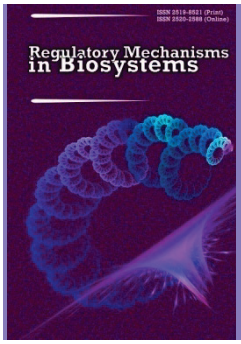

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The translational content and expression of intracellular sensors under endoplasmic reticulum stress in diabetic rats

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Endoplasmic reticulum stress (ER stress) activates the unfolded protein response (UPR) as a defense mechanism for maintaining cellular vital activity. Data on interaction between pathways of UPR-sensors, their role and participation in the recovery of regulatory proteins at constant level are currently being accumulated. An experimental model of type 2 diabetes mellitus (T2DM) was reproduced in male rats fed on 3-month high-fat diet with additional streptozotocin administration. Rats in different groups were treated with metformin, propionate, and their combination for 2 weeks. We analyzed the expression of the GRP78, PERK, IRE-1, and ATF6 genes in gastric glandulocytes using PCR primers. The quantitation of protein content of the GRP78, PERK, IRE-1, and ATF6 genes was performed using western blotting. Statistical intergroup differences were calculated using a one-way ANOVA test followed by Tukey's post-hoc. Comparison of mRNA and protein contents at rest state, in condition of ER stress and drug treatment, as well as the study of correlations between UPR-sensors, provides a basis for the development of the concept of defense mechanism activation in gastric glandulocytes under T2DM conditions. Under stress, chaperone GRP78 plays a crucial role to prevent protein misfolding: it dissociates from ER, enters cisternae and inactivates misfolded proteins. The ATF6 pathway is activated in all conditions. It probably activates transcription of the corresponding content turnover sites in both GRP78 and ATF6. The IRE-1 and PERK pathways are activated in glandulocytes under different circumstances, and they probably target to different regulatory actions. Combination drug treatment in T2DM conditions induces activation of all signaling sensors in the UPR system with simultaneous maximal downregulation of GRP78, causing a balance in the intracellular homeostasis system.

Keywords: glandulocytes; rats; T2DM; metformin; propionate; GRP78; PERK; IRE-1; ATF6; unfolded protein response; gastric fundus.

Introduction

Cells are exposed to numerous internal and external pathological influences that cause a state of functional stress. The long-term or excessive exposure of these factors leads to a stress state occurrence. However, cells have developed specific defense responses, such as compensatory mechanisms in response to stress to maintain its normal life during evolution (Kang et al., 2022).

Endoplasmic reticulum (ER) is the organelle responsible for protein synthesis, post-translational modification, folding, and transport. Accumulation of misfolded proteins ("misfolding") in ER results in ER stress followed by activation of a mechanism that aims to restore the ER homeostasis called the unfolded protein response (UPR) (Kang et al., 2022). UPR activation affects almost all stages: it alters the protein synthesis rate, protein translocation into ER, folding, transport, and induces the removal of misfolding structures by ER-associated protein degradation (ERAD). 78-KDA glucose-regulated protein (GRP78) is a major chaperone that regulates an intracellular homeostasis and acts as ER stress sensor. At rest conditions, it is associated with the following transmembrane signaling enzymes: PERK (PKR-like endoplasmic reticulum kinase), IRE1 (inositol-requiring enzyme-1), and transcription factor ATF6 (activating transcription factor 6) (Wodrich et al., 2022). Coordinated activation of UPR-sensors acts as a quality control mechanism for protein synthesis, but under conditions of prolonged stress, when ER homeostasis cannot be restored, the cell activates intracellular death programs.

The general principle of the system functioning is presented as follows: under stress, BiP/GRP78 is sequestered from the ER membrane into the cistern to binding to misfolded proteins (Ghemrawi & Khair, 2020). The chaperone inactivates unfolded proteins and thereby downregulates proteotoxicity in the cell. At the same time, when the BiP/GRP78 protein is released from the membrane, the transmembrane sensors PERK, IRE-1 and ATF6 are also being released. They activate intranuclear transcription in the corresponding sites of the gene and promote the system of BiP/GRP78 content recovery, express genes of ERAD systems, suppress complete translation and induce apoptosis pathways, if necessary (Shahzad et al., 2020). Data on interaction between pathways of UPR-sensors, their role and participation in the restoring a constant level of regulatory proteins that provide intracellular homeostasis are currently being accumulated (Rozpedek et al., 2017; Sundaram et al., 2018). However, the ways by which ATF6, IRE1, and PERK signaling pathways are interconnected remain unclear. It is also not clear what provides the synthesis of UPR-sensors themselves, maintains their concentration and controls their regulation to steady-state level.

In a cell, the interrelation between a protein and its transcriptome (mRNA) is determined by degradation and translation. These two processes are strictly regulated at both the global and gene-specific levels (de Sousa Abreu et al., 2009). Their imbalance inevitably results in violation: from a pathological condition to a serious illness. However, understanding these processes in regulatory systems is very difficult. During evolution, the gene expression regulation has been optimized at several levels, and their different strategies exist that are characterized by mutual correlations between the rates of transcription, translation, degradation, potentiation (ability to change the transcription rate depending on the circumstances) (de Sousa Abreu et al., 2009; Buccitelli & Selbach, 2020).

The interrelationship between protein and mRNA concentrations is studied under various conditions: steady state, short-term stress response, adaptation, long-term changes, etc. (Cheng et al., 2016; Liu & Aebersold, 2016; Liu et al., 2016; Buccitelli & Selbach, 2020; Natrus et al., 2024). However, a single quantitation of mRNA was noted to be insufficient to predict the protein concentrations at the post-translational level. Additional determining of the change in the concentrations of mRNA and protein encoded by this mRNA under different conditions or over is appropriate (Liu et al., 2016).

The state of long-term persistent hyperglycemia in type 2 diabetes mellitus (T2DM) is considered to be a stressful condition for the body's cells. Our previous works described ER changes investigated in experimental model of T2DM neurons in the ventromedial hypothalamus, gastrocytes, and enterocytes from the small intestine (Natrus et al., 2022; Klys et al., 2023; Sorout et al., 2023; Natrus et al., 2024). The study of intracellular homeostasis with the pharmacological correction is currently ongoing. Along with administration of metformin as the main antidiabetic drug, we studied the effect of propionic acid administration and its combination with metformin.

The goal of our work was to investigate the translational content and degree of expression of GRP78, PERK, IRE-1, and ATF6 genes in fundal gastric glandulocytes of T2DM rats and with the pharmacological correction with metformin, propionate, and their combination treatment.

Materials and methods

Experimental animals and experimental design. The experiments with the animals were conducted in accordance with the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines 2.0 (du Sert et al., 2020), the bioethical norms of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986); "General ethical principles for conducting experiments on animals" (Ukraine, 2001), the Law of Ukraine on the Protection of Animals from Cruelty No. 3447-IV (Ukraine, 2006). The protocol of the experiment on rats was approved at a meeting of the Commission of NMU O. O. Bogomolets, protocol No. 123 of 26.09.2022.

To reproduce the experimental model, T2DM was induced in 24 male Wistar rats (weighing 160–180 grams) fed on a standard balanced diet and water. Initially, the rats were fed on a high-fat diet (HFD) for 3 months (Natrus et al., 2022), and were intravenously injected once with streptozotocin (STZ) at a dose of 25 mg/kg of body weight. Two weeks after STZ injection, the T2DM model was reproduced by measuring rat body parameters, biochemical indexes, and insulin tolerance tests. Untreated healthy rats served as controls. Six rats in the control group were fed on standard diet throughout the whole experiment. T2DM-induced rats were randomly divided into 4 groups: 1) untreated T2DM rats; 2) T2DM rats treated with oral antihyperglycemic agent metformin (Glucophage, Merck Sante, France, 60 mg/kg of body weight); 4) T2DM rats treated with oral propionic acid sodium salt (Propicum®, Flexopharm Brain GmbH & Co, Germany, 60 mg/kg; 5) T2DM rats treated with specified doses of the both drugs (combination treatment). The rats were treated with the drugs for 14 days. The animals were removed from the experiment by decapitation after being sacrificed with intraperitoneal lethal dose of sodium thiopental (200 mg/kg). Samples from the fundal part of the stomach were dissected for further analyses, frozen in liquid nitrogen and stored at –70 °C until the analyses were carried out.

Quantitative real-time PCR analysis. We analyzed the expression of the studied genes using quantitative real-time polymerase chain reaction (PCR). RNA was isolated from frozen gastric fundal samples with an average weight of 0.03 g using the GeneJET RNA Purification Kit (Thermo Fisher Scientific Inc., US) according to the manufacturer's instructions. The concentration and purity of the isolated mRNA were measured with OD260/280 and OD260/230 ratio using DeNovix DS-11 FX+ (DeNovix Inc., USA, 2014). Removal of possible genomic DNA residues from RNA was carried out using DNase I (Thermo Fisher Scientific Inc., USA). Purified RNA from tissue samples was immediately reverse-transcribed into cDNA using RevertAid First Strand kit (Thermo Fisher Scientific Inc., USA). cDNA was synthesized from 5 μg of total RNA by reverse transcription with 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT; 10 mM dNTP Mix; RiboLock RNase Inhibitor (20 U/μL); Random hexamer primers (100 μM) and RevertAid Reverse Transcriptase (200 U/μL). The concentration measurements were performed with DeNovix DS-11 FX+, and the substance was stored for use at –20 °C. Gene expression analysis was carried out using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific Inc., USA) on Applied Biosystems™ 7500 Real-Time PCR Systems (Life Technologies Corporation, USA, 2014). RT-PCR amplification was performed according to the following protocol: the reaction mixture was heated at 95 °C for 10 minutes, repeated for 40 cycles: 95 °C for 15 seconds, and incubated at 60 °C for 60 seconds. Amplification specificity was determined by the melt curve analysis of RT-PCR products. Analysis of expression data was carried out with 7500 Real-Time PCR Software.

In our study, primers were designed using the Primer BLAST software. Table 1 shows the primer sequences.

Table 1

Design of primers used to study the expression of unfolded protein response genes in the fundus of the stomach of diabetic rats

Primer name	Forward primer sequence (5′→3′)	Reverse primer sequence (5′→3′)
GRP78	TCGACTTGGGGACCACCTATTCC	GCCCTGATCGTTGGCTATGATCTC
PERK	CAGAGAAGTGGCAAGAGGAGATGGA	GGGCATCCATTGGGCTAGGG
ATF6	AGCTGGACCAGGTGGTGTCAGAG	CACAGACAGCTCTGCGCTTTGG
IRE-1	GCGATGGACTGGTGGTACT	GTTTGCTCTTGGCCTCTGTC
β-Actin	TGCAGAAGGAGATTACTGCCCTGG	GCTGATCCACATCTGCTGGAAGG

The relative content of transcripts in the studied genes was normalized to the β-actin expression content. The data were calculated using the ΔΔCt method. To reduce the sample variability, all samples were analyzed in triplicate.

Western blot. We analyzed the concentrations of the studied proteins GRP78, PERK, ATF6 and IRE1 using western blotting. Samples of gastric fundus (0.1 g of tissue) were ground in porcelain crucibles under liquid nitrogen. The tissue homogenate was kept for 20 minutes in RIPA lysis buffer (1:9) with the addition of a mixture of protease inhibitors (Sigma, USA), then centrifuged (+4 °C, 16000 g, 45 min), and the supernatant was collected. The aliquot of each lysate containing 70 μg of protein was separated by 10–15% SDS-PAGE. The proteins were transferred to a nitrocellulose membrane (#HATF00010, pore size 0.45 microns, Merck Millipore, USA), then protein-binding sites were blocked with 5% non-fat milk in a phosphate-salt buffer with 0.05% Tween-20 (PBST) for 1 hour. The membranes were incubated overnight at +4 °C with primary GRP78 antibodies (1:2000, #PA5-34941, Invitrogen, USA), PERK (1:250, #PA5-79193, Invitrogen, USA), ATF6 (1:1000, #PA5-85935, Invitrogen, USA), IRE1 (1:250, #PA5-20190, Invitrogen, USA) and β-actin (1:5000, A3854, Sigma-Aldrich, USA). After being washed, the membranes were incubated with HRP-conjugated secondary anti-rabbit IgG (1:10000, #31460, Sigma-Aldrich, USA). The enhanced chemiluminescence reaction with p-coumaric acid and luminol was performed to visualize targeted proteins. The relative contents of GRP78, PERK, ATF6, and IRE1 were normalized to β-actin and quantified using Gel-Pro Analyzer32, v3.1. The data is presented in conventional units as the multiplicity of changes compared to control.

Statistical analysis. Statistical processing of the results was performed using the IBM SPSS Statistics software, version 23.0 (SPSS Inc., USA). The Shapiro-Wilk test was used to evaluate the distribution normality of data. Statistical differences between the groups were analyzed by the one-way ANOVA test followed by Tukey's post-hoc test. The intergroup differences were considered to be statistically significant at $P < 0.05$. All data were obtained on the basis of two or three independent experiments and presented as the mean ± error of the mean (x ± SE). The data were quantitative continuous variables with normal data distribution, therefore

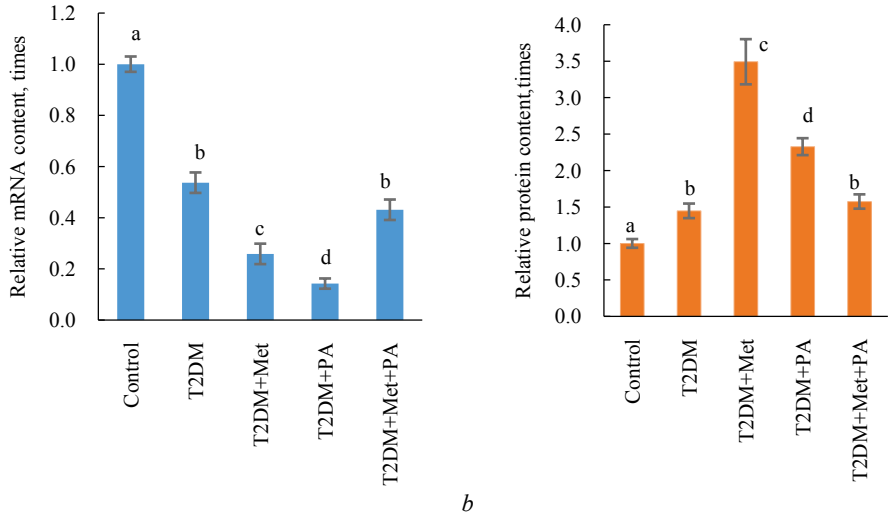
the Pearson correlation coefficient (r) was calculated with P-value corresponding to 95% confidence interval (Petrie & Sabin, 2020).

Results

The relative content of mRNA in GRP78 gene of T2DM rats was 1.86 times lower (P = 0.038) compared to control. But GRP78 protein value was 1.45 times greater than in control rat group (P = 0.042) (Fig. 1a). GRP78 mRNA expression in metformin- and propionate-treated T2DM rats was reduced by 3.87 and 7.02 times, respectively, compared to control (P = 0.005, P = 0.0004, respectively). In these groups, GRP78 protein concentrations exceeded the values in control group by 3.49 and 2.33 times (P = 0.045, P = 0.032). The transcriptional and translational content of GRP78 in combination treatment group were the same as in T2DM rats.

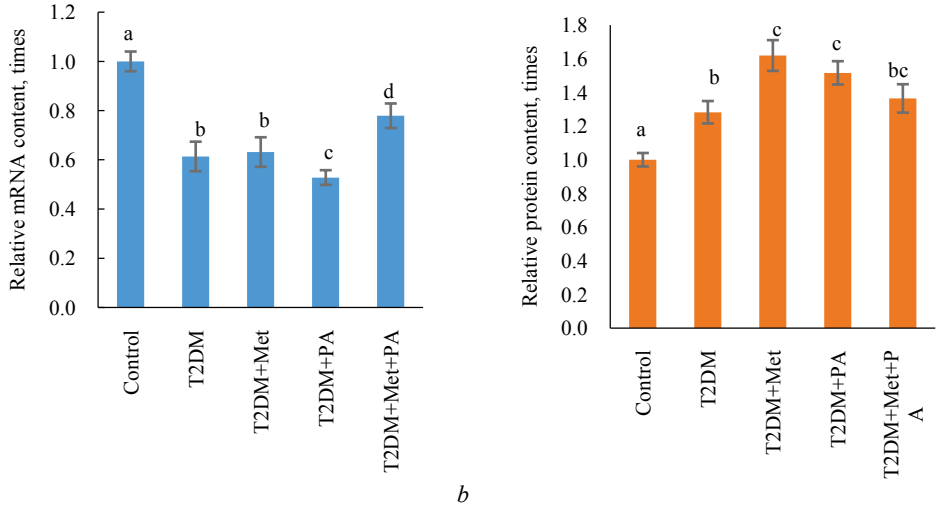
Analysis of ATF6 mRNA value in T2DM showed a decrease in the expression of this gene by 1.63 times; the protein content in gastric homogenates was 1.28 times higher compared to control group (P = 0.043, P = 0.03) (Fig. 2a, 2b). In metformin- and propionate-treated T2DM rats, ATF6 mRNA level was at the indicator of untreated T2DM group, and value of ATF6 protein exceeded the control group by 1.62 and 1.52 times, respectively (P = 0.0026, P = 0.035, respectively). In combined treatment of the drugs, ATF6 protein value exceeded the control by 1.36 times on the background of a 1.28-fold decrease in the mRNA content in this gene (P = 0.04, P = 0.045, respectively).

IRE1 gene expression by the level of mRNA in T2DM was reduced by 1.9 times compared to control (P = 0.003, Fig. 3a). In metformin-treated T2DM group, we found an additional expression downregulation, 3.88 times less than in control group (P = 0.001); the protein content was the same as in the control group (Fig. 3b). In propionate-treated T2DM group, relative content of IRE1 mRNA was 7.04 times lower than in control group (P = 0.002), and concomitant treatment with the drugs resulted in the establishment of this indicator to the value of concentration in T2DM. The protein content in the propionate-treated T2DM group and combined treatment of the drugs content of IRE1 was 1.38 times higher (P = 0.044) compared to control.



a *b*

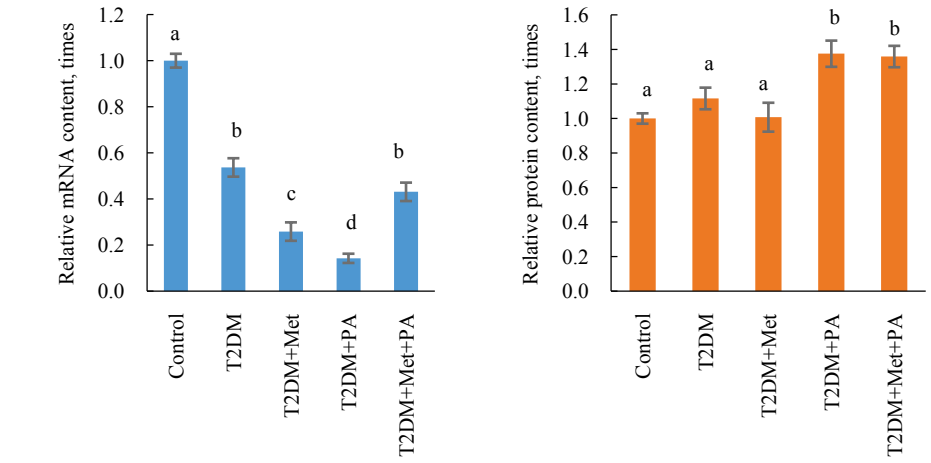
Fig. 1. Relative content of mRNA (*a*) and protein (*b*) GRP78 in gastric fundus from metformin- and PA-treated T2DM rats; all data are presented as the mean ± SE of 3 independent experiments performed in triplicate (n = 6 rats per group); different letters indicate significant different from each other according to one-way ANOVA with Tukey's post hoc test results (P < 0.05)



a *b*

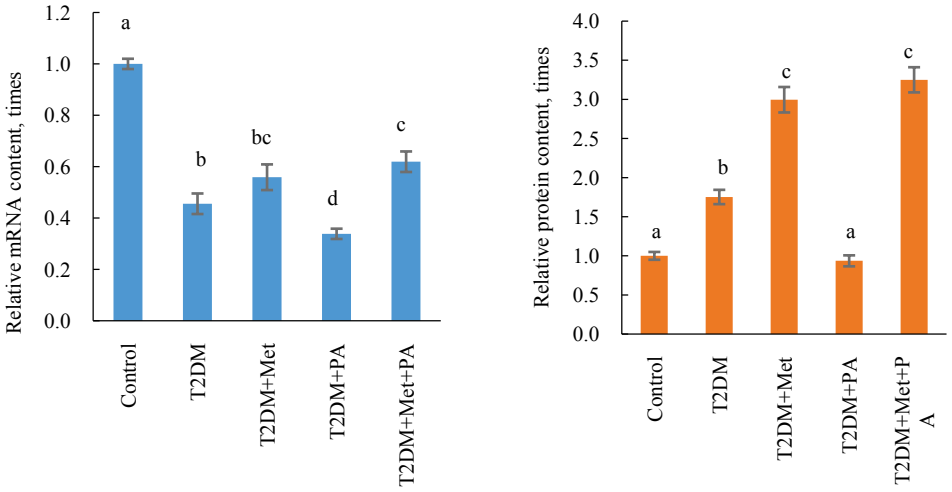
Fig. 2. Relative content of mRNA (*a*) and proteins (*b*) ATF6 in gastric fundus from metformin- and PA-treated T2DM rats; see Fig. 1 for details

Transcriptional value of PERK in the gastric tissue of T2DM rats was 2.20 times lower than in control group (P = 0.032, Fig. 3a). Its indicator was the lowest in PA-treated T2DM group (2.96 times less, P = 0.038 compared with control group). In the metformin-treated T2DM group and in the T2DM group with combination treatment, it was almost the same: less than the control by 1.79 and 1.61 times, respectively (P = 0.044, P = 0.04 respectively). The PERK content in T2DM exceeded the control value by 1.75 times (P = 0.032). The metformin-treated T2DM group and T2DM group with combination treatment showed a 3.00- and 3.25-fold increase in this protein concentration, compared to control (P = 0.003, P = 0.0028, respectively). An unexpected finding was that the PERK protein content in the PA-treated T2DM group was at the level in control group (Fig. 3b).



a *b*

Fig. 3. Relative content of mRNA (*a*) and protein (*b*) IRE1 in gastric fundus from metformin- and PA-treated T2DM rats; see Fig. 1 for details



a *b*

Fig. 4. Relative content of mRNA (*a*) and proteins (*b*) PERK in gastric fundus from metformin- and PA-treated T2DM rats; see Fig. 1 for details

We analyzed the correlations between the mRNA value and protein components in UPR system (Table 2). An inverse relationship between GRP78 protein concentration and its mRNA transcript was expected (r = – 0.459, P = 0.011). The detection of a strong positive relationship between the amount of major chaperone in UPR system, GRP78 and ATP6 (r =0.383, P = 0.037), and inverse relationship between ATP6 protein concentration and GRP78 mRNA transcript (r = – 0.420, P = 0.021) highlights their close interaction in the system. A strong correlation was also found between the amounts of ATP6 and PERK sensors (r = 0.374, P = 0.042), which indicates their synergistic effect in implementing the stress response. The finding of close direct relationships between sensor transcripts was also valuable. Thus, GRP78 mRNA directly correlates with ATP6 mRNA (r = 0.609, P = 0.001), PERK mRNA (r = 0.552, P = 0.002) and IRE1 mRNA (r = 0.511, P = 0.004). We found a positive relationship between PERK mRNA, ATP6 mRNA (r = 0.526, P = 0.003) and IRE1 mRNA (r = 0.403, P = 0.027). IRE1 mRNA content is also directly related with ATP6 mRNA (r = 0.463, P = 0.010). This is schematically shown in Figure 3.

Discussion

The synthesis of proteins in the cell, their proper folding and posttranslational modifications are crucial for maintaining cellular homeostasis. These conditions provide an important role in the folding quality control system. UPR comprises a conservative group of intracellular signaling pathways aimed at restoring the cellular homeostasis during stress caused by the accumulation of unfolded or misfolded proteins in ER (Schroder & Kaufman, 2005; Walter & Ron, 2011; Coleman et al., 2019).

Table 2

Correlation between mRNA and protein contents in UPR system of T2DM rat glandulocytes on the background of T2DM and treatment according to the Pearson coefficient (n = 30)

Indicator content	GRP78 protein	ATF6 protein	PERK protein	IRE1 protein	*GRP78* mRNA	*ATF6* mRNA	*PERK* mRNA	*IRE1* mRNA
GRP78 protein	1	0.383*	0.355	0.059	–0.459*	–0.360	–0.186	–0.274
ATF6 protein	–	1	0.374*	–0.081	–0.420*	–0.353	–0.104	–0.180
PERK protein	–	–	1	0.020	–0.245	–0.082	0.158	–0.172
IRE1 protein	–	–	–	1	–0.098	0.124	–0.196	–0.333
GRP78 mRNA	–	–	–	–	1	0.609**	0.552**	0.511**
ATF6 mRNA	–	–	–	–	–	1	0.526**	0.463**
PERK mRNA	–	–	–	–	–	–	1	0.403*
IRE1 mRNA	–	–	–	–	–	–	–	1

Note: * – P < 0.05; ** – P < 0.01.

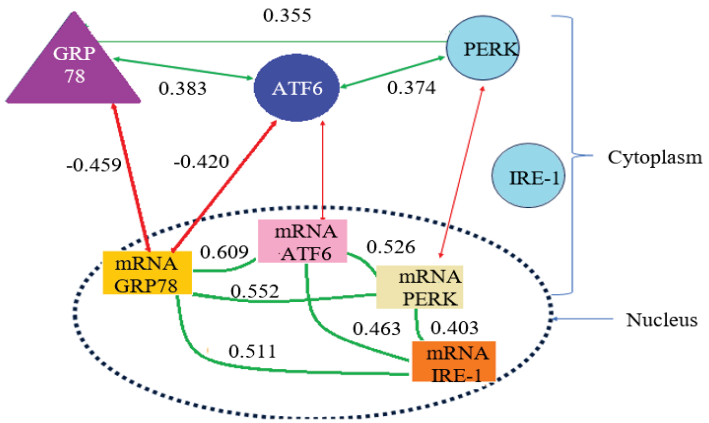


Fig. 3. Schematic representation of the relationship between transcripts and UPR system sensors in gastric glandulocytes: inverse relationship between components is shown in red, and direct correlations are shown in green; the numbers next to the arrows indicate the correlation between the indicators

In the latest study (Deka et al., 2022) on intestinal cells, the authors hypothesized that actually transmembrane sensors in UPR system, after being dissociated with chaperone, trigger the synthesis of pro-inflammatory signaling molecules in the cell (Deka et al., 2022). UPR is thought to provide a wide range of physiological functions. Each individual UPR pathway plays a unique and specialized role in various developmental and metabolic processes (Wu & Kaufman, 2006). And exactly what role a particular signaling pathway in gastric glandulocytes plays under ER stress or in the use of pharmacocorrection still needs to be investigated. Now, we are adding our own contribution to perspective of cooperative relationships in this intracellular signaling system.

The correlation studies in the protein-transcript group are also ongoing. A clearly defined biological paradigm was developed on the existence of a non-trivial relationship between the concentrations of a transcript and its protein (Wethmar et al., 2010; Barrett et al., 2012; Liu et al., 2016). Tang & Amon summed up thoroughly the existing achievements in the study on quantitative assessment of transcripts and proteins (Tang & Amon, 2013). The authors showed the importance of numerous factors that affect these processes. This includes, in particular: translation rate due to the varying complexity of codons and reading of information by the ribosome; modulation of translation rate by binding non-coding RNAs such as microRNA; modulation of protein half-life (as the complex ubiquitin-proteasome pathway or autophagy, which can affect protein concentration levels independently of transcript concentration); delayed protein synthesis because this synthesis is more time- and energy-consuming than mRNA synthesis; protein transport, as its export which spatially disconnects proteins from the transcripts they were synthesized from. Thus, it can be considered proven that a direct comparison of concentration values of protein and mRNA from the same location or from the same cell type may not be appropriate. The vast majority of studies have been conducted using the simplest cell models. There is a clear understanding that the higher cellular organization results in more complex interactions between signaling molecules. Therefore, we did not expect to find a direct correlation between the components in our studies. However, we tried to investigate how the misfolding response link system works in the glandulocyte (a cell that intensively synthesizes proteins) upon stress and in an attempt to mitigate it pharmacologically.

We found the strongest inverse correlation between protein and transcript in GRP78. This characterizes a robust regulatory pathway evolutionarily designed to maintain the required chaperone content. Consequently, the increased chaperone concentration downregulates its transcription in the nucleus of the gastric glandulocyte. The IRE-1 and ATF6 transcription intensity was also inversely dependent on the content of these proteins in the cytoplasm, but the strength of their correlation was less, which reflects the confounding role of other factors in the regulation of their synthesis. The PERK transcription intensity did not depend at all on the corresponding protein concentration. It is theorized that when misfolding proteins are accumulated under stress, ATF6 can be transported to the nucleus after being released from the ER, where it participates in the activation of genes associated with cellular stress adaptation, folding regulators, and lipid synthesis genes, and also enhances the transcription of GRP78 chaperone (Gallagher et al., 2016). However, on the contrary, we found a strong negative correlation between the concentrations of ATF6 and GRP78 mRNA, demonstrating the non-participation of ATF6 in enhancement of the chaperone synthesis. At least, we obtained exactly such results in our experimental model during the study of UPR components specifically in gastric glandulocytes.

Another achievement was the identification of a direct correlation between the cellular content of GRP78, ATF6, and PERK. In which connection, chaperone concentration was strongly correlated with the amount of ATF6, but ATF6 was also strongly associated with PERK value. This suggests that these particular proteins in glandulocytes provide the ER stress sensor system and the appropriate response to participate in its compensation. Being the element of the UPR system, the IRE-1 pathway is barely involved in mitigation of ER stress in gastric glandulocytes. The most interesting finding was the discovery of a very strong correlation between the degree of transcription of all proteins in the system. Probably, activation of sites of elements of the UPR system occurs simultaneously or has common mechanisms in gastric glandulocytes. However, GRP78 is most closely related to ATF6 in glandulocytes, which determines the main pathway for regulation of the maintenance of intracellular homeostasis. Therefore, activation of the chaperone GRP78 and signaling molecule ATF6, as its transmembrane partner, can be assumed as the main defense mechanism to attenuate ER stress in gastric glandulocytes under T2DM conditions, based on the analysis of gene transcription intensity. An increase in both of these components of the system in the cytoplasm occurs due to their dissociation from the ER membrane to inactivate misfolding proteins. The PERK pathway is involved to a lesser extent, probably as a supplemental, and the IRE-1 signal chain in this cascade is often inactive and/or activated only for certain reasons. Nuclear expression of gene sites in the UPR sensors is probably interrelated and has common mechanisms of activation. However, again, the closest relationship is observed between the GRP78 genes and ATF6, and, to a lesser extent, between GRP78 and PERK, ATF6 and PERK. Activation of the IRE-1 protein transcript is also intense at the nuclear level, but this is not due to the involvement of this protein's signaling pathway for implementation of stress response.

To better understand the relationships of signaling molecules in the stress response, we schematically combined the protein content of the UPR system in gastric glandulocytes under various conditions and analyzed the involvement of each pathway (Fig. 4).

ER stress in gastric glandulocytes is characterized by a 1.5-fold increase in GRP78 amount. This is probably a response to an increase in the cellular misfolding proteins for their binding and inactivating. At the same time, we found a 2-fold increase in the PERK content, which indicates dissociation of this specific pair: GRP78 with its transmembrane PERK sensor. To a lesser extent, the ATF6 content increases, and the IRE-1 pathway is not active at all. Under conditions of the treatment with metformin, we observed a maximum (3.5-fold) GRP78 activation and a 3-fold PERK activation. This also indicates the involvement of chaperones in the stress response, their participation in the inactivation of misfolding proteins and maintaining/regulation of the response via the PERK pathway. The ATF6 pathway is also involved in the response, but to a lesser extent; at the same time, the IRE-1 pathway is inactive. Treatment with propionate represents another configuration of the response participants.

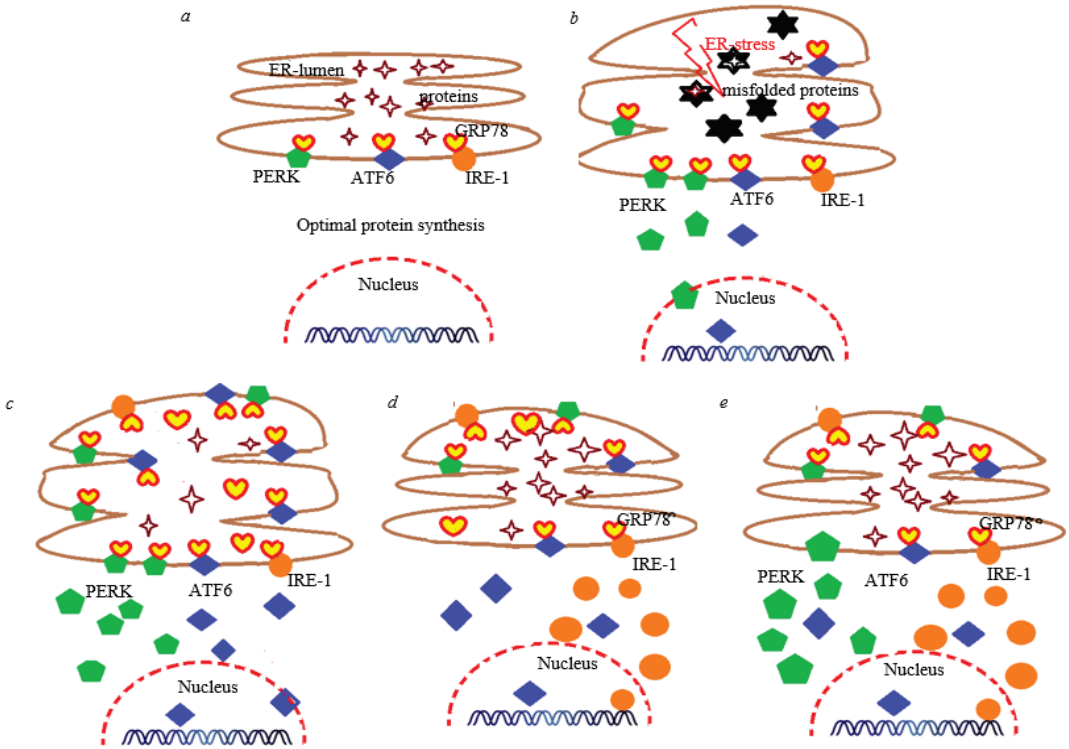


Fig. 4. Schematic representation of proteins ratio in UPR system of rat glandulocytes at resting state (*a*), under T2DM conditions (*b*), and in treatment: metformin (*c*), propionate (*d*), combination (*e*)

Increased GRP78 level occurs mainly due to dissociation of the chaperone from another sensor, IRE-1. Probably, under this condition, the UPR system is regulated via the IRE-1 pathway. The ATF6 sensor is also activated, but to a lesser extent. At the same time, the PERK path is inactive. Combination treatment with metformin and propionate slightly reduces the chaperone content, but its level almost corresponds to the untreated T2DM group. It can be assumed that it aimed to compensate the effect of drugs on the cell. At the same time, the activity of the IRE-1 and PERK pathways are maximized, and ATF6 activation is maintained.

At present, our insights into the UPR system are the following. Under stress, the chaperone GRP78 is essential to prevent misfolding: it dissociates from ER, enters cisternae and inactivates misfolded proteins. The ATF6 pathway is activated in all conditions. It is probably this mechanism which provides information on the cellular chaperone content to the nucleus, and it will activate transcription of the corresponding sites for content turnover of both GRP78 and ATF6 in conditions of the chaperone content reduction (according to the principle of inverse relationship). Taking into account the revealed correlations between these molecules and their related activation under various conditions, we can assume a genetically determined relationship between these sensors. The IRE-1 and PERK pathways are activated in glandulocytes under different circumstances, and probably they target different regulatory actions that are inherent in sensors: they alter the protein synthesis rate, protein translocation to the ER, folding, transport, etc. not only in the UPR system, but also in other cellular mechanisms. According to our observations, combination treatment with drugs in T2DM conditions causes activation of all signaling sensors in the UPR system, but at the same time it minimizes the GRP78 content. And if we consider a high level of chaperone to be a sign of stress (Coleman & Haller, 2019), then the combination treatment with the drugs likely causes maximum equilibrium in the intracellular homeostasis system due to the binding effect of signaling pathways.

Conclusion

The study of the translational content and degree of expression of the GRP78, PERK, IRE-1 and ATF6 genes in fundal gastric glandulocytes from untreated T2DM rats and on the background of pharmacological correction with metformin, propionate and their combination gave rise to a concept of response of the signaling UPR system and an understanding of the involvement of molecular sensors in maintaining intracellular homeostasis in the gastric cells. However, we understand the limitations of this study. Nowadays, there is very little data on these intracellular signaling pathways, especially in T2DM conditions and its treatment, so most of our results look like assumptions. Further accumulation of experimental data will be beneficial for analyzing the stress response in various cells of the body. Therefore, conducting further research of proteins in UPR system in cells of various specialization, functional significance, regenerative potential, etc., the study of ability and participation of sensors in ER stress compensation,will allow us to develop general principles of intracellular mechanisms for implementing adaptation pathways in biological systems.

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