An update on lipotoxic endoplasmic reticulum stress in pancreatic β-cells

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Abstract

The UPR (unfolded protein response) or ER (endoplasmic reticulum) stress response was first described 20 years ago. The field of ER stress has expanded tremendously since, moving from basic biology in yeast to human neurodegenerative, inflammatory, cardiovascular and neoplastic diseases. The ER stress response has also been implicated in diabetes development, affecting both insulin production by pancreatic β-cells and insulin sensitivity in peripheral tissues. In the present mini-review, we focus on recent progress in the field of ER stress in pancreatic β-cells. Recent advances in the understanding of lipotoxic ER stress and β-cell recovery from ER stress are discussed.

ER (endoplasmic reticulum) stress signalling

The ER stress response is an important adaptive cellular signalling response in cells with high secretory activity. It aims to balance secretory protein synthesis in the ER against ER protein folding capacity. An imbalance between ER folding needs and capacity leads to activation of the ER stress response, also known as the UPR (unfolded protein response). This cellular response aims to restore ER homeostasis by attenuating global protein translation, up-regulating ER chaperones (thereby increasing ER folding capacity), and degrading misfolded proteins [1,2]. These co-ordinated transcriptional and translational responses (schematically represented in Figure 1) are initiated by the ER stress transducers IRE (inositol requiring ER-to-nucleus signal kinase) 1, ATF (activating transcription factor) 6 and PERK [PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase]. These three ER membrane proteins are inactive when bound to the ER chaperone BiP (immunoglobulin heavy-chain-binding protein), also known as GRP (glucose-regulated protein) 78 or HSPA5 (heat-shock protein A5). Dissociation of BiP from its luminal side leads to their activation and downstream ER stress signalling. Active IRE1 alternatively splices XBP (X-box-binding protein) 1 mRNA. XBP1s (spliced XBP1) induces genes involved in ER expansion, protein folding and misfolded protein degradation [3–5].

Active ATF6 translocates to the Golgi, where it is cleaved from the membrane by S1P (site-1 protease) and S2P (site-2 protease) [6] and then induces transcription of ER chaperones such as BiP. PERK phosphorylates the eIF (eukaryotic initiation factor) 2α, thereby preventing ternary complex formation and suppressing global protein translation initiation as a means to decrease the functional demand on the ER. In parallel, translation of selected proteins, such as ATF4, is facilitated and downstream CHOP [C/EBP (CCAAT/enhancer-binding protein) homologous protein], also known as GADD (growth-arrest and DNA-damage-inducible protein) 153 or DDIT3 (DNA damage inducible transcript 3) [7], is induced [5]. ATF4 and CHOP up-regulate expression of GADD34, which associates with PP-1 (protein phosphatase 1) to dephosphorylate eIF2α in a negative-feedback loop. The integrated result of the ER stress response is attenuation of protein translation, up-regulation of ER folding capacity and degradation of irreversibly misfolded proteins. When this response fails to restore ER homeostasis, apoptosis is triggered.

ER stress in diabetes

The ER stress response has been implicated in diabetes development, affecting both insulin production by pancreatic β-cells and insulin sensitivity in peripheral tissues. Its role in diabetes has been the subject of a comprehensive review [1]. In this mini-review, we therefore focus on very recent progress in the field of ER stress in pancreatic β-cells.

Evidence for β-cell ER stress in diabetes

Insulin deficiency is a requisite for the development of diabetes and may result from pancreatic β-cell dysfunction and/or apoptosis. The importance of ER stress signalling in human β-cell failure and diabetes is supported by the discovery of juvenile forms of human diabetes owing to mutations in the UPR transducer PERK [8] or the wolframin ER Ca2+ channel encoded by WFS1 (Wolfram syndrome 1)
Figure 1 | ER stress signalling
IRE1, ATF6 and PERK are the three main ER stress transducers. They are inactive due to binding to the ER chaperone BiP, which is a key regulator of the ER stress response. When proteins misfold in the ER lumen, BiP dissociates from the ER stress transducers, leading to their activation. Because protein folding in the ER is Ca\textsuperscript{2+}-dependent, proteins can misfold when the SERCA pump is inhibited and ER Ca\textsuperscript{2+} stores are depleted. This is an important mechanism by which NEFAs trigger ER stress in \( \beta \)-cells [29]. The IRE1 endoribonuclease splices XBP1 mRNA [XBP1t (total XBP1)], allowing its translation. XBP1s is a transcription factor regulating expression of ER genes involved in protein folding and export and ERAD (ER-associated protein degradation). IRE1 also activates JNK. ATF6 induces ER chaperones, such as BiP. PERK phosphorylates eIF2\( \alpha \), thereby inhibiting translation initiation and reducing the arrival of newly synthesized proteins in the ER. Translation of some proteins such as ATF4 is facilitated. ATF4 induces expression of the pro-apoptotic transcription factor CHOP, and 4E-BP1, which contributes further to suppression of translation initiation. The translational inhibition is relieved through ATF4- and CHOP-mediated up-regulation of GADD34 and PP-1-mediated dephosphorylation of eIF2\( \alpha \). In the case of prolonged and excessive ER stress, apoptosis will be triggered. NEFAs cause apoptosis through JNK, CHOP and the ER-specific caspase 12. Modified from [29] with permission of the Company of Biologists.

[9]. Specific to the \( \beta \)-cell, mutations in the insulin gene itself were recently described to cause neonatal forms of diabetes [10–12]. The mutations probably cause insulin malfolding, as has been described in the Akita [13] and Munich [14] mice that harbour amino acid substitutions in the insulin-2 gene impairing disulfide bond formation. Despite the expression of normal insulin in the mice, diabetes ensues. These mouse and human mutations thus act in a dominant fashion: the mutant insulin protein triggers an ER stress response, impairing cell function and leading to massive \( \beta \)-cell loss. The insulin mutations are a common cause of neonatal diabetes, but they are rare in MODY (maturity-onset diabetes of the young) and do not seem to contribute to Type 2 diabetes [15].

Different genetic factors are implicated in \( \beta \)-cell dysfunction in Type 2 diabetes, and these are now starting to be elucidated [16,17]. Among the Type 2 diabetes genes identified so far, only WFS1 is known to play a role in the ER stress response [18]. Several studies have demonstrated increased expression of ER stress markers in \( \beta \)-cells from Type 2 diabetic patients [19–21] and expansion of the ER [22], which is a hallmark of the ER stress response. This ER stress signalling might be related to the environmental causes of \( \beta \)-cell failure in Type 2 diabetes, such as high saturated fat food intake (see below for a discussion of lipotoxic ER stress in \( \beta \)-cells). Whether and how gene–environment interactions contribute to the pathogenesis of diabetes remains to be clarified, but it is noteworthy that individuals with a family history of Type 2 diabetes seem to be particularly susceptible to fatty-acid-induced \( \beta \)-cell dysfunction [23].

Lipotoxic ER stress and apoptosis in pancreatic \( \beta \)-cells
Elevated circulating NEFA [non-esterified (‘free’) fatty acid] levels predict and probably contribute to \( \beta \)-cell dysfunction in Type 2 diabetes, and NEFAs trigger \( \beta \)-cell apoptosis in vitro (reviewed in [24]). The saturated NEFA palmitate and, to a lesser extent, the unsaturated NEFA oleate induce apoptosis in FACS-purified primary rat \( \beta \)-cells and the insulin-producing cell line INS-1E [25,26]. The equimolar combination of oleate and palmitate is not toxic to pancreatic \( \beta \)-cells, and the adverse effects of diet may thus be quantitative as well as qualitative, by modifying the saturated/unsaturated NEFA balance. As the circulating NEFA composition is rapidly reflected in cellular NEFA content, an increased dietary supply of saturated NEFAs may contribute to \( \beta \)-cell apoptosis in vivo.
Pancreatic β-cells exposed to NEFAs have dilated ER and undergo apoptosis

INS-1E cells were treated for 15 h with vehicle (ethanol) (A) or 0.5 mM oleate (B). Oleate-treated cells have cytoplasmic triacylglycerol droplets and distended ER (arrows). Rat primary β-cells were treated with vehicle (ethanol) (C) or 0.25 mM oleate (D) for 2 days, or with 0.25 mM palmitate (E) for 8 days. In the oleate-treated β-cell, the accumulation of lipid droplets (LD) is accompanied by ER dilatation (arrows in D). The palmitate-treated β-cell is undergoing apoptosis, with chromatin condensation and nuclear fragmentation. (F) A human β-cell treated with 0.25 mM oleate for 6 days is undergoing apoptosis. Arrows in (E) and (F) indicate chromatin condensation. Under all conditions, the culture medium contained 1 % BSA as described in [27]. Pictures were taken at ×3000 magnification. GC, Golgi complex; IG, insulin granule; LD, lipid droplet; M, mitochondrion; N, nucleus.

Following our electron microscopy observation that NEFAs cause ER distension in β-cells (illustrated in Figure 2), we demonstrated that NEFAs trigger ER stress in a β-cell line [26], and several studies have since demonstrated that palmitate, and, to a lesser extent, oleate, activate an ER stress response in insulin-producing INS-1(E) and MIN6 cells [21,27,28]. Palmitate markedly activates the IRE1, PERK and ATF6 pathways, while the unsaturated oleate leads to milder PERK and IRE1 activation and comparable ATF6 signalling. In addition to the work in β-cell lines, we recently demonstrated palmitate-induced ER stress in FACS-purified primary rat β-cells and human islets [29]. The NEFA-induced ER stress response was not modified by high glucose, suggesting that ER stress in primary β-cells is mostly lipotoxic,
and not glucolipotoxic. The lack of a major impact of high glucose on ER stress in primary β-cells highlights the capacity of these cells to deal with augmented functional demand (see the Pancreatic β-cell recovery from ER stress section below). These observations also suggest that irreversible ER stress, and consequent apoptosis, will only be triggered in β-cells when key regulatory functions of the ER are impaired. In line with this idea, IFN-γ (interferon γ) and high glucose contribute to CPA (cyclosporic acid), a reversible blocker of SERCA (sarcoplasmic/endooplasmic reticulum Ca$^{2+}$-ATPase)-induced apoptosis in INS-1E cells, by decreasing expression of ER chaperones and other ‘defence’ pathways [29,30]. It is noteworthy that the ER stress markers observed in vivo in islets obtained from Type 2 patients [19–22] can be reproduced in vitro by exposure of human islets to NEFAs, but not to high glucose [29]. This, and the fact that increased NEFA levels precede, by many years, hyperglycaemia in obese individuals progressing to Type 2 diabetes, suggest that lipotoxicity may be more relevant than glucotoxicity in the pathogenesis of β-cell loss in Type 2 diabetes.

We examined how NEFAs trigger ER stress in β-cells. Palmitate led to an early and sustained depletion of ER Ca$^{2+}$ stores, which can trigger ER stress by impairing protein folding [29]. The degree of ER Ca$^{2+}$ depletion might lead to a differential activation of ER stress pathways by the different NEFAs, with PERK requiring a more severe depletion compared with IRE1, and ATF6 being readily activated by saturated and non-saturated NEFAs alike. The activation of the latter branch may be protective, as overexpression of ATF6-dependent BiP attenuated ER stress and protected β-cells against palmitate in one study [21], although not in another [31]. In line with this possibility, an equimolar combination of palmitate and oleate, which is not toxic to β-cells, induced BiP expression, but failed to induce PERK activation or major eIF2α phosphorylation [29]. In addition to these classical ER stress transducers, we observed that OASIS (old astrocyte specifically induced substance) is expressed in β-cells (M. Cnop and D.L. Eizirik, unpublished work). Resembling ATF6, OASIS is cleaved by S1P and S2P in response to ER stress, and its cytoplasmic domain acts as a transcriptional activator in the nucleus [32]. Whether OASIS plays a role in β-cell physiology and ER homeostasis remains to be examined.

As an alternative approach to protect β-cells from ER stress, chemical chaperones have been used. These molecules facilitate protein folding and have previously been used to alleviate ER stress and insulin resistance in peripheral tissues [33]. In INS-1 cells, the chemical chaperone 4-phenylbutyric acid was shown to restore ER morphology and reduce eIF2α phosphorylation by palmitate [34], but other ER stress markers were not examined.

Because deficient PERK–eIF2α signalling is deleterious to β-cells, we explored the potential protective effects of salubrinal, a selective inhibitor of eIF2α dephosphorylation [35]. Unexpectedly, up-regulation of the PERK–eIF2α pathway by salubrinal markedly potentiated the deleterious effects of palmitate and oleate [27] and even rendered the oleate and palmitate mixture toxic to β-cells (Figure 3). The synergistic activation of the PERK branch and the marked induction of ATF4 and CHOP exacerbated NEFA-induced β-cell apoptosis (Figure 3), but had little effect on apoptosis induced by other ER stressors, such as cytokines (Figure 3), CPA or thapsigargin [27]. PERK is involved in the execution of NEFA-induced β-cell apoptosis through induction of the transcription factor CHOP, as we demonstrated using an RNA interference approach [29]. ATF3 did not seem to play a role in NEFA-induced β-cell apoptosis [29], whereas it has been shown to be pro-apoptotic under basal conditions, and contribute to cytokine- and nitric-oxide-induced β-cell death [19,36]. Additional pro-apoptotic signals include palmitate-induced JNK (c-Jun N-terminal kinase) activation, possibly downstream of IRE1 [29] (see also Figure 1). We also observed NEFA-mediated activation of caspase 12, which is specifically activated under conditions of ER stress, and downstream caspase 3/7 in β-cells [29].

**Figure 3** Salubrinal selectively sensitizes β-cells to NEFAs
Primary rat β-cells were cultured for 72 h in the presence or absence of oleate or an equimolar combination of oleate and palmitate (0.5 mM NEFA in the presence of 1% BSA, as described in [27]) or cytokines (50 units/ml IL-1β and 500 units/ml IFN-γ), without (open bars) or with 75 μM salubrinal (black bars). The percentage of apoptotic β-cells was determined following staining with the DNA-binding dyes propidium iodide and Hoechst 33342. Based on data from [27]. Results are means ± S.E.M. for 4–15 experiments. *P < 0.05, **P < 0.001 for the comparison with controls, +P < 0.05, ++P < 0.001 for the comparison with the same experimental condition without salubrinal, by ANOVA followed by paired Student’s t test.

**Translational suppression in β-cells under ER stress**

The suppression of global protein translation is part of the physiological ER stress response, and allows a decrease in the arrival of newly synthesized proteins in the ER. Under conditions of severe ER stress, the role of translational suppression in β-cell survival is controversial. The potentiation of NEFA toxicity by salubrinal, an inhibitor of eIF2α dephosphorylation, could indicate that translation inhibition is poorly tolerated by β-cells [27]. In a study using the GLP-1 (glucagon-like peptide-1) receptor agonist...
exendin-4, translational suppression by thapsigargin was relieved by GADD34 up-regulation and PP-1-mediated eIF2α dephosphorylation, and this was associated with enhanced β-cell survival [37]. During ER stress, translation in β-cells is inhibited via a second mechanism: ATF4 transcriptionally up-regulates 4E-BP (eIF4E-binding protein) 1 [38]. By sequestering eIF4E, 4E-BPs reduce the initiation of cap-dependent translation, as the 5′ end of mRNAs is no longer recognized and bound to the eIF4F complex [39]. The 4E-BP1 induction seems to prolong suppression of translation in ER stressed β-cells. Deletion of this mechanism of translational control increased sensitivity of MIN6 cells to thapsigargin or tunicamycin, and worsened β-cell demise and diabetes in Akita and WFS1−/− mice [38]. In the absence of 4E-BP1, the enhanced translation of CHOP was suggested to contribute to β-cell apoptosis. Thus eIF2α and 4E-BP1 may both be involved in the differential regulation of translation of pro- and anti-apoptotic proteins in pancreatic β-cells. Translational control plays a major role in the selective regulation of protein expression that takes place during cell stress and apoptosis [39], and it remains to be clarified how this level of regulation operates in β-cells exposed to severe ER stress. Differential regulation of survival and death signals in the course of ER stress and during β-cell recovery is also present at the transcriptional level, as discussed below.

**Pancreatic β-cell recovery from ER stress**

To clarify some of the mechanisms involved in the process of recovery from ER stress, we recently performed a time-course microarray analysis in INS-1E cells exposed to the reversible SERCA blocker CPA for up to 12 h, including an additional group of cells treated for 6 h and then allowed to recover without CPA for 3 h [40]. CPA, shown previously to induce severe β-cell ER stress and apoptosis [30,41], modified expression of approx. 190 genes in at least one of the time points studied. The two groups of genes most affected by CPA were those involved in cellular responses to ER stress, which were up-regulated, and those related to differentiated β-cell functions, which were down-regulated. After the 3 h recovery period, most genes returned to basal levels, as is the case for the pro-apoptotic transcription factors ATF3 and CHOP. On the other hand, expression of the ER chaperones BiP and GRP94 remained elevated (Figure 4). This, together with the fast decrease in expression of pro-apoptotic genes, may explain why β-cells that have endured a severe ER stress promptly recover after removal of the stressor, without reaching the ‘point of no return’ for cell death.

CPA markedly inhibited the expression of mRNAs encoding insulin-1 and -2 (Figure 4); a similar inhibition was observed in INS-1E and primary rat β-cells exposed to thapsigargin or IFN-γ and IL-1β (interleukin 1β) [40]. In Drosophila, ER stress induces a rapid degradation of mRNAs targeted for translation at the ER through the IRE1 endoribonuclease activity [42]. This degradation decreases production of non-vital proteins at the ER and complements other UPR mechanisms, such as PERK activation, in decreasing the functional load in the ER. Since insulin is the most prevalent mRNA in β-cells, we reasoned that a similar mechanism could be operative during β-cell ER stress. Indeed, the CPA-mediated early degradation of insulin-1 and -2 occurred in parallel to IRE1 activation and in the absence of altered insulin promoter activity [40], and recent observations indicate that partial inactivation of IRE1 prevents insulin mRNA depletion during ER stress [43]. We therefore suggest that (i) early and massive degradation of insulin mRNA, (ii) prolonged up-regulation of ER chaperones, and (iii) immediate down-regulation of pro-apoptotic genes upon removal of the source of ER stress all contribute to β-cell survival.

**Figure 4 | Time course of β-cell gene expression during ER stress and recovery**

INS-1E cells were exposed to the reversible SERCA blocker CPA (25 mM) for 2 or 6 h or 6 h followed by a 3 h recovery period (without CPA). Gene expression data were assessed by APOCHIP microarray or by real-time PCR (for XBP1s) and are expressed as fold change compared with control. (A) CPA-induced ER stress caused a sharp increase in the expression of the pro-apoptotic genes ATF3 and CHOP, and in the transcription factors ATF4 and XBP1s. Upon arrest of the ER stress, however, the expression of these genes returned to basal levels. Note that the scale for XBP1s is different. (B) CPA-induced chaperones BiP, calnexin, GRP94, Sec61 and PDI (protein disulfide-isomerase) increased progressively during ER stress and, in contrast with the pro-apoptotic genes (in A), remained elevated after the 3 h recovery period. (C) ER stress caused a marked decrease in insulin mRNA expression, which was only partially restored after the 3 h recovery period. The decrease in insulin expression is mirrored by the increase in XBP1s (indicating IRE1α activation; A). Based on data from [40].
during and after ER stress. Future studies should evaluate whether similar changes are present in the course of physiological/pathophysiological fluctuations of the UPR in β-cells.

Conclusions
ER stress in pancreatic β-cells is likely to contribute to β-cell failure in Type 2 and other forms of diabetes, and our understanding of ER stress signalling in β-cells has significantly progressed in recent years [1]. Depending on the context, different ER stress signalling responses prevail. ER stress elicited by saturated or non-saturated NEFAs, cytokines, mutant pro-insulin accumulation and synthetic agents such as thapsigargin or tunicamycin will have different properties and outcomes, i.e. β-cell survival or apoptosis. In addition, the response varies according to cell types, and may have specific characteristics in β-cells. A better understanding of the mechanisms regulating the survival/death balance will help to pave the way for the development of novel approaches to prevent pancreatic β-cell loss in diabetes.

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