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ER Stress in Diabetic Peripheral Neuropathy: A New Therapeutic Target

Phillipe D. O'Brien,[1] Lucy M. Hinder,[1] Stacey A. Sakowski,[2] and Eva L. Feldman[1]

Abstract

Significance: Diabetes and other diseases that comprise the metabolic syndrome have reached epidemic proportions. Diabetic peripheral neuropathy (DPN) is the most prevalent complication of diabetes, affecting ~50% of diabetic patients. Characterized by chronic pain or loss of sensation, recurrent foot ulcerations, and risk for amputation, DPN is associated with significant morbidity and mortality. Mechanisms underlying DPN pathogenesis are complex and not well understood, and no effective treatments are available. Thus, an improved understanding of DPN pathogenesis is critical for the development of successful therapeutic options. ***Recent Advances:*** Recent research implicates endoplasmic reticulum (ER) stress as a novel mechanism in the onset and progression of DPN. ER stress activates the unfolded protein response (UPR), a well-orchestrated signaling cascade responsible for relieving stress and restoring normal ER function. ***Critical Issues:*** During times of extreme or chronic stress, such as that associated with diabetes, the UPR may be insufficient to alleviate ER stress, resulting in apoptosis. Here, we discuss the potential role of ER stress in DPN, as well as evidence demonstrating how ER stress intersects with pathways involved in DPN development and progression. An improved understanding of how ER stress contributes to peripheral nerve dysfunction in diabetes will provide important insight into DPN pathogenesis. ***Future Directions:*** Future studies aimed at gaining the necessary insight into ER stress in DPN pathogenesis will ultimately facilitate the development of novel therapies. *Antioxid. Redox Signal.* 21, 621–633.

Introduction

DISEASES THAT COMPRISE the metabolic syndrome, including obesity, atherosclerosis, and diabetes mellitus, have reached epidemic proportions. In 2012, the International Diabetes Federation reported that over 370 million people worldwide have diabetes (38). In the US, 1.9 million new diabetes cases were diagnosed in 2010, adding to the existing cases for a total of 25 million Americans, or over 8% of the population (12). A further 33% of the US population is affected by prediabetes, a condition characterized by elevated glucose levels and impaired glucose tolerance, and associated with a high risk of developing diabetes (15, 25).

Diabetes is a complex metabolic disorder affecting carbohydrate, lipid, protein, and electrolyte metabolism. Type 1 diabetes is due to impaired insulin signaling resulting from pancreatic islet cell death, while type 2 diabetes is due to insulin resistance in metabolically active tissues. Chronic hyperglycemia in diabetes invokes the onset of macrovascular (heart disease, stroke, and peripheral arterial disease) and microvascular (nephropathy, retinopathy, and neuropathy) complications. Diabetic peripheral neuropathy (DPN) is the most prevalent microvascular complication, affecting ~50% of diabetic patients (19). The consequences of DPN include chronic pain or loss of sensation, recurrent foot ulcerations, and amputation (20). Mechanisms underlying the pathogenesis of DPN are complex and despite over 30 years of intensive research, no mechanism-based treatment has proved effective in the treatment of DPN in man (20, 96). An improved understanding of DPN pathogenesis is critical for the development of successful therapeutic options. Recent research implicates endoplasmic reticulum (ER) stress as a novel mechanism in the onset and progression of DPN (56, 57).

[1]Department of Neurology, University of Michigan, Ann Arbor, Michigan.
[2]A. Alfred Taubman Medical Research Institute, University of Michigan, Ann Arbor, Michigan.

ER stress is associated with the development of various diseases, including neurodegenerative disorders (53) and more recently the metabolic syndrome (3, 68, 76, 80, 105). In this review, we summarize evidence supporting the potential role of ER stress in the pathogenesis of DPN. We first introduce the ER stress response and then present evidence of ER stress in DPN. Finally, we discuss the important intersection of ER stress pathways with other established signaling mechanisms associated with peripheral nerve injury in DPN. Understanding how ER stress contributes to peripheral nerve dysfunction in diabetes will provide important insight into DPN pathogenesis and may identify novel therapeutic targets.

The ER Stress Response

The ER is a membranous network that extends from the nuclear envelope toward the periphery of the cell. It is required for protein packaging and lipid biosynthesis, and acts as an intracellular calcium store and as a sensor of cellular stress. All secreted and membrane proteins must undergo specific post-translational modifications and appropriate protein folding within the ER before they are fully functional and targeted to their final destination. The ER has an intricate quality control system to ensure accuracy of protein folding and post-translational modifications, with the capacity to adapt to homeostatic disturbances caused during periods of cellular stress or at times when increased demands on protein production occur. Pathological cellular stressors that disrupt ER homeostasis include increases in unsaturated fatty acids or cholesterol, altered redox status, nutrient deprivation, elevated glucose, and perturbation of calcium homeostasis. These stressors result in the accumulation of unfolded or misfolded proteins within the luminal space of the ER, resulting in ER stress and activation of the unfolded protein response (UPR), a well-orchestrated signaling cascade responsible for relieving stress and restoring normal ER function (17). This is accomplished by (i) attenuating protein translation, (ii) upregulating the synthesis of chaperones and enzymes that assist in protein folding, and (iii) promoting protein degradation *via* ER-associated protein degradation (ERAD) (78).

Responding to stress: the UPR

The distinct signaling pathways of the UPR are mediated by three sensors that detect disturbances in the luminal environment of the ER: protein kinase-R-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1α (IRE1α) (Fig. 1). Under native conditions, the chaperone binding immunoglobulin protein (BiP), also known as glucose regulating protein (GRP)78, is bound to each sensor to prevent their activity. During periods of ER stress, however, BiP dissociates from the sensors and preferentially binds to misfolded or unassembled proteins within the ER, resulting in the activation of the UPR (6, 58, 79). Dissociation of BiP from PERK results in dimerization and autophosphorylation of the kinase to activate the PERK pathway and decrease protein influx into the ER. This translational attenuation is achieved by PERK-mediated phosphorylation of eukaryotic initiation factor 2α (eIF2α) that in turn blocks the guanine nucleotide exchange activity of eIF2B that is required for eIF2α cycling and continued protein synthesis (32, 44). Repression of global protein synthesis resulting from limited eIF2α activity results in favored translation of mRNAs, including activating transcription factor 4 (*Atf4*) (104). Translocation of ATF4 to the nucleus upregulates expression of proteins and chaperones required to restore ER homeostasis (33). Dissociation of BiP from the transcription factor ATF6 results in translocation of ATF6 to the Golgi apparatus where it undergoes sequential proteolysis by site 1 and site 2 proteases. The cytosolic fragment of ATF6 translocates to the nucleus where it induces transcription of molecular chaperone proteins, similar to ATF4 (34, 77). Finally, dissociation of BiP from IRE1α results in IRE1α dimerization and autophosphorylation (109). Following autophosphorylation, IRE1α endoribonuclease activity cleaves X-box binding protein 1 (*Xbp1*) mRNA to remove a 26-nucleotide intron. Spliced X-box binding protein 1 (*sXbp1*) is translated and in turn XBP1 translocates to the nucleus to initiate transcription of chaperone proteins and proteins involved in ERAD. Together, these adaptive mechanisms of the UPR function to attenuate mild to moderate ER stress to restore normal ER function (78).

Responding to stress: apoptosis

During times of extreme or chronic stress, the capacity of the UPR is overwhelmed, and the resulting failure to alleviate ER stress triggers apoptotic processes (Fig. 2) (28). In addition to splicing *Xbp1* mRNA, activated IRE1α is capable of triggering cell death *via* its association with tumor necrosis factor receptor-associated factor 2 (TRAF2) and apoptosis signal-regulating kinase 1 (ASK1). During ER stress, the adapter protein TRAF2 is recruited to the kinase domain of IRE1α, followed by ASK1, and the formation of the IRE1α/TRAF2/ASK1 heterooligomeric complex then recruits c-Jun-N-terminal kinase (JNK) and activates the JNK signaling pathway, promoting the establishment of a proapoptotic environment (93). The PERK-mediated branch of the UPR also contributes to stress-induced apoptosis. PERK activation and phosphorylation of eIF2α leads to upregulation of *Atf4*, which subsequently induces the transcription of C/EBP homologous protein (*Chop*). CHOP is ubiquitously expressed at low levels during normal cellular homeostasis; however, persistent ER stress leads to robust CHOP expression. $Chop^{-/-}$ cells are protected from ER stress-induced apoptosis (113), and CHOP has been associated with inherited demyelinating disorders, including Charcot–Marie–Tooth 1B disease (16, 73), further highlighting the significance of this pathway. CHOP contributes to apoptosis *via* upregulation of ER oxidase 1α (*Ero1*α), which subsequently activates the ER calcium channel inositol 1,4,5-triphosphate (IP3) to trigger the release of calcium stores into the cytosol (47). This ER stress-induced release of calcium results in inner mitochondrial membrane depolarization, cytochrome c release, and initiation of cell death processes (90). CHOP also activates growth arrest and DNA damage 34 (GADD34), which dephosphorylates eIF2α, thereby promoting the recovery of protein synthesis that was repressed by PERK (16, 59). Finally, in mice, the UPR-induced activation of apoptosis is further complimented by cleavage of procaspase-12 within the ER to alternatively initiate the caspase signaling cascade (66, 88).

ER Stress in Disease

Dysregulated ER stress responses have been implicated in various degenerative disorders, such as Parkinson's disease,

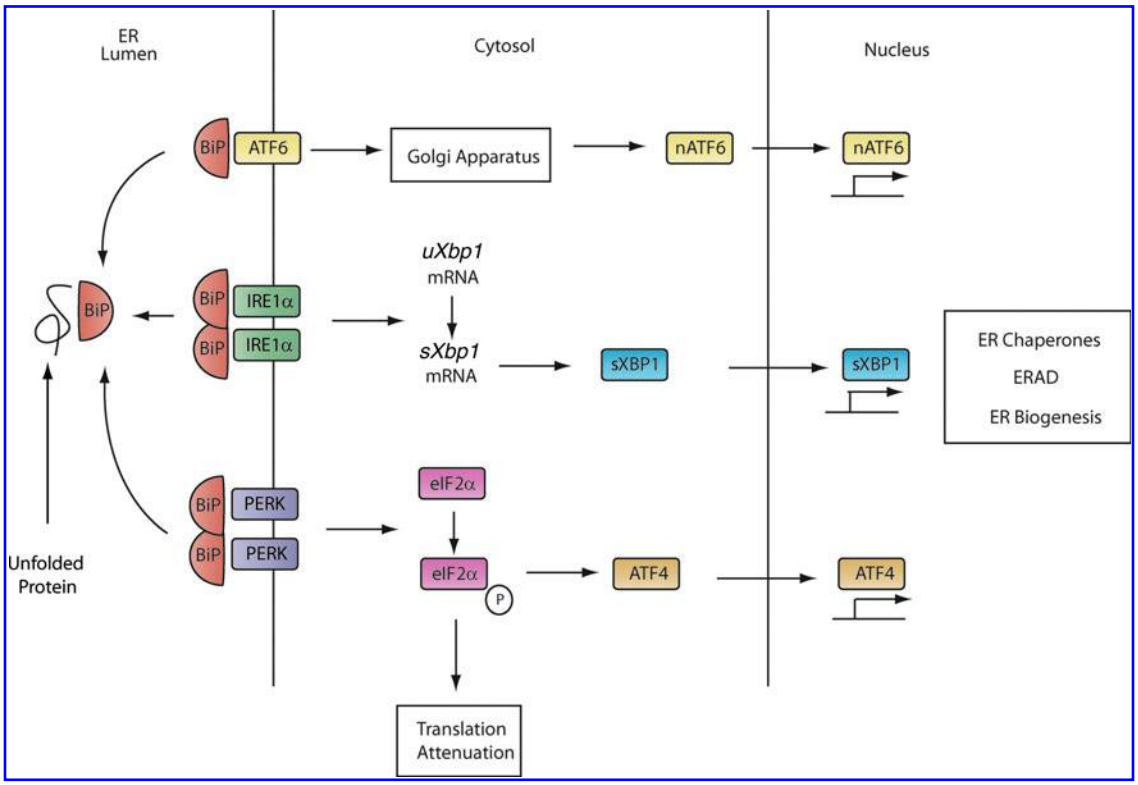

FIG. 1. The UPR signaling pathway. During normal cellular homeostasis, the ER chaperone BiP, also known as GRP78, is associated with the ER transmembrane sensors PERK, eIF2α, and ATF6. The ER stress response is activated when BiP dissociates from the ER stress sensors and binds to misfolded proteins, thus activating the three arms of the UPR. Dimerization and autophosphorylation of PERK phosphorylates eIF2α, which halts protein synthesis and activates ATF4 translocation to the nucleus, where ATF4 initiates the transcription of genes involved in restoring homeostasis. IRE1α autophosphorylation promotes the splicing of *Xbp1*. Once spliced and activated, sXBP1 translocates to the nucleus where it initiates transcription of genes leading to the expression of molecular chaperones and components of the ERAD system. Following dissociation from BiP, ATF6 translocates to the Golgi apparatus where it is cleaved by specific proteases, after which, fragmented ATF6 moves to the nucleus and initiates transcription of molecular chaperone proteins. Together, these pathways alleviate stress and restore homeostasis by halting protein translation, upregulating synthesis of molecular chaperones, and activating ERAD. ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BiP, binding immunoglobulin protein; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; GRP, glucose regulating protein; IRE1α, inositol-requiring enzyme 1α; PERK, protein kinase-R-like ER kinase; XBP1, X-box binding protein 1; UPR, unfolded protein response. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Alzheimer's disease, multiple sclerosis, Charcot–Marie–Tooth 1B, and amyotrophic lateral sclerosis (53, 73, 80, 83). In recent years, a large body of literature has also been documented linking ER stress to diseases of the metabolic syndrome, thus supporting the premise that ER stress may be a critical player in DPN pathogenesis (Table 1) (3, 68, 76, 105).

ER stress in the metabolic syndrome

Diseases of the metabolic syndrome include obesity, atherosclerosis, and diabetes. Obesity-induced ER stress has been identified in mice with diet-induced obesity, a model of prediabetes, and in type 2 diabetes leptin-deficient *ob/ob* mice, as evidenced by elevated levels of phosphorylated PERK and eIF2α in liver and adipose tissue compared with lean controls (68). This association was further confirmed in man. Reductions in *Bip* and *sXbp1* as well as increases in phosphorylated eIF2α and JNK are present in adipose tissue of obese patients (29), and increased eIF2α phosphorylation, ATF4, and CHOP are evident in liver tissue of obese patients (74). Increased ER stress is also manifest in diabetic pathophysiology. Pancreatic β cells undergo UPR-induced apoptosis due to sustained insulin signaling (21), UPR-dependent insulin resistance in liver and muscle is associated with activation of the JNK signaling pathway (24), and diabetes-induced ER stress has been linked to diabetic nephropathy, retinopathy, and cognitive decline (14, 80, 89, 112). More recently, however, evidence has arisen implicating a role for ER stress in peripheral nerve injury and DPN (56, 57).

ER stress in DPN

DPN is primarily a sensory polyneuropathy. Despite being described as the most common and devastating complication associated with diabetes, there are no effective therapies. The main reason for lack of treatment options is an incomplete understanding of disease pathogenesis. DPN development and progression occur within the heterogeneous environment of the peripheral nerve and involve a complex interplay

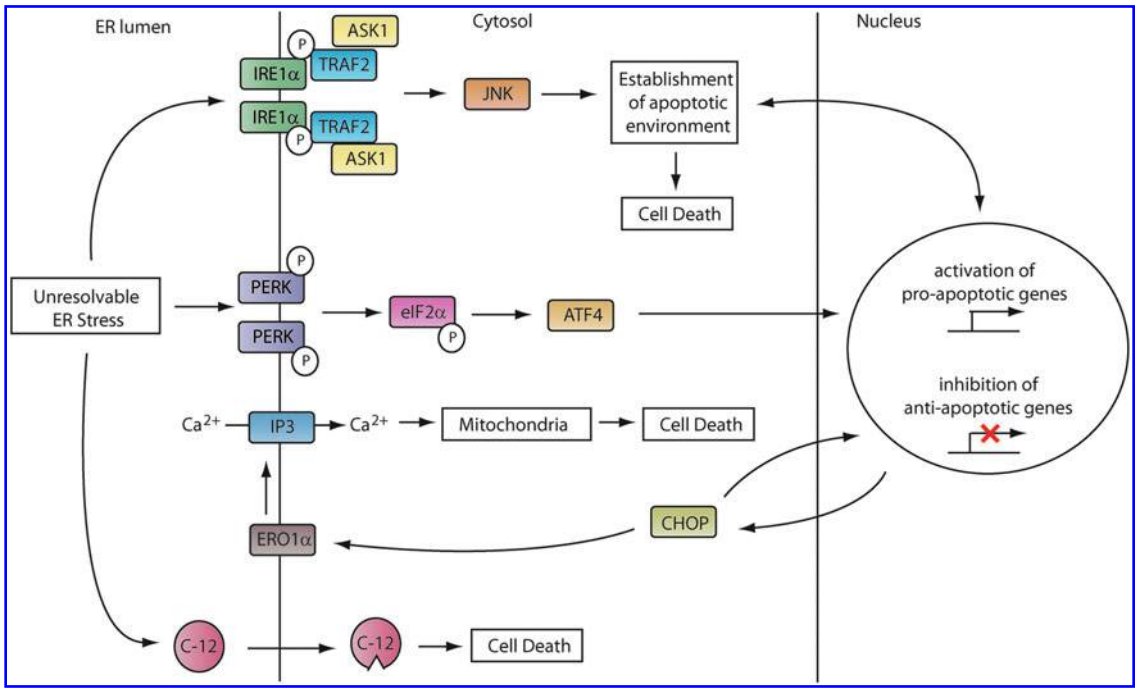

FIG. 2. ER stress-associated apoptosis. Under conditions of unresolvable ER stress, apoptotic pathways are activated. IRE1α forms a multioligomer complex with TRAF2 and ASK1, and in turn recruits and activates JNK, which promotes cell death by establishing a proapoptotic environment. The PERK/eIF2α pathway is also capable of activating apoptosis *via* ATF4-mediated upregulation of CHOP, which activates expression of proapoptotic genes and downregulates the expression of antiapoptotic genes. CHOP also induces the expression of ERO1α, which activates the ER calcium release channel, IP3, resulting in increased cytosolic calcium that triggers mitochondrial-associated cell death. Alternatively, activation and cleavage of ER-bound caspase-12 can trigger apoptosis *via* UPR-independent mechanisms. ASK1, apoptosis signal-regulating kinase 1; CHOP, C/EBP homologous protein; ERO1α = ER oxidase 1α; IP3, inositol 1,4,5-triphosphate; JNK, c-Jun-N-terminal kinase; TRAF2, tumor necrosis factor receptor-associated factor 2. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

between the nerve and surrounding cells and tissues. A decreased blood flow to the nerves (108), hyperglycemia (35, 99, 101), dyslipidemia (98, 99), and a lack of insulin signaling (42) contribute to DPN pathogenesis. These factors induce multiple pathogenic processes, such as low-grade inflammation, elevated sorbitol aldose reductase signaling, protein kinase C activation, advanced glycated end products, oxidative stress, and mitochondrial dysfunction (96), which culminate in the physiologic and morphologic changes associated with DPN.

A role of ER stress in DPN is being increasingly considered based on *in vivo* evidence supporting ER stress involvement in the initiation and progression of DPN in both type 1 and type 2 diabetic rodent models. Furthermore, studies using ER chaperone proteins, which assist protein folding in the ER by preventing newly synthesized polypeptide chains and assembled subunits from aggregating into nonfunctional structures, is an emerging therapeutic approach aimed at restoring ER function (22). Oral administration of trimethylamine oxide (TMAO), a chemical chaperone known to alleviate ER stress, to Zucker fatty (*fa/fa*) rats decreased protein expression of BiP/GRP78 in the sciatic nerve, and improved nerve conduction velocities and behavioral responses to mechanical and thermal stimuli (56). C57B6 mice fed a high-fat diet (HFD) also displayed an improved neuronal phenotype when treated with salubrinal, a compound that enhances eIF2α phosphorylation (56). Neuropathy severity was also attenuated in streptozotocin (STZ)-treated rats treated with TMAO (57). Finally, C57B6 $Chop^{-/-}$ mice injected with STZ displayed improved nerve function and increased expression of the folding proteins BiP/GRP78 and GRP94 in peripheral nerves compared with wild-type STZ-injected mice, further suggesting a role for ER signaling in DPN development (57). Together, these data support a link between ER stress and DPN, as restoration of ER function by administration of chemical chaperones or ER stress inhibitors alleviates ER stress and improves peripheral nerve function (56, 57).

Although these studies confirm the role of ER stress in DPN, certain considerations must be made when evaluating outcomes following systemic administration of chemical chaperones. As both hyperglycemia and dyslipidemia are known contributors to DPN (10, 96, 98), improved neuronal phenotypes could be attributed to enhanced UPR function in pancreatic β cells rather than direct effects on the UPR in the peripheral nerve. Administration of the chemical chaperone phenyl butyric acid (PBA) or overexpression of oxygen regulated protein 150 (ORP150) improves pancreatic β cell function and enhances insulin secretion, which in turn improves the metabolic profile of the animal (67, 69). Similarly, insulin sensitivity in adipose and hepatic tissue may be restored following chaperone treatment (24). This must also be considered in DPN studies. TMAO treatment in Zucker *fa/fa* rats improved blood glucose and triglyceride levels, and impaired glucose tolerance was attenuated in high fat-fed mice treated with salubrinal (56). Thus, systemic

Table 1. ER Stress in Diseases Related to the Metabolic Syndrome

Tissue/cell	*Disease model*	*ER stress response*	*Ref.*
Obesity/atherosclerosis			
Liver	Prediabetic HFD-fed mice; type 2 diabetic *ob/ob* mice	ER stress promotes JNK-induced phosphorylation of IRS1 and a resulting decrease in insulin gene expression	(68)
	HFD-fed *Lcat*$^{-/-}$ mice	Cholesterol contributes to diet-induced obesity and insulin resistance through an ER stress-mediated pathway	(31, 49)
Macrophage	Mouse peritoneal macrophages	Cholesterol loading depletes calcium stores and activates ER-dependent apoptosis	(23, 74)
Adipose tissue	HFD-fed mice; 3T3-L1 preadipocytes	Hypoxia increases the expression of *Bip* and *Chop* mRNA	(36)
Diabetes			
Pancreas	INS-1 and rat pancreatic islet cells	High glucose treatment increases *Chop* and *Bip* mRNA expression	(29, 103)
	Pancreatic β cells	Chronic high glucose exposure causes ER stress, hyperactivation of IRE1α, and suppression of insulin expression; pancreatic islets show increased IRE1α phosphorylation and increased *sXbp1* mRNA expression	(54)
	db/db pancreatic islets; type 2 diabetic patient pancreatic sections; insulin secreting MIN6 cells	ER stress identified in palmitate-treated MIN6 cells and islets from *db/db* mice; elevated BiP and CHOP are evident in pancreatic sections from type 2 diabetic patients	(46)
Diabetic complications			
Kidney	Type 1 diabetic STZ rats	Elevated BiP, CHOP, JNK, and caspase-12 is evident in kidney homogenates	(55)
	Human diabetic nephropathy kidney biopsies; renal epithelial cells	Diabetic nephropathy patients exhibit increased *BiP* and *sXbp1*; hyperglycemia induces *Xbp1* and *Bip* in renal epithelial cells	(52)
	Cultured murine podocytes	Palmitate-induced lipotoxicity increased BIP and CHOP levels, and decreased levels of the antiapoptotic protein Bcl-2	(89)
Eye	Akita mice; oxygen-induced retinopathy mice	Increased levels of *Bip*, mRNA; eIF2α, and ATF4 protein present in mouse retina; tunicamycin increased BiP and ATF4 in mouse retina	(48)
	Type 1 diabetic STZ mice; rat Muller retinal cells	STZ-treated mice exhibit elevated ATF4 in Muller cells of the retina; high glucose induces ATF4 in cultured rat retinal Muller cells	(112)
Nerve	Immortalized Schwann cells	Palmitate-induced lipotoxicity upregulates *Chop*, *Bip*, and *Xbp1*; ER stress response is magnified with increasing levels of glucose	(70)
	Zucker *fa/fa* rats	Elevated BiP and GRP94 protein expression in sciatic nerve	(56)
	Type 1 diabetic STZ rats; STZ-treated *ChoP*$^{-/-}$ mice	Phosphorylated PERK, eIF2α, IRE1α, BiP, and ERO1α identified in sciatic nerve, and increased CHOP, ERO1α, and BiP identified in spinal cord of STZ-treated rats; *Chop* knockout in STZ diabetic mice improves DPN phenotypes	(57)

ATF4, activating transcription factor 4; BiP, binding immunoglobulin protein; CHOP, C/EBP homologous protein; DPN, diabetic peripheral neuropathy; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERO1α, ER oxidase 1α; GRP, glucose regulating protein; HFD, high-fat diet; IRE1α, inositol-requiring enzyme 1α; IRS1, insulin receptor substrate 1; JNK, c-Jun-N-terminal kinase; PERK, protein kinase-R-like ER kinase; STZ, streptozotocin; XBP1, X-box binding protein 1; sXBP1, spliced X-box binding protein 1.

administration or overexpression of chaperones may mask the beneficial effects on the nerve by indirectly improving the metabolic profile. To circumvent these issues, direct neuronal delivery of chaperones without impacting systemic glycemia may be warranted to elucidate the role of ER stress on nerve function in DPN. However, TMAO treatment in STZ-injected rats improved DPN phenotypes despite maintained hyperglycemia (57), suggesting that therapies targeting ER stress responses may have direct efficacy in the peripheral nerve.

Molecular Mechanisms of ER Stress and DPN

Although the previous studies validate the contention that ER stress is present in DPN, mechanistic studies are required to elucidate the involvement and implications of ER stress in DPN development and progression. Given the heterogeneous environment of the peripheral nerve, vascular endothelial cells, macrophages, glial cells, and nerve cells may all potentially contribute to DPN pathogenesis (Fig. 3B). Therefore, determining the precise localization of ER stress in peripheral nerve and characterization of UPR pathways in these cells is necessary to comprehend what role ER stress plays in DPN. Furthermore, the multitude of physiological mechanisms that are potentially involved in DPN pathogenesis, including hyperglycemia, dyslipidemia, inflammation, oxidative stress, and altered calcium signaling (Fig. 3A), all add a level of complexity to studies aimed at understanding the connections between ER stress and DPN. In this review,

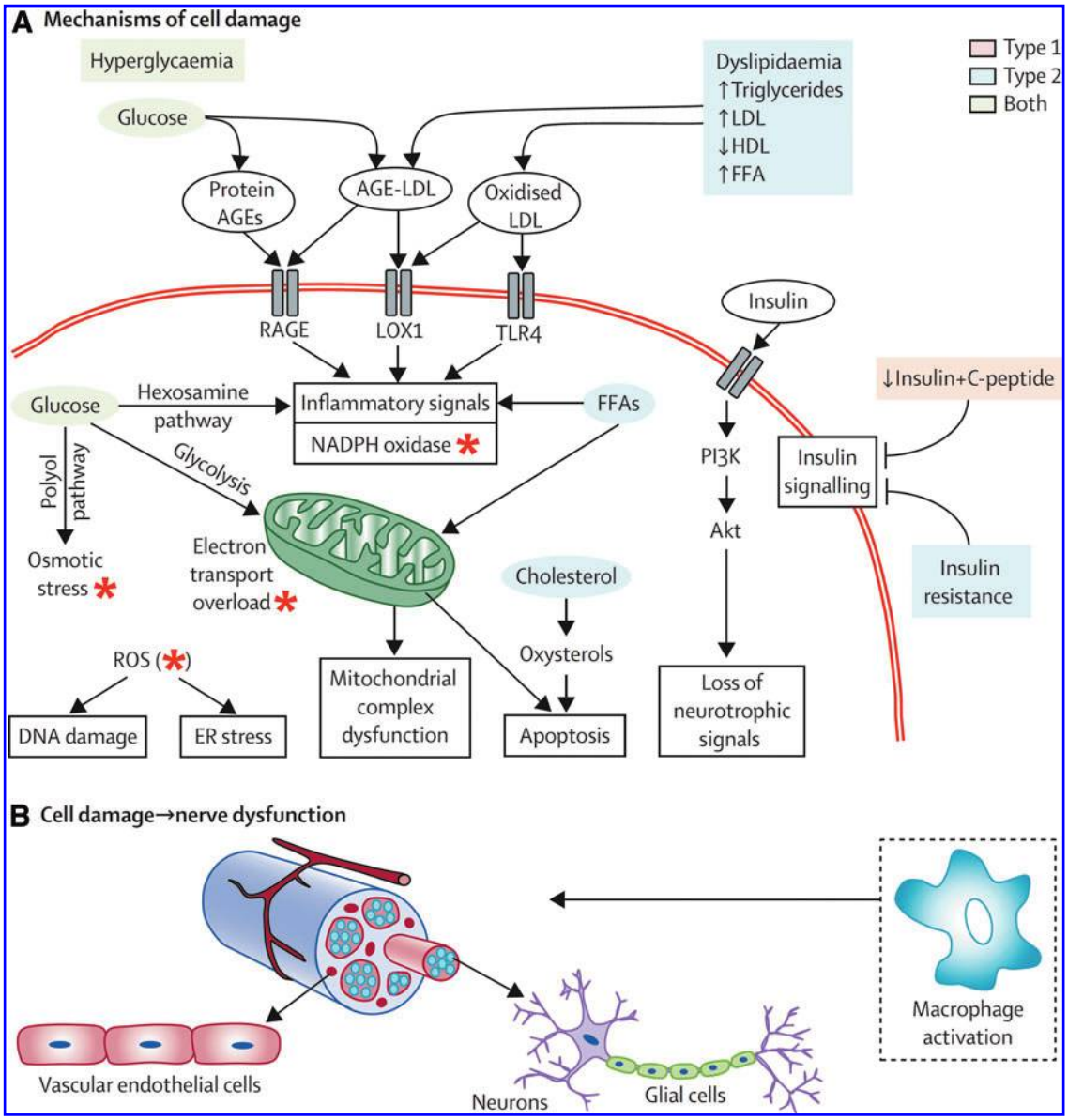

FIG. 3. Mechanisms of diabetic neuropathy. Factors linked to type 1 diabetes (orange), type 2 diabetes (blue), and both (green) cause DNA damage, ER stress, mitochondrial complex dysfunction, apoptosis, and loss of neurotrophic signaling **(A)**. This cell damage can occur in neurons, glial cells, and vascular endothelial cells, as well as triggering macrophage activation, all of which can lead to nerve dysfunction and neuropathy **(B)**. The relative importance of the pathways in this network will vary with the cell type, disease profile, and time. AGE, advanced glycation end products; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FFA, free fatty acids; ROS, reactive oxygen species (red star); PI3K, phosphatidylinositol-3-kinase; LOX1, oxidized LDL receptor 1; RAGE, receptor for advanced glycation end products; TLR4, Toll-like receptor 4. Reprinted from: The Lancet Neurology, Vol. 11, Callaghan *et al.*, Diabetic neuropathy: clinical manifestations and current treatments, Pages 521–534, Copyright (2012), with permission from Elsevier. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

we discuss evidence demonstrating how ER stress intersects with these pathways to provide insight into the possible involvement of ER stress in DPN pathogenesis.

Hyperglycemia

Evidence from studies examining ER stress in diabetic complications-prone tissues supports the contention that hyperglycemia can impact ER stress and the integrated stress response (14, 45). Diabetic retinopathy studies demonstrate that high-glucose treatment of retinal endothelial cells results in increased levels of CHOP, ATF4, and phosphorylated PERK and eIF2α, upregulation of inflammatory cytokines, including tumor necrosis factor α (TNFα), and increased expression of vascular endothelial growth factor (VEGF) (14). This link is further supported by studies demonstrating improvements in retinal inflammation and vascular leakage with *Atf4* knockdown in STZ-injected mice (14). Markers of UPR signaling are also significantly upregulated in diabetic cardiomyopathy. Increased expression of phosphorylated PERK and eIF2α, ATF4, CHOP, and ATF6 is observed in the myocardium of spontaneous diabetic Torii rats, a model of nonobese type 2 diabetes (45). Finally, lipotoxicity-induced ER stress responses, including decreases in ER calcium levels and expression of CHOP, XBP1, and BiP/GRP78, are exacerbated in immortalized Schwann cells (iSCs) in the presence of high glucose (70); however, glucose exposure in the absence of lipotoxicity did not affect cell viability or ER calcium levels. In addition, along these lines, diabetic patients with DPN who receive intensive glucose therapy do not show favorable improvements in nerve function (10), suggesting that glucose-independent processes also contribute to DPN.

Dyslipidemia

Dyslipidemia is strongly associated with DPN pathophysiology by both epidemiological and basic research (2, 98, 99, 105), and elevated levels of fatty acid and cholesterol-induced ER stress have been implicated in metabolic diseases (37, 46, 50, 65, 89). Therefore, it is important to understand how abnormal lipid content can provoke ER stress in DPN. Cell-based approaches have determined that elevated concentrations of free fatty acids (FFA) can trigger the UPR in supporting cells of the peripheral nerve. Using iSCs, investigators have demonstrated that lipotoxicity associated with the saturated fatty acid palmitate (PA) promotes iSC dysfunction and cell death *via* the UPR signaling pathway, as evidenced by increased expression of BiP, CHOP, and XBP1 (70). These studies also demonstrated that activation of the UPR precedes the generation of reactive oxygen species (ROS), mitochondrial depolarization, and apoptosis, suggesting that the ER stress response occurs upstream of these processes (70). Interestingly, oleate, a monounsaturated fatty acid, abolishes PA-induced ER stress and lipotoxicity in C2C12 myoblasts, indicating that the composition of fatty acid-derived phospholipids within the ER is an important determinant of UPR activation (72).

Cholesterol has also been implicated in ER stress, as the ER is incredibly sensitive to perturbations in free cholesterol levels given its particularly low native cholesterol content (8). In macrophages, cholesterol loading depletes ER luminal calcium stores and triggers UPR activation, the expression of CHOP, and caspase-3-mediated apoptosis (23). Moreover, unlike esterified cholesterol, the insertion of free cholesterol into the lipid bilayer of macrophages can cause disturbances in the physical properties of the membrane, which may in turn activate the UPR. Along these lines, Schwann cells play a significant role in myelination and are incredibly sensitive to perturbation in membrane composition, suggesting that dyslipidemia-induced ER stress in Schwann cells may play a role in DPN pathogenesis. ER stress is involved in multiple myelin-related disorders (16, 51, 73, 83), and recent evidence has identified abnormalities in the sciatic nerve of STZ-treated rats that include imbalances in myelin's phospholipid, fatty acid, and cholesterol content (13). Thus, an altered lipid profile brought on by disturbances in lipid homeostasis has important consequences in Schwann cell biology by affecting membrane fluidity and function (1, 13). This may be especially important during remyelination following nerve injury in diabetes if myelin generation is compromised due to elevated/abnormal lipid substrates. However, the role of lipid homeostasis and ER stress in DPN remains relatively unexplored and further studies addressing this relationship are warranted.

Insulin signaling

Although hyperglycemia resulting from dysfunctional insulin signaling in pancreatic β cells and metabolically active tissues is a key contributor to nerve injury in DPN, recent evidence also suggests that impaired insulin signaling in peripheral neurons may also be an important factor in nerve degeneration. The insulin receptor (IR) and insulin receptor substrate 1 (IRS1) are widely expressed in cell bodies and axons of peripheral neurons (30, 86, 87), where they are activated by insulin to provide vital neurotrophic support to the nerve (9, 91, 107). In STZ-injected rats, intrathecal low-dose insulin treatment attenuated DPN-associated reductions in nerve function and reversed atrophy of myelinated sural nerve sensory axons (9). Similarly, insulin resistance has been observed in dorsal root ganglia from db/db type 2 diabetic mice (42) and in hypothalamic neurons in response to PA-induced lipotoxicity (62). Whereas the exact mechanisms linking ER stress and insulin resistance in peripheral nerves must still be elucidated, several mechanisms linking ER stress and hepatic and adipose insulin resistance have been proposed (24). One theory suggests that UPR signaling directly suppresses IR signaling through hyperactivation of JNK and subsequent phosphorylation of IRS1 (39, 62, 93). Alternatively, induction of lipogenic and glyconeogenic genes downstream of UPR signaling could result in abnormal activation of these pathways and ultimately promote insulin resistance (24). Further studies are required to comprehend how ER stress contributes to insulin resistance in DPN.

Inflammation

ER stress-induced UPR signaling is associated with the production of numerous proinflammatory cytokines (50). In macrophages, excess free cholesterol induces TNFα and interleukin-6 (IL-6) production *via* activation of JNK/NFκB and CHOP, respectively (37, 110). Recent studies also provide evidence that inflammation-mediated ER stress is important in Schwann cell-based nerve injury. Studies investigating spinal cord injury in rats demonstrate that

upregulation of TNFα, following sciatic nerve crush injury, effectively triggers the UPR in Schwann cells (61). Along with increased BiP/GRP78 expression, TNFα induction of CHOP expression led to Schwann cell apoptosis, demyelination, and nerve degeneration (61), suggesting that inflammatory mediators may play an important role in DPN disease progression *via* ER stress pathways. Upregulation of low-grade inflammation (41, 81) and elevation of cytokines, such as TNFα (26, 106), in patients with DPN further emphasize the importance of additional studies focused on determining whether these inflammatory factors contribute to ER stress-induced nerve pathology.

Oxidative stress

Oxidative stress is a major mechanism of hyperglycemia-induced DPN in humans and rodents (97, 99, 102). During normal cellular metabolism in mitochondria, ROS are formed as by-products of electron transfer to molecular oxygen and are important signaling molecules in biological processes such as autophagy, phagocytosis, and inflammation (95). However, increased ROS production and compromised endogenous antioxidant defenses in diabetes lead to oxidative stress, a pro-oxidant state resulting in injurious oxidation of proteins and lipids (97, 99, 102). Indeed, hyperglycemia leads to increased ROS and cellular apoptosis in cultured dorsal root ganglia neurons (102). Furthermore, the ER is both a source of increased ROS generation and is affected by increased cellular ROS (4). Although it is less well characterized than in mitochondria, the ER has its own electron transport system that traffics reducing equivalents within the lumen of the ER and transfers electrons to molecular oxygen, resulting in concomitant ROS production (60). Thus, ROS generation is a natural by-product of ER oxidative protein folding and accounts for $\sim$25% of the total cellular ROS generation (92). Under times of increased protein load, ER ROS generation can significantly increase (60). Given the potential for high levels of ROS in the cell and ER, oxidative stress may significantly impact the ER stress response in DPN.

During normal physiological conditions, endogenous antioxidant mechanisms are upregulated to scavenge free radicals and prevent cellular injury (4). During ER stress, activation of PERK signaling increases NF-E2-related factor 2 (NRF2) activation and subsequent transcription of antioxidant and detoxification enzyme genes. This response is evident in cultured dorsal root ganglion neurons and in Schwann cells in response to acute hyperglycemic and oxidative (hydrogen peroxide) stress (100). Prolonged hyperglycemia, on the other hand, attenuates dorsal root ganglion neuron antioxidant levels and activities (100), increasing the significance of ER ROS generation under disease conditions. Similarly, the glutathione antioxidant activity in the ER is impacted by oxidative stress. During normal ER protein folding (4, 60) and during the UPR response to correct misfolded proteins (60), glutathione is converted from its reduced form (GSH) to an oxidized form (GSSG); however, excessive UPR activation can deplete the GSH:GSSG ratio, decreasing its antioxidant capacity. Chronic stress-induced UPR-mediated CHOP elevation also depletes the glutathione antioxidant capacity and increases ROS production, further escalating cellular oxidative stress levels (5, 63). Along these lines, decreasing ER stress and CHOP expression with TMAO chaperone protein treatment for 12 weeks following STZ-induced diabetes in rats attenuates lipid and protein oxidation in the sciatic nerve, and is coincident with improvements in electrophysiological, behavioral, and structural neuropathy parameters in treated diabetic animals (57). Further work from the same group using *Chop* knockout mice confirmed that a CHOP-mediated ER stress response is involved in sciatic nerve oxidative stress and the development of DPN (57), and is consistent with reports that pancreatic β cell CHOP deletion decreases oxidative damage in a number of mouse models of diabetes (82).

In addition to normal protein folding and the UPR, sources of ROS outside the ER may also trigger the ER stress response (*i.e.*, the mitochondrial respiratory chain, increased NADPH oxidase activity, and inflammatory processes). Although these sources of ROS in diabetes and their contribution to peripheral nerve dysfunction are relatively well characterized, their interactions with the ER remain largely unexplored (60) and require future attention.

Calcium signaling

The ER is a major store for intracellular calcium, and calcium levels are three- to four-times greater in the ER lumen than in the cytosol (85); thus, factors that perturb calcium homeostasis activate the UPR. Experimentally, calcium stores in the ER can be depleted using the calcium ionophore A23167 or thapsigargin to block uptake of calcium from the cytosol (7, 18). This depletion impairs calcium-dependent chaperones and folding enzymes and results in the accumulation of misfolded proteins and subsequent activation of the UPR (11, 27, 75, 84). Disruptions of ER calcium homeostasis are involved in various forms of neuropathology (94), and evidence for disrupted ER calcium homeostasis is evident in diabetic animal models. Dorsal root ganglia sensory neurons from type 1 diabetic STZ-injected rats exhibit reduced calcium levels and diminished calcium uptake by the ER (111). Similarly, comparison of lumbar and cervical dorsal root ganglia neurons from diabetic STZ rats revealed that altered calcium dynamics are more prominent in lumbar neurons, the ganglia that are affected first in DPN (40, 43). Additional implications for a role of ER calcium homeostasis in DPN stems from the recent microarray analysis of peripheral nerve tissue from diabetic mice. Differentially expressed genes (DEGs) related to Ca^{2+} transport are highly downregulated in 24-week db/db mice; however, no significant increase is observed in UPR-related DEGs despite large changes in calcium (71). Experimental validation is required to confirm these data, but it is plausible that calcium-induced ER stress may induce UPR-independent cellular stress. Studies in human and mouse macrophages demonstrate that ER stress activates the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome, and subsequent inflammatory responses independent of the UPR (64), and calcium release from the ER can impact mitochondrial membrane depolarization and increase oxidative stress, which could contribute to neuronal injury (27). Further examination into the relationship between calcium signaling in DPN is required.

Conclusion

Despite recent advances in our understanding of DPN pathophysiology, few therapies exist for the management of

DPN. Given the recent association of ER stress with DPN in diabetic animal models, the association of ER stress with pathways involved in DPN pathogenesis and the insight gained from studies examining interventions that target ER stress pathways, the development of therapeutic approaches that enhance ER function (*i.e.*, increase chaperone availability) and decrease UPR-activated cell death should be considered. The role of ER stress in DPN, however, remains largely unexplored and several key questions must still be answered. For instance, what induces ER stress in the diabetic milieu of the peripheral nerve? What cells in the peripheral nerve are affected by ER stress, and are all affected cells equally susceptible to ER stress? Furthermore, which UPR pathways are implicated in DPN? Finally, what is the interplay between ER stress and other components of DPN pathogenesis, such as calcium storage and transport, oxidative stress, myelination and insulin signaling? As an abundance of information on other signaling pathways in DPN etiology exists, it is crucial to understand these interactions with the ER and elucidate how they contribute to disease. With additional insight into cell-specific contributions of the UPR to DPN pathogenesis, we hope to ultimately discover mechanism-based therapies that can prevent this injury cascade and ameliorate the signs and symptoms of DPN.

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Address correspondence to:
Dr. Eva L. Feldman
Department of Neurology
University of Michigan
109 Zina Pitcher Place
5017 AAT-BSRB
Ann Arbor, MI 48109

E-mail: efeldman@umich.edu

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Abbreviations Used

AGE = advanced glycation end products
ASK1 = apoptosis signal-regulating kinase 1
ATF4 = activating transcription factor 4
ATF6 = activating transcription factor 6
BiP = binding immunoglobulin protein
CHOP = C/EBP homologous protein
DEG = differentially expressed gene
DPN = diabetic peripheral neuropathy
eIF2α = eukaryotic initiation factor 2α
ER = endoplasmic reticulum
ERAD = ER-associated protein degradation
ERO1α = ER oxidase 1α
FFA = free fatty acid
GRP = glucose regulating protein
GSH, GSSG = glutathione (reduced, oxidized)
HDL = high-density lipoprotein
HFD = high-fat diet
IL-6 = interleukin-6
IP3 = inositol 1,4,5-triphosphate
IR = insulin receptor
IRE1α = inositol-requiring enzyme 1α
IRS1 = insulin receptor substrate 1
iSCs = immortalized Schwann cells
JNK = c-Jun-N-terminal kinase
LCAT = lecithin-cholesterol acyltransferase
LDL = low-density lipoprotein
LOX1 = oxidized LDL receptor 1
NLRP3 = NOD-like receptor family, pyrin domain containing 3
NRF2 = NF-E2-related factor 2
ORP150 = oxygen regulated protein 150
PA = palmitate
PBA = phenyl butyric acid
PERK = protein kinase-R-like ER kinase
PI3K = phosphatidylinositol-3-kinase
RAGE = receptor for advanced glycation end products
ROS = reactive oxygen species
STZ = streptozotocin
sXBP1 = spliced X-box binding protein 1
TLR4 = Toll-like receptor 4
TMAO = trimethylamine oxide
TNFα = tumor necrosis factor α
TRAF2 = tumor necrosis factor receptor-associated factor 2
UPR = unfolded protein response
XBP1 = X-box binding protein 1

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