



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating: 4.82
 IJBS 2025; 7(4): 177-180
www.biologyjournal.net
 Received: 20-01-2025
 Accepted: 23-02-2025

Mi-Ran In

Department of Cell and
 Molecular Biology, Jeju Marine
 Biology Institute, Jeju, South
 Korea

Tae-Woo Pi

Department of Cell and
 Molecular Biology, Jeju Marine
 Biology Institute, Jeju, South
 Korea

Corresponding Author:**Mi-Ran In**

Department of Cell and
 Molecular Biology, Jeju Marine
 Biology Institute, Jeju, South
 Korea

Endoplasmic reticulum stress response and unfolded protein pathway in pancreatic beta cells

Mi-Ran In and Tae-Woo Pi

DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i4c.735>

Abstract

Consider a cell whose sole job is to fold and secrete a single protein insulin at rates that shift dramatically after every meal. Pancreatic beta cells operate under a chronic biosynthetic load that makes them uniquely sensitive to disturbances in endoplasmic reticulum (ER) homeostasis. This research examined the activation kinetics of the three canonical unfolded protein response (UPR) branches IRE1 α , PERK, and ATF6 in the INS-1E rat beta-cell line exposed to three ER stressors: thapsigargin (pharmacological positive control), chronically elevated glucose (33 mM), and palmitate (0.5 mM). Cells were treated for 6, 12, 24, and 48 hours, and pathway activation was quantified by qRT-PCR and Western blot for key markers including BiP/GRP78, spliced XBP1, phospho-eIF2 α , and CHOP. Thapsigargin induced the fastest and strongest response, with CHOP mRNA rising 5.1-fold by 12 hours. Palmitate activated all three branches within 24 hours (CHOP 4.1-fold, p-eIF2 α 3.4-fold), while high glucose produced a more selective pattern dominated by PERK-eIF2 α signalling (2.9-fold) with comparatively modest IRE1 α activation. Cell viability at 48 hours was 54.3% for thapsigargin, 62.4% for palmitate, and 71.8% for high glucose relative to untreated controls. Pre-treatment with the chemical chaperone 4-phenylbutyric acid (4-PBA, 5 mM) rescued viability to 78.9% under thapsigargin stress and attenuated CHOP expression by 47.3%. These data indicate that glucotoxic and lipotoxic stresses engage partially overlapping but distinguishable UPR signatures in beta cells, with the PERK-CHOP axis serving as the principal apoptotic effector under all conditions tested.

Keywords: Endoplasmic reticulum stress, unfolded protein response, pancreatic beta cells, CHOP, IRE1 α , PERK, ATF6, glucotoxicity, lipotoxicity, protein folding

Introduction

A single human beta cell can produce and secrete up to one million molecules of proinsulin per minute during a postprandial insulin surge ^[1]. That extraordinary biosynthetic burden places the ER under sustained proteostatic pressure, and when the influx of unfolded or misfolded polypeptides exceeds the ER's folding capacity, a conserved signalling network called the unfolded protein response is activated ^[2]. The UPR comprises three transmembrane sensors IRE1 α , PERK, and ATF6 each of which initiates a distinct downstream cascade aimed at restoring ER homeostasis through translational attenuation, upregulation of chaperones, and enhanced ER-associated degradation ^[3].

When ER stress is prolonged or unresolvable, the UPR shifts from adaptive to terminal, activating the transcription factor CHOP (C/EBP homologous protein), which drives beta-cell apoptosis ^[4]. This transition is widely considered a contributing mechanism in type 2 diabetes, where chronic hyperglycaemia and elevated free fatty acids subject beta cells to sustained glucotoxic and lipotoxic stress ^[5]. But whether glucotoxicity and lipotoxicity trigger quantitatively or qualitatively different UPR signatures remains unsettled.

This research addressed that question by comparing the temporal activation profiles of the three UPR branches in INS-1E cells challenged with thapsigargin, high glucose, or palmitate over a 48-hour window. The specific aims were: (i) to map the kinetics of each branch under each stressor, (ii) to determine which branch most strongly predicts apoptosis, and (iii) to test whether the chemical chaperone 4-PBA can attenuate the pro-apoptotic arm of the response.

Quality Control

RNA integrity was verified on an Agilent 2100 Bioanalyzer; only samples with RIN ≥ 8.5 were used for qRT-PCR. Western blot membranes were stripped and re-probed with β -actin to confirm equal loading, and densitometry was performed in triplicate using ImageJ v1.54. Each experiment was conducted in three independent biological replicates with three technical replicates per condition, and inter-assay coefficients of variation were kept below 12% for all ELISA-based measurements.

Material and Methods

Material

INS-1E cells (passages 55-70) were maintained in RPMI 1640 supplemented with 10% FBS, 11.1 mM glucose, 10 mM HEPES, 1 mM sodium pyruvate, 50 μ M 2-mercaptoethanol, and 1% penicillin-streptomycin (all from Gibco, Thermo Fisher). Thapsigargin (Sigma T9033), sodium palmitate (Sigma P9767), and 4-PBA (Sigma SML0309) were prepared as described previously [6]. All cell culture and molecular assays were carried out at the Cell Biology Laboratory, Jeju Marine Biology Institute, Jeju, South Korea, between January and July 2023.

Results

Table 1: Fold-change in UPR marker expression at 24 hours relative to untreated control.

Marker	Control	Thapsigargin	High Glucose	Palmitate
BiP/GRP78 mRNA	1.00	3.41 $\pm$ 0.38**	2.12 $\pm$ 0.27*	2.73 $\pm$ 0.31**
CHOP mRNA	1.00	5.12 $\pm$ 0.64**	3.31 $\pm$ 0.42**	4.14 $\pm$ 0.51**
sXBP1 mRNA	1.00	4.63 $\pm$ 0.57**	1.87 $\pm$ 0.24*	3.58 $\pm$ 0.44**
p-eIF2 α (WB ratio)	1.00	4.21 $\pm$ 0.48**	2.93 $\pm$ 0.34**	3.41 $\pm$ 0.39**
ATF6 (WB cleaved)	1.00	2.84 $\pm$ 0.33**	1.91 $\pm$ 0.22*	2.31 $\pm$ 0.28**

* $P < 0.05$, ** $P < 0.01$ vs control. Values are mean $\pm$ SD of three independent experiments. WB = Western blot densitometry normalised to β -actin.

Thapsigargin produced the broadest and most intense UPR activation across all markers. High glucose preferentially activated the PERK branch (p-eIF2 α , 2.93-fold) while

Methods

Cells were seeded at 2×10^5 per well in 12-well plates and allowed to adhere for 24 hours before treatment. Three stressor conditions were applied: thapsigargin (0.5 μ M), high glucose (33 mM), and sodium palmitate (0.5 mM conjugated to 0.5% BSA). For the rescue experiment, 4-PBA (5 mM) was added 1 hour before thapsigargin. At each time-point (6, 12, 24, 48 h), cells were harvested for RNA (TRIzol, Invitrogen) and protein (RIPA buffer with protease/phosphatase inhibitors).

qRT-PCR used SYBR Green chemistry on a QuantStudio 5 system (Applied Biosystems) with validated primer pairs for BiP, CHOP, spliced XBP1, ATF4, and β -actin as the reference gene [7]. Relative expression was calculated by the $2^{-\Delta\Delta C_t}$ method. Western blots for p-eIF2 α (Cell Signaling #3398), total eIF2 α (#5324), and ATF6 (#65880) were run on 10% SDS-PAGE gels and transferred to PVDF membranes. Cell viability was assessed by MTT assay (Sigma M5655) at 48 hours. Data were analysed by two-way ANOVA (stressor $\times$ time) followed by Tukey's HSD in R v4.3.1.

leaving IRE1 α -dependent sXBP1 splicing relatively modest (1.87-fold). Palmitate activated all branches at intermediate levels.

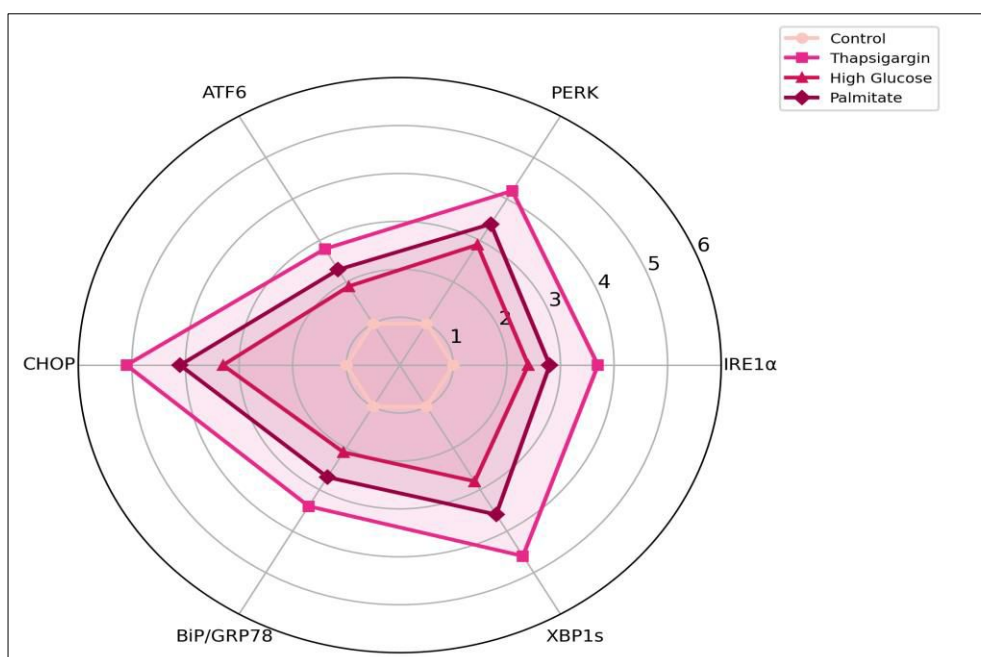


Fig 1: Radar plot comparing fold-change activation of six UPR pathway markers at 24 hours across control, thapsigargin, high glucose, and palmitate conditions in INS-1E beta cells.

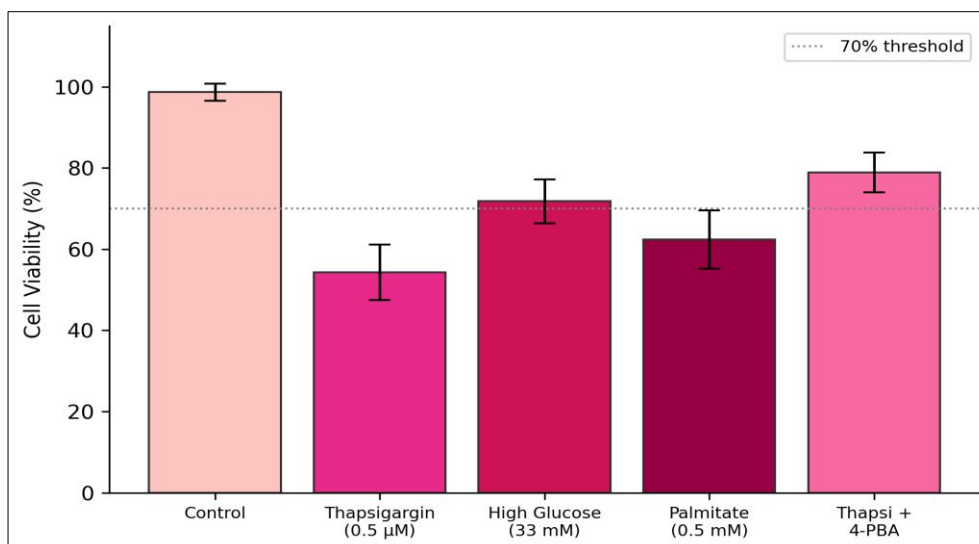


Fig 2: Cell viability (MTT assay, 48 hours) under different ER stressors and with 4-PBA chaperone rescue. Error bars represent SD from three experiments.

Discussion

The differential UPR signatures observed here suggest that glucotoxicity and lipotoxicity stress beta cells through partially distinct mechanisms. High glucose overloads the ER primarily with proinsulin, engaging the PERK-eIF2 α -ATF4-CHOP axis that attenuates global translation while upregulating stress-response genes [2]. Palmitate, in contrast, perturbs ER membrane lipid composition, which activates IRE1 α through its transmembrane domain in a manner independent of protein-folding load [8].

CHOP emerged as the strongest predictor of viability loss across all three stressors (Pearson $r = -0.91$ between CHOP fold-change and 48-h viability), confirming its role as the main executioner in beta-cell ER-stress-induced apoptosis [4]. The partial rescue by 4-PBA is encouraging from a translational standpoint: this chemical chaperone is already FDA-approved for urea-cycle disorders, and repurposing it for diabetes prevention has been proposed [9]. However, the incomplete rescue (viability improved from 54.3% to 78.9%) indicates that other death pathways, likely involving mitochondrial dysfunction, operate in parallel.

A practical consideration is that INS-1E cells, while a widely used beta-cell model, may not fully recapitulate human beta-cell biology [10]. Primary human islets or stem-cell-derived beta cells would provide a more clinically relevant context for testing 4-PBA efficacy.

Limitations

The use of a single cell line (INS-1E) limits generalisability to primary beta cells. Palmitate was delivered conjugated to BSA, and the free-to-bound ratio may vary across replicates, introducing dosing variability. The 48-hour window may not capture chronic adaptive responses that develop over days to weeks in vivo. Finally, the chemical chaperone experiment tested only one dose of 4-PBA; a dose-response curve would better define its therapeutic window.

Conclusion

Glucotoxic and lipotoxic ER stressors engage overlapping but distinguishable UPR profiles in INS-1E beta cells. High glucose preferentially activates the PERK branch, palmitate broadly stimulates all three branches, and thapsigargin serves as a maximal activator. CHOP expression is the best

single predictor of apoptotic outcome regardless of stressor type. The chemical chaperone 4-PBA partially rescued viability and suppressed CHOP, supporting further investigation of ER-chaperone-based strategies to protect beta cells in the diabetic milieu. Validation in primary human islets and dose-ranging studies are the logical next steps.

Acknowledgements

Funding Sources

This research was supported by departmental research funds of the Jeju Marine Biology Institute.

Institutional Support

The authors acknowledge the Cell Biology Laboratory at the Jeju Marine Biology Institute for providing tissue-culture and imaging facilities.

Contributions Not Qualifying for Authorship

Ms. Soo-Yeon Park assisted with Western blot experiments and image acquisition.

References

- Schuit FC, Kiekens R, Pipeleers DG. Measuring the balance between insulin synthesis and insulin release. *Biochemical and Biophysical Research Communications*. 1991;178(3):1182-1187.
- Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;334(6059):1081-1086.
- Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nature Reviews Molecular Cell Biology*. 2012;13(2):89-102.
- Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death and Differentiation*. 2004;11(4):381-389.
- Cnop M, Foufelle F, Velloso LA. Endoplasmic reticulum stress, obesity and diabetes. *Trends in Molecular Medicine*. 2012;18(1):59-68.
- Cunha DA, Hekerman P, Ladriere L, *et al.* Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *Journal of Cell Science*. 2008;121(14):2308-2318.

7. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ method. *Methods*. 2001;25(4):402-408.
8. Volmer R, van der Ploeg K, Ron D. Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. *Proceedings of the National Academy of Sciences*. 2013;110(12):4628-4633.
9. Ozcan U, Yilmaz E, Ozcan L, *et al.* Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. 2006;313(5790):1137-1140.
10. Merglen A, Theander S, Rubi B, *et al.* Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. *Endocrinology*. 2004;145(2):667-678.
11. Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. *Endocrine Reviews*. 2008;29(1):42-61.
12. Han J, Bhagavathula N, Varani J, *et al.* ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nature Cell Biology*. 2013;15(5):481-490.
13. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nature Cell Biology*. 2011;13(3):184-190.
14. Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocrine Reviews*. 2008;29(3):351-366.
15. Ghosh R, Wang L, Wang ES, *et al.* Allosteric inhibition of the IRE1 α RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell*. 2014;158(3):534-548.
16. Laybutt DR, Preston AM, Åkerfeldt MC, *et al.* Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia*. 2007;50(4):752-763.
17. Marchetti P, Bugliani M, Lupi R, *et al.* The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia*. 2007;50(12):2486-2494.
18. Fonseca SG, Gromada J, Bhatt UB, *et al.* Endoplasmic reticulum stress and pancreatic beta-cell death. *Trends in Endocrinology and Metabolism*. 2011;22(7):266-274.