prednisolone for the indicated times (A,C,E) or for 20h in the presence (black bars) or absence (open bars) of 1 µM of RU486 (B,D,F). A,E. Time-dependent effects of prednisolone were determined on IRE1α and XBP1s by immunoblots and densitometric quantifications. The results are expressed in % of those from vehicle (DMSO)-treated cells at the same time-point. C. Time-dependent effect of prednisolone on XBP1s mRNA expression was determined using real time PCR, normalized for HPRT expression using the ΔCt-method and expressed in arbitrary unit. G. Representative immunoblots for IRE1α, XBP1s and IRβ protein expression are shown. B,F. Densitometric quantifications of IRE1α and XBP1s immunoblots were performed and the results expressed in arbitrary units. D. XBP1s mRNA expression was determined and expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs vehicle.
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reduced 8-12 h following the addition of prednisolone (Fig. 6a-b). This prednisolone-induced dephosphorylation of PERK and eIF2α was prevented by pre-treatment with RU486 (Fig. 6c-e). The prednisolone-induced inactivation of the PERK/eIF2α pathway was accompanied by decrease in mRNA levels of CHOP and TRIB3 downstream target genes (data not shown).

Figure 6. Effects of prednisolone and RU486 on PERK/eIF2α pathway in INS-1E cells. INS-1E cells were incubated with vehicle (0.5% v/v DMSO) or 700 nM prednisolone for the indicated times (A,C) or for 20 h in the presence (black bars) or absence (open bars) of 1 µM of RU486 (B,D,E). A,C. Time-dependent effects of prednisolone were determined on pPERK/Thr980, peIF2α-Ser51 and IRβ by immunoblots and densitometric quantifications. The results are expressed in % of those from vehicle (DMSO)-treated cells at the same time-point. E. Representative immunoblots for pPERK/Thr980, peIF2α-Ser51 and IRβ protein expression are shown. B,D. Densitometric quantifications of pPERK/Thr980 and peIF2α-Ser51 immunoblots were performed and the results expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs vehicle.
Prednisolone increases expression of distal markers of ER-stress pathways and induces apoptosis: Prednisolone did not affect mRNA or protein expression of the ER-chaperone BIP (data not shown). However, using microarray profiling analysis, we found that prednisolone induced a time-dependent upregulation of the ER-associated protein degradation (ERAD) components EDEM1 and MAN1A1 (Fig. 7a-b). Furthermore, we also observed a significant increase in calpain 10 mRNA expression within 4 h after the addition of prednisolone to INS-1E cells (Fig. 7c). This protease is involved in the ER-stress-induced apoptosis via delayed activation of the caspases 3 and 12 [17]. Accordingly, a significant GR-mediated increase in cleaved caspase-3 was found in INS-1E cells treated for 20 h with prednisolone (Fig. 7d-e).

Figure 7. Effects of prednisolone on distal markers of ER stress and apoptosis in INS-1E cells. INS-1E cells were incubated with vehicle (0.5% v/v DMSO) or 700 nM prednisolone for the indicated times (A-D) or for 20 h in the presence (black bars) or absence (open bars) of 1 µM of RU486 (E-F). A-C. Time-dependent effect of prednisolone on EDEM1, MAN1A1 and calpain 10 mRNA expression was determined using Affymetrix profiling. D. Time-dependent effect of prednisolone was determined on cleaved caspase 3 by immunoblots and densitometric quantification was performed. The results are expressed in % of those from vehicle (DMSO)-treated cells at the same time-point. E. Representative immunoblots for cleaved caspase 3 and IRβ protein expression are shown. F. Densitometric quantification of cleaved caspase 3 immunoblots was performed and the results expressed in arbitrary units. All the results are expressed as means ± SEM (n=4). *, p<0.05 vs vehicle.

DISCUSSION

In the present study we report that chronic exposure to prednisolone abrogates GSIS, reduces insulin biosynthesis and induces markers of apoptosis in the insulin-secreting INS-1E cells. Interestingly, our in vitro results provide for the first time evidence that these effects
of prednisolone on beta-cell dysfunction are tightly associated with atypical GR-mediated modulation of the ER stress-induced UPR, as illustrated by concomitant activation of ATF6 and IRE1α and inactivation of PERK/eIF2α signaling pathways.

In response to perturbations of ER homeostasis in beta cells, a coordinated program is activated leading to upregulation of genes enhancing the ER protein processing capacity, such as protein folding, trafficking, and degradation [7-9]. ATF6-dependent transcriptional induction is regulated by ER stress-induced trafficking of ATF6 from the ER to the Golgi where the protein is cleaved by proteases to release a cytosolic active fragment. Although we were not able to detect an effect of prednisolone on ATF6 cleavage, the GR-mediated upregulation of the well-characterized ATF6 downstream target gene XBP1 suggests that this pathway is activated. Of note, prednisolone had no significant effect on XBP1 protein expression (data not shown), suggesting that the XBP1 mRNA is rapidly cleaved into XBP1s by IRE1α. Interestingly, nutrient or chemical ER stress-mediated activation of ATF6 has been shown to impair insulin expression and secretion in INS-1E cells, at least in part via suppression of PDX1 expression [18]. In addition to mitigating ER stress by increasing the transcription of key proteins involved in the regulation of ER folding capacity, IRE1α also lowers ER protein load by degrading mRNA of ER-targeted proteins [19]. In beta cells, IRE1α was shown to be directly involved in the degradation of insulin mRNA in response to ER stress [20-22]. Furthermore, accumulation of the transcription factor XBP1s has also been linked to beta-cell dysfunction by reducing both insulin and PDX1 expression, and promoting apoptotic beta-cell death [23]. An additional explanation for the rapid decrease in insulin mRNA level induced by prednisolone could be the presence of a GC responsive element in the promoter region of the gene, which acts as a negative regulatory site [24, 25]. However, although this could contribute to the rapid downregulation of insulin expression, it can not explain the similar rapid decline in PDX1 mRNA observed. Interestingly, in our experimental conditions, the prednisolone-induced reduction of PDX1 and insulin mRNA levels preceded the onset of the ER-stress response. Therefore, it is possible that the ER-stress response is triggered by an increased demand for insulin synthesis caused by the initial decline in insulin and PDX1 mRNA levels. Furthermore, PDX1 deficiency also enhances beta-cell susceptibility to ER stress-associated apoptosis [26].

Another cellular strategy to alleviate ER stress is the reduction in protein influx by reducing protein translation, a process which is classically mediated by activation of PERK and subsequent phosphorylation of its downstream target eIF2α [7-9]. One of our most puzzling results is that prednisolone, by contrast to its activating effect on ATF6 and IRE1α pathways, decreases the phosphorylation of PERK and eIF2α, suggesting a GR-mediated increase in the
activity of protein phosphatase(s) involved in the dephosphorylation of these key proteins. Although we did not find any change in protein phosphate 1 expression (data not shown), which has been implicated in the dephosphorylation of eIF2α [7-9], we cannot exclude more subtle enzymatic regulation in response to prednisolone and/or involvement of upstream phosphatases targeting PERK. It should however be mentioned that the exact mechanism(s) by which the PERK/eIF2α pathway is regulated during ER stress is still not entirely clear. For example, similar counter-intuitive observations were recently made in activated B cells where phosphorylation of the PERK/eIF2α pathway was reduced during ER stress and did not correlate with cellular protein synthesis rate [27]. In addition, loss of PERK function has also been reported not to affect protein synthesis but rather to impair ER-to-Golgi protein trafficking and proteasomal degradation [28].

In relation to signaling pathways attenuating protein translation, crosstalk between GR and the nutritional sensor mammalian of rapamycin (mTOR) has been recently highlighted in muscle, identifying Regulated in Development and DNA damage responses 1 (REDD1), an upstream negative regulator of mTOR, as a direct GR-targeted gene [29]. Interestingly, in our conditions, prednisolone induced significant upregulation of REDD1 and concomitantly reduced mTORC1 activity in INS-1E cells (MML/DMO, unpublished data), suggesting that modulation of this pathway could contribute to the GR-mediated reduction in insulin biosynthesis by decreasing global protein synthesis. Further experiments are still required to clarify this point.

One of the limitations of our findings could eventually be the concentration of prednisolone used in the present study (700 nM), which is ~3-4 fold higher than the plasma therapeutic level reported in humans treated with the drug [30]. However, significant effects of prednisolone on insulin secretion/biosynthesis and ER stress signaling pathways are already present at lower concentrations (data not shown). To note, similar time- and dose-dependent GR-mediated effects were also observed in INS-1E cells treated with dexamethasone, another GC compound (MML/DMO, unpublished data).

In a recent clinical study, we have reported that treatment with the glucagon-like peptide-1 (GLP-1) receptor agonist exenatide (exendin-4) prevented prednisolone-induced beta-cell dysfunction in healthy men [31]. Although the underlying molecular mechanism(s) remain(s) to be elucidated, it is striking that exendin-4 was previously shown to decrease genetically- or pharmacologically-induced ER stress and to improve beta-cell function and survival in mice, isolated islets and INS-1E cells [32, 33]. In addition, exendin-4 prevented GC-induced apoptosis in INS-1E cells [6]. Taken together, it is therefore tempting to suggest that the beneficial effect of exenatide evidenced on beta cell functions in humans could be ascribed,
at least in part, to a reduction in prednisolone-induced pancreatic ER stress [31]. Further in vitro studies are required to investigate whether exendin-4 is able to prevent prednisolone-induced ER stress and beta-cell dysfunction.

In conclusion, we report here that prednisolone increases ER stress in INS-1E cells, promoting activation of selective UPR signaling pathways which leads to decreased insulin expression. Taken together, our data suggest that the inhibition of GSIS could be ascribed, at least in part, to a prednisolone-induced decrease in insulin biosynthesis resulting from GR-mediated impairment in ER homeostasis and apoptotic cell death.

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REFERENCES


### SUPPLEMENTARY FILES

**Supplementary Table 1. List of Antibodies**

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**Supplementary Table 2. Primer sequences**

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