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Cortisol controls endoplasmic reticulum stress and hypoxia dependent regulation of insulin receptor and related genes expression in HEK293 cells

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Objective. Glucocorticoids are important stress-responsive regulators of insulin-dependent metabolic processes realized through specific changes in genome function. The aim of this study was to investigate the impact of cortisol on insulin receptor and related genes expression in HEK293 cells upon induction the endoplasmic reticulum (ER) stress by tunicamycin and hypoxia.

Methods. The human embryonic kidney cell line HEK293 was used. Cells were exposed to cortisol (10 μM) as well as inducers of hypoxia (dimethyloxalylglycine, DMOG; 0.5 mM) and ER stress (tunicamycin; 0.2 μg/ml) for 4 h. The RNA from these cells was extracted and reverse transcribed. The expression level of *INSR*, *IRS2*, and *INSIG2* and some ER stress responsive genes encoding XBP1n, non-spliced variant, XBP1s, alternatively spliced variant of XBP1, and DNAJB9 proteins, was measured by quantitative polymerase chain reaction and normalized to ACTB.

Results. We showed that exposure of HEK293 cells to cortisol elicited up-regulation in the expression of *INSR* and *DNAJB9* genes and down-regulation of XBP1s, XBP1n, IRS2, and INSIG2 mRNA levels. At the same time, induction of hypoxia by DMOG led to an up-regulation of the expression level of most studied mRNAs: XBP1s and XBP1n, IRS2 and INSIG2, but did not change significantly *INSR* and *DNAJB9* gene expression. We also showed that combined impact of cortisol and hypoxia introduced the up-regulation of INSR and suppressed XBP1n mRNA expression levels. Furthermore, the exposure of HEK293 cells to tunicamycin affected the expression of IRS2 gene and increased the level of XBP1n mRNA. At the same time, the combined treatment of these cells with cortisol and inductor of ER stress had much stronger impact on the expression of all the tested genes: strongly increased the mRNA level of ER stress dependent factors XBP1s and DNAJB9 as well as INSR and INSIG2, but down-regulated IRS2 and XBP1n.

Conclusion. Taken together, the present study indicates that cortisol may interact with ER stress and hypoxia in the regulation of ER stress dependent *XBP1* and *DNAJB9* mRNA expression as well as INSR and its signaling and that this corticosteroid hormone modified the impact of hypoxia and especially tunicamycin on the expression of most studied genes in HEK293 cells. These data demonstrate molecular mechanisms of glucocorticoids interaction with ER stress and insulin signaling at the cellular level.

Keywords: cortisol, tunicamycin, hypoxia, XBP1s, insulin receptor, IRS2, INSIG2, DNAJB9, HEK293

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Glucocorticoids are primary stress hormones, which are involved in the control of metabolism, development, stress, and inflammatory responses through multiple mechanisms of the regulation of gene transcription within a physiological context (Zanchi et al. 2010; Kadmiel and Cidlowski 2013; Ratman et al. 2013; de Guia and Herzig 2015; Frank et al. 2021a,b). The diverse array of biological activity of glucocorticoids is realized through interaction with the specific for glucocorticoids receptor. This receptor molecule represents a steroid hormone-activated transcription factor that binds to various glucocorticoid response elements to up- or down-regulate the transcription of thousands of genes (Burnstein and Cidlowski 1989; Hayashi et al. 2004; Psarra and Sekeris 2011; Frank et al. 2021b). The glucocorticoid receptor function is modulated by various coregulators (Subramaniam et al. 1999; Windahl et al. 1999; McKay and Cidlowski 2000; Kurihara et al. 2002; Dvorak et al. 2008; Qin et al. 2015; Kubli et al. 2019; Leonardi et al. 2019).

It is very important to note that glucocorticoids strongly interact with insulin receptor signaling pathways through various mechanisms (Minchenko and Germanyuk 1984; Uchoa et al. 2014; Vitellius et al. 2018). Glucocorticoids usually counteract insulin-mediated regulation of gene expression (Buyse et al. 2001; van Raalte et al. 2012). The glucocorticoids affect insulin sensitivity events resulting from the resistance to insulin action at the level of skeletal muscle, adipose tissue, and liver, described as the known mechanisms of glucocorticoid hormone-mediated insulin resistance, and provide an update of the contributions of glucocorticoids to understanding the pathophysiology of insulin resistance (Yuen et al. 2013; Beaupere et al. 2021; Kokkinopoulou et al. 2021). Previously, we have demonstrated that insulin controls the metabolism of glucocorticoids and that the metabolism of these hormones in diabetes is strongly suppressed (Minchenko and Tronjko 1988). This phenomenon possibly contributes to the enhanced sensitivity of metabolic processes in diabetes to glucocorticoid hormones.

Binding of insulin or other ligands to insulin receptor (INSR), which is a member of the receptor tyrosine kinase family of proteins, activates the insulin signaling pathway, which mediates the pleiotropic actions of insulin through phosphorylation of several intracellular substrates including insulin receptor substrates (IRS) and other signaling intermediates. The insulin signaling pathway plays a key role in metabolic regulation and growth control (Payankaulam et al. 2019). In addition, to the classical roles associated with cell-surface receptors, new data are consistent with nuclear (genomic) role for both INSR and IGF1R. The increase of INSR chromatin binding by insulin has a major basic and translational impact on the fields of metabolism, diabetes, and cancer (Sarfstein and Werner 2013; Hancock et al. 2019). Importantly, insulin receptor stimulation induces transcription of genes encoding important enzymes of glycolysis and lipogenesis and inhibits genes encoding essential enzymes of gluconeogenesis. Overall, the activation or inhibition of insulin-responsive transcription factors is an important aspect of orchestrating a wide range of insulin-induced changes in the biochemistry and physiology of insulin-responsive tissues (Thiel et al. 2021). Furthermore, insulin induced gene *INSIG2* mediates activation of the PERK signaling pathway of ER stress (Watanabe et al. 2021). These data indicate the dependence of one of the signaling pathways of ER stress on insulin.

Endoplasmic reticulum (ER) stress and hypoxia are important factors for support homeostasis in health and disease and play also an important role in cancerogenesis (Minchenko et al. 2002; Moenner et al. 2007; Denko 2008; Auf et al. 2010, 2013; Bravo et al. 2013; Lenihan and Taylor 2013; Manie et al. 2014; Chevet et al. 2015; Batie and Rocha 2020). Previously, we have shown that hypoxia controls the expression of most genes through HIF transcription factor and that hypoxic regulation of numerous genes is significantly modified by inhibition of ERN1/IRE1a (Minchenko et al. 2013, 2015, 2016a, b, 2017, 2019; Tsymbal et al. 2017). These data have clearly demonstrated that ER stress controls the hypoxic regulation of gene expression. There are data indicating that glucocorticoids can also participate in tumor growth and that glucocorticoid receptor (NR3C1) expression is controlled by ERN1 signaling pathway of ER stress (Pufall 2015; Thomas et al. 2015; Minchenko et al. 2016b; Cao et al. 2019; Kubli et al. 2019; Leonardi et al. 2019). Previously we have also shown that INSR and some related proteins are controlled by ER stress (Minchenko et al. 2013). It is very important to find interconnection of glucocorticoids, which are primary stress hormones, and insulin signaling with ER stress and hypoxia, which accompany most pathological processes, and identify possible molecular mechanisms. In order to test the effect of low dose of tunicamycin (0.2 μg/ml) on human embryonic kidney cell line HEK293, we studied its impact on the expression of ER stress dependent genes encoding XBP1 and DNAJB9, including alternative spliced variant of XBP1 (XBP1s). XBP1s is strongly

up-regulated upon ER stress and responsible for inducing the expression of hundreds of genes, but is also controlled by specific protein kinases and phosphatases (Shen et al. 2002; Acosta-Alvear et al. 2007; Papaioannou et al. 2022). Recently it was shown that RtcB, the tRNA ligase responsible for XBP1 mRNA splicing, is tyrosine-phosphorylated by c-Abl and dephosphorylated by PTP1B (Papaioannou et al. 2022). Moreover, the phosphorylation of RtcB perturbs its interaction with ERN1, thereby attenuating XBP1 mRNA splicing.

The aim of this study was to examine the impact of cortisol on the expression of insulin receptor and related genes encoding IRS2 and INSIG2 as well as some ER stress dependent proteins XBP1 and DNAJB9 [DnaJ heat shock protein family (Hsp40) member B9] upon hypoxia and induction of ER stress by tunicamycin in HEK293 cells for clarification of possible molecular mechanisms of the interaction of glucocorticoids with ER stress and hypoxia at cellular level.

Materials and Methods

Cell culture and treatment. The human embryonic kidney cell line HEK293 (CRL-1573) were obtained from ATCC (USA) and grown in low glucose Dulbecco's modified Eagle's minimal essential medium (DMEM; Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco) and streptomycin (0.1 mg/ml; Gibco) at 37°C in incubator with 5% CO_2. Cells were exposed to cortisol (10 μM) as well as to inducers of hypoxia (dimethyloxalylglycine, DMOG; 0.5 mM) and ER stress (tunicamycin, 0.2 μg/ml) for 4 h. Cortisol was received from Sigma-Aldrich (St. Louis, MO, USA) as hydrocortisone-water soluble BioReagent suitable for cell culture (cyclodextrin-encapsulated hydrocortisone, H0396). Tunicamycin was also received from Sigma-Aldrich.

RNA extraction and cDNA synthesis. The RNA from HEK293 cells was extracted using TRIZOL reagent as described previously (Auf et al. 2013; Rudnytska et al. 2021). The RNA pellets were washed with 75% ethanol and dissolved in the nuclease-free water. For an additional purification, the RNA samples were re-precipitated with 95% ethanol containing sodium acetate and the pellets were again dissolved in nuclease-free water. The RNA quantity and spectral characteristics were measured using NanoDrop One (Thermo Scientific). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Rudnytska et al. 2021).

Real-time quantitative polymerase chain reaction. The expression levels of INSR, IRS2, INSIG2, DNAJB9, and XBP1 (both alternative spliced and not spliced variants) mRNAs as well as ACTB mRNA were measured in HEK293 cells by quantitative polymerase chain reaction using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Polymerase chain reaction was performed in triplicate. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The pair of primers specific for each studied gene was received from Sigma-Aldrich and Metabion Int. AG (Martinstried, Germany) and used

Table 1
Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide number in sequence	GeneBank accession number
INSR	Insulin receptor	F: 5'- caacctcggcctcattgaag R: 5'- ccctgagtgatggtgaggtt	1672–1691 1875–1856	NM_000208.4
IRS2	Insulin receptor substrate 2	F: 5'- ctgcagctcatgaacatccg R: 5'- gacgattggctcttactgcg	1248–1267 1456–1437	NM_003749.3
INSIG2	Insulin induced gene 2	F: 5'- acactggctgcactatccat R: 5'- cattgccagttgtcgaccaa	615–634 845–826	NM_016133.4
DNAJB9 (ERdj4)	DnaJ heat shock protein family (Hsp40) member B9 (endoplasmic reticulum DnaJ homolog 4)	F: 5'- ctgtggaggagcagcagtag R: 5'- ccttcttgatttggcgctct	198–217 373–354	NM_012328.3
XBP1n	X-box binding protein 1 (not spliced variant)	F: 5'- gctgagtccgcagcactcag R: 5'- caataccgccagaatccatg	527–572 627–608	NM_005080.4
XBP1s	X-box binding protein 1 (alternative spliced variant)	F: 5'- ctgctgagtccgcagcaggt R: 5'- caataccgccagaatccatg	529–548 627–608	NM_005080.4
ACTB	beta-actin	F: 5'- ggacttcgagcaagagatgg R: 5'- agcactgtgttggcgtacag	747–766 980–961	NM_001101

for quantitative polymerase chain reaction (Table 1).

The amplification product quality was analyzed by melting curves and by electrophoresis in 3% agarose gel. An analysis of quantitative PCR was performed using special computer program "Differential Expression Calculator". The mRNA expressions values were normalized to the level of ACTB mRNA and represented as a percentage of control (100 %).

Statistical analysis. All values are expressed as mean±SEM from triplicate measurements performed in 4 independent experiments. Statistical analysis was performed according to Student's t-test using Excel program as described previously (Rudnytska et al. 2021). A value of $p<0.05$ was considered to be statistically significant.

Results

We investigated the impact of cortisol (hydrocortisone-water soluble BioReagent; 10 μM for 4 h) on the expression of INSR (insulin receptor), IRS2 (insulin receptor substrate 2), INSIG2 (insulin-induced gene 2), DNAJB9 (DnaJ heat shock protein family (Hsp40) member B9), also known as ERdj4 (endoplasmic reticulum DnaJ homolog 4), and XBP1 (both X-box binding protein 1 alternative spliced and non-spliced variants) mRNA in human embryonic kidney cell line HEK293 treated by inducer of hypoxia (DMOG, 0.5 mM) and by inducer of ER stress (tunicamycin, 0.2 μg/ml) for 4 h. In order to test the effect of low dose of tunicamycin on human embryonic kidney cell line HEK293, we studied the expression of ER stress dependent genes *XBP1* and *DNAJB9*. Figure 1 demonstrates that tunicamycin at low dose increases the expression of XBP1n mRNA (non-spliced variant XBP1; +49%) and that inducer of hypoxia has similar effect on this mRNA expression (+51%). At the same time, the expression level of alternative spliced variant of XBP1 (XBP1s) does not change significantly in HEK293 cells treated by low dose of tunicamycin, but it is increased in the cells treated by hypoxia (+14%) (Figure 2). We also determine the impact of tunicamycin on the expression of gene encoding DNAJB9, which is an ER stress inducible protein. As shown in Figure 3, exposure of HEK293 cells to tunicamycin as well as to DMOG does not change significantly the expression of gene encoding DNAJB9 in HEK293 cells.

Additionally, we found that exposure of HEK293 cells with cortisol leads to down-regulation of both XBP1n and XBP1s mRNA expression (–13 and –14%, correspondingly) and up-regulation of DNAJB9 (+41%) (Figures 1–3). At the same time, we also shown that joint effect of tunicamycin with cortisol introduced much stronger changes in the expression levels of all these genes especially XBP1s (+1366%) and DNAJB9 (+305%) in HEK293 cells (Figures 2 and 3). Moreover, a sharp increase in the expression level of XBP1s in cells after joint treatment by tunicamycin with cortisol was accompanied by decrease of XBP1n mRNA (–55%) in relation to the cells treated by tunicamycin only (Figures 1 and 2). Thus, cortisol

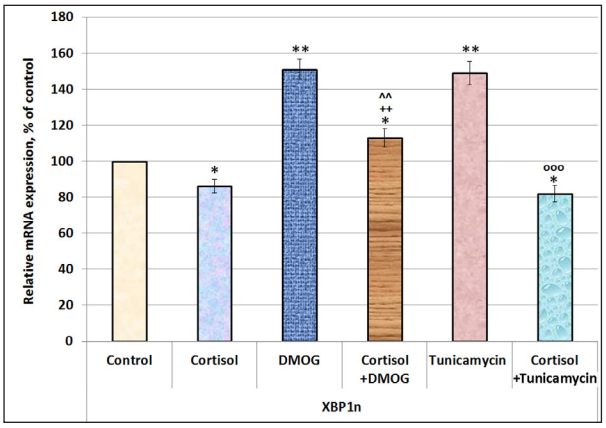


Figure 1. The impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG) on the expression of X-box binding protein 1, alternative not spliced variant (*XBP1n*) in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; ^ – compared to DMOG; + – compared to cortisol; 1, 2, and 3 symbols – $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

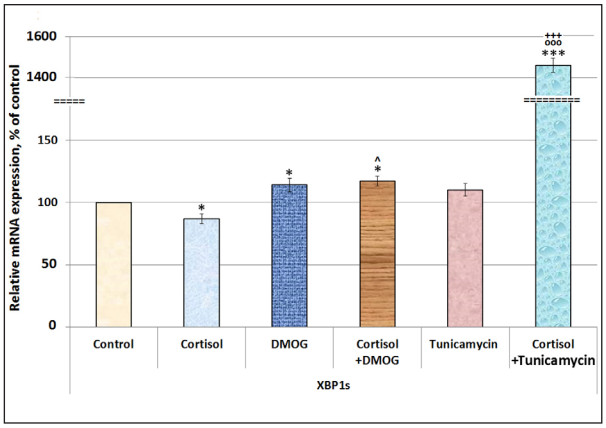


Figure 2. The impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG) on the expression of X-box binding protein 1, alternative spliced variant (*XBP1s*) in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; ^ – compared to DMOG; + – compared to cortisol; 1, 2, and 3 symbols – $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

strongly increased effect of tunicamycin on the expression levels of XBP1s and DNAJB9 mRNAs. At the same time, cortisol does not change the impact of DMOG on the expression levels of XBP1s and DNAJB9 in HEK293 cells after joint treatment by DMOG with cortisol, but significantly suppressed XBP1n mRNA expression (–25%) in relation to the cells treated by DMOG only (Figures 1–3).

Thereafter, we tested the impact of tunicamycin and DMOG on the expression level of INSR mRNA in HEK293 cells. It was shown that the exposure of these cells with DMOG does not significantly change the expression level of INSR mRNA, however, treatment of cells with tunicamycin introduces small but statistically significant increase in this mRNA level (+13%) as compared to control cells (Figure 4). Furthermore, treatment of HEK293 cells by cortisol also lead to increased expression of INSR mRNA (+15%), but more significant changes were observed in these cells after treatment of cells by cortisol in combination with DMOG (+41%) or tunicamycin (+44%) (Figure 4). It is possible that cortisol increases sensitivity of insulin receptor gene expression to hypoxia and ER stress.

We also studied the expression of insulin receptor dependent genes *IRS2* and *INSIG2* in HEK293 cells treated with DMOG and shown that hypoxia up-regulates these gene expressions (+22 and +17%, correspondingly), but tunicamycin does not change significantly the expression of INSIG2 gene and up-regulate IRS2 (+13%) as compared to control cells (Figure 5 and 6). At the same time, treatment of HEK293 cells by cortisol in combination with tunicamycin completely eliminates the impact of tunicamycin; however, significantly increases the expression level of INSIG2 mRNA in relation to the appropriate control groups: both tunicamycin (+19%) and cortisol (+97%) only (Figure 5 and 6). Furthermore, treatment of HEK293 cells by cortisol in combination with DMOG introduced changes in the expression levels IRS2 mRNA (+18%) as compared to control cells, which was similar to that introduced by DMOG only (Figure 5). At the same time, in case of INSIG2 mRNA, its expression is down-regulated (–12%) after joint treatment of HEK293 cells by cortisol with DMOG in relation to the treatment by DMOG only, but it is much higher (+56%) than in cells treated by cortisol only (Figure 6). Therefore, treatment of HEK293 cells by cortisol up-regulates the expression level of INSR and decreases IRS2 and INSIG2 mRNAs. Furthermore, cortisol increases the sensitivity of *INSR* gene to hypoxia and ER stress in HEK293 cells, but decreases ER stress dependent control of *IRS2* gene showing suppression of insulin receptor-mediated signaling.

Discussion

In this work, we investigated the impact of cortisol (hydrocortisone-water soluble BioReagent; 10 μM for 4 h) on the expression of mRNA encoding insulin receptor (INSR), IRS2, insulin induced

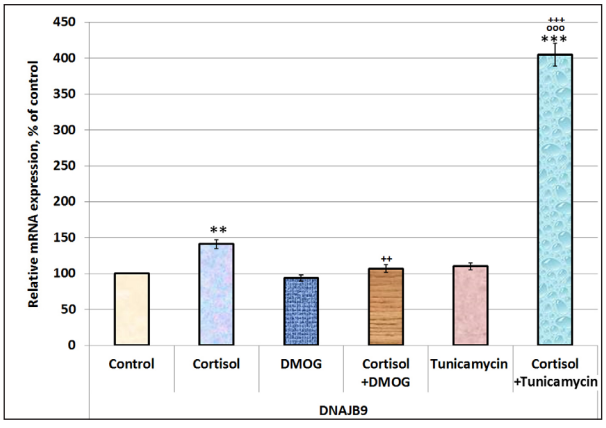


Figure 3. The impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG) on the expression of DnaJ heat shock protein family (Hsp40) member B9 (*DNAJB9*) in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; + – compared to cortisol; 1, 2, and 3 symbols – p<0.05, p<0.01, and p<0.001, respectively.

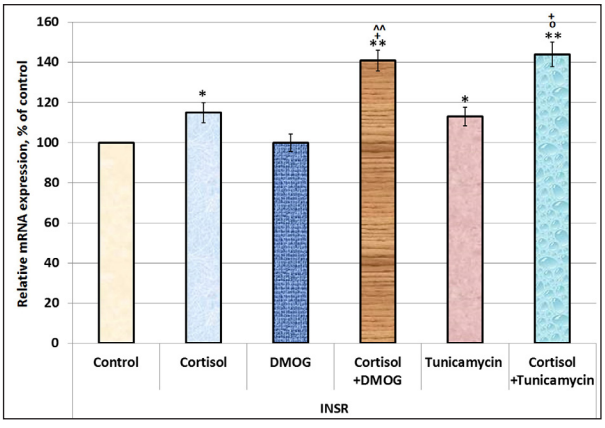


Figure 4. The impact of cortisol, tunicamycin (ER stress inducer), and dimethyloxalylglycine (DMOG, hypoxia inducer) on the expression of insulin receptor (*INSR*) gene in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; ^ – compared to DMOG; + – compared to cortisol; 1, 2, and 3 symbols – p<0.05, p<0.01, and p<0.001, respectively.

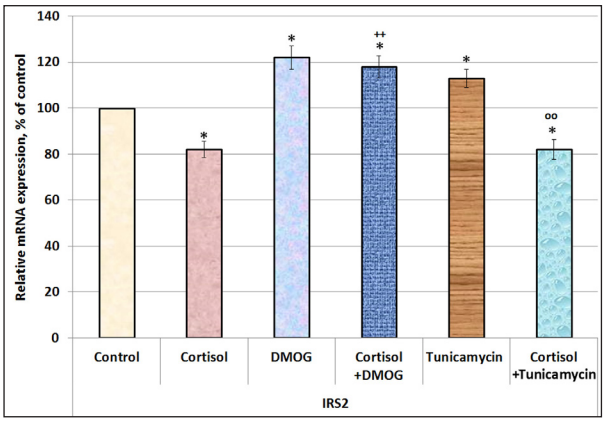


Figure 5. The impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG) on the expression of insulin receptor substrate 2 (*IRS2*) gene in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; + – compared to cortisol; 1, 2, and 3 symbols – p<0.05, p<0.01, and p<0.001, respectively.

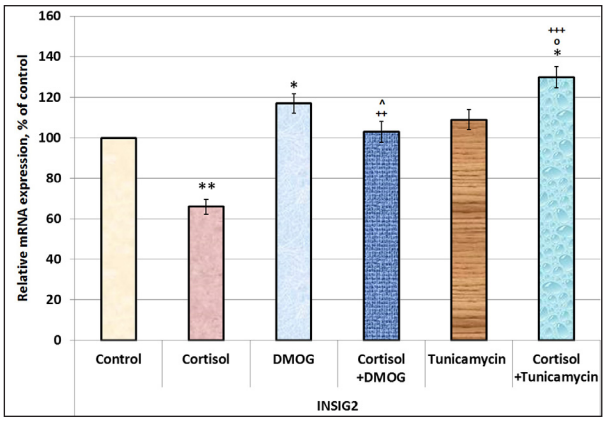


Figure 6. The impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG) on the expression of insulin induced gene 2 (*INSIG2*) in the human embryonic kidney cell line HEK293. Values of this mRNA level were normalized to the ACTB mRNA level; n=4. * – compared to control; o – compared to tunicamycin; ^ – compared to DMOG; + – compared to cortisol; 1, 2, and 3 symbols – p<0.05, p<0.01, and p<0.001, respectively.

gene 2 (INSIG2), DnaJ heat shock protein family (Hsp40) member B9 (DNAJB9 or ERdj4), and two variants (alternative spliced and basic, non-spliced) of transcription factor XBP1 in human embryonic kidney cell line HEK293 treated by inducer of hypoxia DMOG (0.5 mM) and inducer of ER stress tunicamycin (0.2 μg/ml) for 4 h. Results of this study are summarized in Figure 7. In order to test the effect of this low dose of tunicamycin on this human embryonic cell line, we studied the expression of ER stress dependent genes encoding two variants of XBP1 (XBP1n and XBP1s) and DNAJB9 and shown that only XBP1n mRNA is up-regulated at this experimental condition. There are data that tunicamycin introduced ER stress and enhanced alternative splicing XBP1 but this effect of tunicamycin varies in different cells and is time- and dose-dependent (Auf et al. 2013; Logue et al. 2018; Papaioannou et al. 2022). Furthermore, in different cancer cell lines, the level of XBP1s is highly variable (Papaioannou et al. 2022). It is possible that XBP1s cannot be a clear indicator of ER stress. In this work, we used not tumor but embryonic cells, which might be the reason why they were resistant to low dose of tunicamycin.

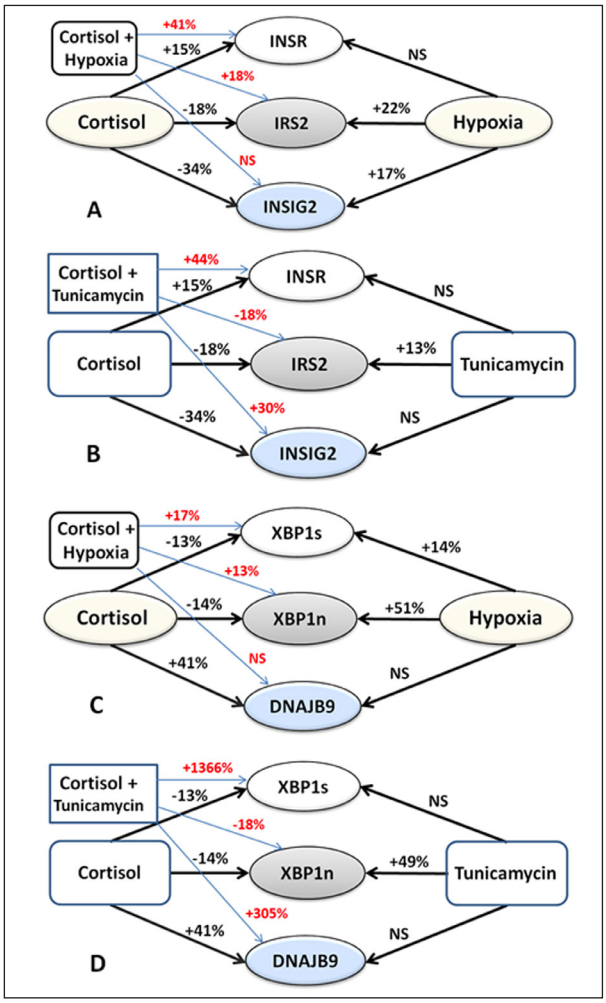


Figure 7. Schematic representation of the impact of cortisol, tunicamycin, and dimethyloxalylglycine (DMOG, hypoxia) separately or in combination on the expression of INSR, IRS2, and INSIG2 (**A** and **B**) as well as on DNAJB9, XBP1s, and XBP1n (**C** and **D**) mRNAs in the human embryonic kidney cell line HEK293.

The unique result of this study is the detection of a sharp enhancing effect of glucocorticoid hormone on the induction of alternative splicing of transcription factor XBP1 by low dose of tunicamycin, although cortisol itself reduced its expression. Moreover, we showed that cortisol also strongly enhanced the expression of DNAJB9 and we do not know yet whether this was due to changes in XBP1s or through other signaling pathways of ER stress. DNAJB9 is a very important co-chaperone, which binds of HSPA5/BiP and stimulates its intrinsic ATPase activity, thereby providing the energy necessary for (re-)folding of proteins, or for targeting of misfolded proteins to the degradation pathway (Fischer and Prols 2021).

The strengthening effect of cortisol on the impact of tunicamycin was also revealed when studying the expression level of INSR in HEK293 cells. Moreover, this glucocorticoid hormone also increased the effect of hypoxia on insulin receptor mRNA expression (Figures 7A and B). At the same time, cortisol down-regulated the expression of IRS2 and INSIG2 in HEK293 cells, but enhanced the impact of tunicamycin on INSIG2 expression only. We also showed that joint treatment of HEK293 cells by cortisol with tunicamycin leads to suppression of this ER stress inducer action on IRS2 mRNA expression. The results agree well with numerous data concerning biological activity of glucocorticoid hormones, which is realized through interaction with specific for glucocorticoids receptor and regulation the transcription of thousands of genes, including insulin receptor signaling pathways through various mechanisms (Minchenko and Germanyuk 1984; Burnstein and Cidlowski 1989; Hayashi et al. 2004; Psarra and Sekeris 2011; Uchoa et al. 2014; Vitellius et al. 2018; Frank et al. 2021b). It is interesting to note that glucocorticoids usually counteract with insulin-mediated regulation of gene expression and affect insulin sensitivity (Buyse et al. 2001; van Raalte et al. 2012; Yuen et al. 2013; Beaupere et al. 2021; Kokkinopoulou et al. 2021). Although we obtained an increase in the mRNA expression level of the insulin receptor under the action of cortisol in combination with tunicamycin, the effect of this hormone on signaling pathways of the insulin receptor is suppressive (Figure 7). Thus, our results clearly demonstrate that cortisol counteracts with insulin-mediated regulation of IRS2 and INSIG2 mRNA expression and strongly suppresses the impact of tunicamycin on IRS2. However, detailed molecular mechanism of the participation of cortisol in the stress-dependent regulation of gene expression requires further more detailed studies.

Conclusion

The present study indicated that cortisol as a primary stress hormone interacts with ER stress signaling pathways and hypoxia in the regulation of ER stress dependent *XBP1* and *DNAJB9* gene expressions as well as INSR, its substrate IRS2 and insulin induced protein 2 and that cortisol modified the impact of hypoxia and especially tunicamycin on the expression of most studied genes in HEK293 cells. These data demonstrate some molecular mechanisms of the interaction of glucocorticoids with ER stress, hypoxia, and insulin signaling at the cellular level.

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Conflict of Interest: *The authors reported no conflict of interest.*

References

Acosta-Alvear D, Zhou Y, Blais A, Tsikitis M, Lents NH, Arias C, Lennon CJ, Kluger Y, Dynlacht DD. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Molecular Cell 27, 53–66, 2007.

Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Bouchecareilh M, Favereaux A, Maitre M, Gaiser T, von Deimling A, Czabanka M, Vajkoczy P, Chevet E, Bikfalvi A, Moenner M. A shift from an angiogenic to invasive phenotype induced in malignant glioma by inhibition of the unfolded protein response sensor IRE1. Proc Natl Acad Sci U S A 107, 15553–15558, 2010.

Auf G, Jabouille A, Delugin M, Guerit S, Pineau R, North S, Platonova N, Maitre M, Favereaux A, Vajkoczy P, Seno M, Bikfalvi A, Minchenko D, Minchenko O, Moenner M. High epiregulin expression in human U87 glioma cells relies on IRE1alpha and promotes autocrine growth through EGF receptor. BMC Cancer 13, 597, 2013.

Batie M, Rocha S. Gene transcription and chromatin regulation in hypoxia. Biochem Soc Trans 48, 1121–1128, 2020.

Beaupere C, Liboz A, Feve B, Blondeau B, Guillemain G. Molecular mechanisms of glucocorticoid-induced insulin resistance. Int J Mol Sci 22, 623, 2021.

Bravo R, Parra V, Gatica D, Rodriguez AE, Torrealba N, Paredes F, Wang ZV, Zorzano A, Hill JA, Jaimovich E, Quest AF, Lavandero S. Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. Int Rev Cell Mol Biol 301, 215–290, 2013.

Burnstein KL, Cidlowski JA. Regulation of gene expression by glucocorticoids. Annu Rev Physiol 51, 683–699, 1989.

Buyse M, Viengchareun S, Bado A, Lombes M. Insulin and glucocorticoids differentially regulate leptin transcription and secretion in brown adipocytes. FASEB J 15, 1357–1366, 2001.

Cao H, Xu Z, Wang J, Cigliano A, Pilo MG, Ribback S, Zhang S, Qiao Y, Che L, Pascale RM, Calvisi DF, Chen X. Functional role of SGK3 in PI3K/Pten driven liver tumor development. BMC Cancer 19, 343, 2019.

Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. Cancer Discov 5, 586–597, 2015.

de Guia RM, Herzig S. How do glucocorticoids regulate lipid metabolism? Adv Exp Med Biol 872, 127–144, 2015.

Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer 8, 705–713, 2008.

Dvorak Z, Vrzal R, Pavek P, Ulrichova J. An evidence for regulatory cross-talk between aryl hydrocarbon receptor and glucocorticoid receptor in HepG2 cells. Physiol Res 57, 427–435, 2008.

Fischer L, Prols F. The function of the co-chaperone ERdj4 in diverse (patho-)physiological conditions. Daverkausen-Cell Mol Life Sci 79, 9, 2021.

Frank F, Liu X, Ortlund EA. Glucocorticoid receptor condensates link DNA-dependent receptor dimerization and transcriptional transactivation. Proc Natl Acad Sci U S A 118, e2024685118, 2021a.

Frank F, Ortlund EA, Liu X. Structural insights into glucocorticoid receptor function. Biochem Soc Trans 49, 2333–2343, 2021b.

Hancock ML, Meyer RC, Mistry M, Khetani RS, Wagschal A, Shin T, Ho Sui SJ, Naar AM, Flanagan JG. Insulin receptor associates with promotors genome-wide and regulates gene expression. Cell 177, 722–736.e22, 2019.

Hayashi R, Wada H, Ito K, Adcock IM. Effects of glucocorticoids on gene transcription. Eur J Pharmacol 500, 51–62, 2004.

Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci 34, 518–530, 2013.

Kokkinopoulou I, Diakoumi A, Moutsatsou P. Glucocorticoid receptor signaling in diabetes. Int J Mol Sci 22, 11173, 2021.

Kubli SP, Bassi C, Roux C, Wakeham A, Gobl C, Zhou W, Jafari SM, Snow B, Jones L, Palomero L, Thu KL, Cassetta L, Soong D, Berger T, Ramachandran P, Baniasadi SP, Duncan G, Lindzen M, Yarden Y, Herranz C, Lazaro C, Chu MF, Haight J, Tinto P, Silvester J, Cescon DW, Petit A, Pettersson S, Pollard JW, Mak TW, Pujana MA, Cappello P, Gorrini C. AhR controls redox homeostasis and shapes the tumor microenvironment in BRCA1-associated breast cancer. Proc Natl Acad Sci USA 116, 3604–3613, 2019.

Kurihara I, Shibata H, Suzuki T, Ando T, Kobayashi S, Hayashi M, Saito I, Saruta T. Expression and regulation of nuclear receptor coactivators in glucocorticoid action. Mol Cell Endocrinol 189, 181–189, 2002.

Lenihan CR, Taylor CT. The impact of hypoxia on cell death pathways. Biochem Soc Trans 41, 657–663, 2013.

Leonardi DB, Anselmino N, Brandani JN, Jaworski FM, Paez AV, Mazaira G, Meiss RP, Nunez M, Nemirovsky SI, Giudice J, Galigniana M, Pecci A, Gueron G, Vazquez E, Cotignola J. Heme oxygenase 1 impairs glucocorticoid receptor activity in prostate cancer. Int J Mol Sci 20, E1006, 2019.

Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, Chevet E, Dwyer RM, Oommen A, Legembre P, Godey F, Madden EC, Leuzzi B, Obacz J, Zeng Q, Patterson JB, Jager R, Gorman AM, Samali A. MKC8866 Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. Nat Commun 9, 3267, 2018.

McKay LI, Cidlowski JA. CBP (CREB binding protein) integrates NF-kappaB (nuclear factor-kappaB) and glucocorticoid receptor physical interactions and antagonism. Mol Endocrinol 14, 1222–1234, 2000.

Manie SN, Lebeau J, Chevet E. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 3. Orchestrating the unfolded protein response in oncogenesis: an update. Am J Physiol Cell Physiol 307, C901–C907, 2014.

Minchenko AG, Germanyuk YL. Effect of hydrocortisone on the expression of mitochondrial genes in the liver of normal and alloxan diabetic rats. Endocrinol Exper 18, 3–18, 1984.

Minchenko AG, Tronjko ND. Subcellular distribution of 3H-hydrocortisone and its metabolites in the liver and kidneys of normal and alloxan diabetic rats. Endocrinol Exper 22, 19–28, 1988.

Minchenko AG, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead VE, Caro J. Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. J Biol Chem 277, 6183–6187, 2002.

Minchenko DO, Kharkova AP, Hubenia OV, Minchenko OH. Insulin receptor, IRS1, IRS2, INSIG1, INSIG2, RRAD, and BAIAP2 gene expressions in glioma U87 cells with ERN1 loss of function: effect of hypoxia and glutamine or glucose deprivation. Endocr Reg 47, 15–26, 2013.

Minchenko DO, Kharkova AP, Tsymbal DO, Karbovskyi LL, Minchenko OH. Expression of insulin-like growth factor binding protein genes and its hypoxic regulation in U87 glioma cells depends on ERN1 mediated signaling pathway of endoplasmic reticulum stress. Endocr Reg 49, 73–83, 2015.

Minchenko DO, Kharkova AP, Halkin OV, Karbovskyi LL, Minchenko OH. Effect of hypoxia on the expression of genes encoded insulin-like growth factors and some related proteins in U87 glioma cells without IRE1 function. Endocr Reg 50, 43–54, 2016a.

Minchenko DO, Riabovol OO, Tsymbal DO, Ratushna OO, Minchenko OH. Inhibition of IRE1 affects the expression of genes encoded glucocorticoid receptor and some related factors and their hypoxic regulation in U87 glioma cells. Endocr Reg 50, 127–136, 2016b.

Minchenko DO, Riabovol OO, Ratushna OO, Minchenko OH. Hypoxic regulation of the expression of genes encoded estrogen related proteins in U87 glioma cells: effect of IRE1 inhibition. Endocr Reg 51, 8–19, 2017.

Minchenko DO, Tsymbal DO, Riabovol OO, Viletska YM, Lahanovska YO, Sliusar MY, Bezrodnyi BH, Minchenko OH. Hypoxic regulation of EDN1, EDNRA, EDNRB, and ECE1 gene expressions in IRE1 knockdown U87 glioma cells. Endocr Reg 53, 250–262, 2019.

Moenner M, Pluquet O, Bouchecareilh M, Chevet E. Integrated endoplasmic reticulum stress responses in cancer. Cancer Res 67, 10631–10634, 2007.

Papaioannou A, Centonze F, Metais A, Maurel M, Negroni L, Gonzalez-Quiroz M, Mahdizadeh SJ, Svensson G, Zare E, Blondel A, Koong AC, Hetz C, Pedeux R, Tremblay ML, Eriksson LA, Chevet E. Stress-induced tyrosine phosphorylation of RtcB modulates IRE1 activity and signaling outputs. Life Science Alliance 5, e202201379, 2022.

Payankaulam S, Raicu AM, Arnosti DN. Transcriptional regulation of *INSR*, the insulin receptor gene. Genes (Basel) 10, 984, 2019.

Psarra AM, Sekeris CE. Glucocorticoids induce mitochondrial gene transcription in HepG2 cells: role of the mitochondrial glucocorticoid receptor. Biochim Biophys Acta 1813, 1814–1821, 2011.

Pufall MA. Glucocorticoids and cancer. Adv Exp Med Biol 872, 315–333, 2015.

Qin J, Chen JX, Zhu Z, Teng JA. Genistein inhibits human colorectal cancer growth and suppresses miR-95, Akt and SGK1. Cell Physiol Biochem 35, 2069–2077, 2015.

Ratman D, Vanden Berghe W, Dejager L, Libert C, Tavernier J, Beck IM, De Bosscher K. How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering. Mol Cell Endocrinol 380, 41–54, 2013.

Rudnytska OV, Khita OO, Minchenko DO, Tsymbal DO, Yefimova YV, Sliusar MY, Minchenko OH. The low doses of SWCNTs exhibit a genotoxic effect on the normal human astrocytes by disrupting the functional integrity of the genome. Curr Res Toxicol 2, 64–71, 2021.

Sarfstein R, Werner H. Minireview: nuclear insulin and insulin-like growth factor-1 receptors: a novel paradigm in signal transduction. Endocrinology 154, 1672–1679, 2013.

Shen Y, Meunier L, Hendershot LM. Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. J Biol Chem 277, 15947–15956, 2002.

Subramaniam N, Treuter E, Okret S. Receptor interacting protein RIP140 inhibits both positive and negative gene regulation by glucocorticoids. J Biol Chem 274, 18121–18127, 1999.

Thiel G, Guethlein LA, Rossler OG. Insulin-responsive transcription factors. Biomolecules 11, 1886, 2021.

Thomas AL, Coarfa C, Qian J, Wilkerson JJ, Rajapakshe K, Krett NL, Gunaratne PH, Rosen ST. Identification of potential glucocorticoid receptor therapeutic targets in multiple myeloma. Nucl Recept Signal 13, e006, 2015.

Tsymbal DO, Minchenko DO, Hnatiuk OS, Luzina OY, Minchenko OH. Effect of hypoxia on the expression of a subset of proliferation related genes in IRE1 knockdown U87 glioma cells. Adv Biol Chem 7, 195–210, 2017.

Uchoa ET, Aguilera G, Herman JP, Fiedler JL, Deak T, de Sousa MB. Novel aspects of glucocorticoid actions. J Neuroendocrinol 26, 557–572, 2014.

van Raalte DH, Brands M, Serlie MJ, Mudde K, Stienstra R, Sauerwein HP, Kersten S, Diamant M. Angiopoietin-like protein 4 is differentially regulated by glucocorticoids and insulin in vitro and in vivo in healthy humans. Exp Clin Endocrinol Diabetes 120, 598–603, 2012.

Windahl SH, Treuter E, Ford J, Zilliacus J, Gustafsson JA, McEwan IJ. The nuclear-receptor interacting protein (RIP) 140 binds to the human glucocorticoid receptor and modulates hormone-dependent transactivation. J Steroid Biochem Mol Biol 71, 93–102, 1999.

Vitellius G, Trabado S, Bouligand J, Delemer B, Lombes M. Pathophysiology of glucocorticoid signaling. Ann Endocrinol (Paris) 79, 98–106, 2018.

Watanabe Y, Sasaki T, Miyoshi S, Shimizu M, Yamauchi Y, Sato R. Insulin-induced genes INSIG1 and INSIG2 mediate oxysterol-dependent activation of the PERK-eIF2α-ATF4 axis. J Biol Chem 297, 10098, 2021.

Yuen KC, Chong LE, Riddle MC. Influence of glucocorticoids and growth hormone on insulin sensitivity in humans. Diabet Med 30, 651–663, 2013.

Zanchi NE, Filho MA, Felitti V, Nicastro H, Lorenzeti FM, Lancha AH Jr. Glucocorticoids: extensive physiological actions modulated through multiple mechanisms of gene regulation. J Cell Physiol 224, 311–315, 2010.