Endothelin-1–Induced Endoplasmic Reticulum Stress in Disease

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ABSTRACT

The accumulation of unfolded proteins in the endoplasmic reticulum (ER) represents a cellular stress induced by multiple stimuli and pathologic conditions. Recent evidence implicates endothelin-1 (ET-1) in the induction of placental ER stress in pregnancy disorders. ER stress has previously also been implicated in various other disease states, including neurodegenerative disorders, diabetes, and cardiovascular diseases, as has ET-1 in the pathophysiology of these conditions. However, to date, there has been no investigation of the link between ET-1 and the induction of ER stress in these disease states. Based on recent evidence and mechanistic insight into the role of ET-1 in the induction of placental ER stress, the following review attempts to outline the broader implications of ET-1–induced ER stress, as well as strategies for therapeutic intervention based around ET-1.

Endoplasmic Reticulum Stress

The endoplasmic reticulum (ER) is a multifunctional organelle involved in the synthesis, folding, and post-translational modification of membrane and secretory proteins. In addition, the ER also serves as a reservoir of calcium ions (Ca$^{2+}$) (Zhang and Kaufman, 2008). In the ER lumen, Ca$^{2+}$ is buffered by calcium-binding proteins. Many of these proteins also serve as molecular chaperones involved in the folding and quality control of ER proteins, and their functional activity alters with changes in Ca$^{2+}$ concentration (Michalak et al., 1998). Growth factors, hormones, and stimuli that perturb cellular energy levels, nutrient availability, or redox status all affect ER calcium storage. Loss of ER Ca$^{2+}$ homeostasis suppresses post-translational modifications of proteins in the ER. As a result, misfolded proteins accumulate, provoking ER stress response pathways known collectively as the unfolded protein response (UPR) (Brostrom and Brostrom, 2003).

The UPR is induced to restore ER homeostasis. It reduces the burden of new proteins entering the ER lumen through attenuation of translation, enhances the protein folding capacity by increasing ER chaperone proteins [glucose-regulated protein78 (GRP78) and GRP94] and folding enzymes, and promotes degradation of remaining unfolded or misfolded proteins through increased capacity of the cytosolic ubiquitin-proteasome system (Brostrom and Brostrom, 2003; Yung et al., 2008; Zhang and Kaufman, 2008). The UPR is initiated by three ER-localized protein sensors as follows: double-stranded RNA-dependent protein kinase-like ER kinase (PERK), inositol-requiring 1$\alpha$ (IRE1$\alpha$), and activating transcription factor 6 (ATF6) (Fig. 1). In resting cells, all three ER-stress sensors are maintained in an inactive state through association with the abundant ER chaperone immunoglobulin-heavy chain-binding protein, also referred to as GRP78. During ER stress, GRP78 is sequestered through binding to unfolded or misfolded polypeptide chains, which leads to the release and, consequently, activation of the ER stress sensors (Zhang and Kaufman, 2008). The release of GRP78 results in activation of PERK through PERK homodimerization and transphosphorylation. This is the most immediate response of the UPR. Activated PERK then phosphorylates the eukaryotic initiation factor 2$\alpha$ (eIF2$\alpha$) subunit, which inhibits nonessential protein synthesis (Zhang and Kaufman, 2008). This helps promote cell survival by preventing

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ABBREVIATIONS: A$\beta$, amyloid-\$\beta$ peptides; ATF6, activating transcription factor 6; BQ788, N-cis-2,6-dimethylpiperidinocarbonyl-L-methylleucyl-o-1-methoxy carbonyltryptophanyl-o-norleucine; CHOP, CCAAT/enhancer-binding protein homologous protein; EAE, experimental autoimmune encephalomyelitis; ECE-1, endothelin-converting enzyme-1; eIF2$\alpha$, eukaryotic initiation factor 2$\alpha$; ER, endoplasmic reticulum; ET-1, endothelin-1; ETA, endothelin A; ETB, endothelin B; GRP78 and -94, glucose-regulated protein78 and -94; IL, interleukin; IP3, inositol 1,4,5-triphosphate; IRE1$\alpha$, inositol-requiring 1$\alpha$; IUGR, intrauterine growth restriction; MMP-2, matrix metalloprotease-2; MS, multiple sclerosis; NO, nitric oxide; PERK, double-stranded RNA-dependent protein kinase-like ER kinase; PLC, phospholipase C; ROS, reactive oxygen species; SLV306, 2-[3(S)-[1-2(R)-[ethoxycarbonyl]-4-phenoxybutyl]cyclopentan-1-ylcarboxamido]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl]acetic acid; U73122, 1-(6-((17-3-methoxyestr-1,3,5(10)-tri en-17-yl(arnino)-hexyl)-1H-pyrrole-2,5-dione; UPR, unfolded protein response; WRS, Wolcott-Rallison syndrome; XBP-1, X-box–binding protein 1.
the influx of more nascent polypeptides into an already saturated ER lumen. The release of GRP78 also allows IRE1α to dimerize, activating both its protein kinase and endoribonuclease activity. IRE1α then initiates the removal of a 26-base intron from mRNA encoding X-box–binding protein 1 (XBP-1), resulting in a translational frameshift and transcription of an XBP-1 isoform with potent activity as a transcription factor, which activates the expression of UPR target genes. The third pathway in the UPR is mediated by ATF6. The luminal domain of ATF6 is responsible for sensing unfolded proteins, whereas the cytoplasmic portion of ATF6 has a DNA-binding domain containing the basic-leucine zipper motif and a transcriptional activation domain. In resting cells, GRP78 binds to the luminal domain of ATF6 and hinders the Golgi-localization signal (Yoshida, 2007). Under conditions of ER stress, the release of GRP78 from ATF6 allows ATF6 to translocate to the Golgi apparatus, where it is cleaved into an active cytosolic form. Active ATF6 then migrates to the nucleus and activates the transcription of more UPR target genes (Zhang and Kaufman, 2008). Essentially, cleaved ATF6 and active XBP-1 function in parallel pathways to induce the transcription of genes encoding ER chaperones and enzymes that promote protein folding. A final pathway of the UPR is responsible for degradation of any remaining unfolded or misfolded proteins. The ER-associated degradation machinery recognizes and translocates these proteins to the cytosol, where they are ubiquitinated and degraded by the cytosolic proteasome (Yoshida, 2007). Collectively, these pathways help resolve the protein folding defect and restore homeostasis in the ER. However, if this fails, apoptosis is then initiated to protect the organism by eliminating the stressed cells that produce misfolded or malfunctioning proteins (Schröder and Kaufman, 2005). ER stress–induced apoptosis is mainly mediated by CCAAT/enhancer-binding protein homologous protein (CHOP), a transcription factor that induces the expression of proapoptotic factors and inhibits transcription of antiapoptotic factors, such as Bcl-2 (Zhang and Kaufman, 2008).

Malfunction of ER stress responses due to aging, genetic mutations, or environmental factors can result in various diseases, such as diabetes, cardiovascular diseases, and neurodegenerative disorders (Yoshida, 2007; Kim et al., 2008). ER stress has also been implicated in the pathophysiology of pregnancy disorders (Yung et al., 2008; Burton et al., 2009; Jain, 2012).

**ER Stress in Pregnancy Disorders: Pre-eclampsia and Intrauterine Growth Restriction**

Pre-eclampsia and intrauterine growth restriction (IUGR) are major causes of clinical morbidity and mortality in pregnant women and prenatal infants, affecting between 3% and 10% of all pregnancies worldwide (Roberts and Hubel, 1999). A study by Yung et al. (2008) demonstrated that protein synthesis inhibition and ER stress play a key role in the pathophysiology of both IUGR and pre-eclampsia. Increased phosphorylation of eIF2α is found to inhibit protein synthesis and reduce cell proliferation in pathologic placentas. Increased p-eIF2α levels in the placenta lead to suppression of protein kinase B (AKT) and proteins/kinases in the mammalian target of rapamycin pathway (Yung et al., 2008). Reduced activity in this pathway results in reduced phosphorylation of 4E-binding protein 1, which is then able to bind
the translation initiation factor eIF4E and inhibit cap-dependent translation (Hay and Sonenberg, 2004). ER stress is also proposed to contribute to inflammation in pregnancy disorders. Following activation of the PERK pathway, upregulation of chaperone proteins occurs as part of the ATF6- and IRE1α-induced stress response. The ER produces reactive oxygen species (ROS) as a byproduct of protein folding, which would be increased under conditions of ER stress during repeated attempts to refold misfolded proteins. ROS produced under conditions of ER stress contribute to an inflammatory response. Increased apoptosis in human term placentas has also been implicated in both pre-eclampsia and IUGR (Ishihara et al., 2002). As explained earlier, if the UPR fails to restore ER homeostasis, then apoptosis is induced, which only occurs under severe conditions of ER stress. Yung et al. (2008) found increased levels of CHOP in both pre-eclamptic and IUGR placentas compared with normal control subjects. A study by Ishihara et al. (2002) also showed elevated apoptosis levels in pre-eclampsia. Collectively, these studies demonstrate evidence of ER stress in pregnancy disorders. A recent study by Jain et al. (2012) identified endothelin-1 (ET-1) as a key stimulus of this stress.

Role of ET-1 in ER Stress

Endothelins are a family of vasoactive peptides that have key physiologic functions, which include acting as modulators of the vascular tone, tissue differentiation, and cell proliferation (Nelson et al., 2003). The family of endothelins comprises three isoforms with 21 amino acids each (ET-1, ET-2, ET-3). ET-1 is the most abundant member of the family of endothelins (Struck et al., 2005) and is synthesized and secreted by a diverse range of cells. ET-1 exerts its effects by binding to the endothelin A (ETA) and endothelin B (ETB) receptors, two highly homologous cell-surface proteins that belong to the G protein–coupled receptor superfamily (Karet and Davenport, 1994). Upon binding G protein–coupled receptors, ET-1 triggers signaling cascades in a wide variety of target tissues. A recent study by Jain et al. (2012) demonstrated that ET-1 acting through the ETB receptor activates the phospholipase C (PLC)/inositol 1,4,5-triphosphate (IP3) pathway to induce Ca2+ release from the ER, which stimulates ER stress in placental tissue. ET-1 induced an increase in p-eIF2α levels and upregulation of the chaperone proteins, GRP78 and GRP94, which are all markers of ER stress (Jain et al., 2012). This action of ET-1 on the induction of ER stress was confirmed by the use of inhibitors acting at different stages of the proposed pathway (Fig. 2).

The study by Jain et al. (2012) also found increased ET-1 levels in both pregnancy disorders, pre-eclampsia and IUGR, which was in concurrence with previous clinical data (Fiore et al., 2005). Deficient conversion of maternal spiral arteries (a key underlying feature of pregnancy disorders) that produces an ischemia/reperfusion–type insult was demonstrated to be a source of the elevated levels of ET-1. Hence, ET-1 was proposed to be an important stimulus of ER stress in pregnancy disorders. Many of the molecules that ET-1 was shown to activate have also been found to be activated in other tissues and cell types, but this was the first report of their activation by ET-1 in relation to the induction of ER stress. Given the presence and activation of these molecules in other tissue and indeed other disease states, including neurodegenerative diseases, broader implications for ET-1–induced ER stress are suggested over and above the effects in pregnancy.

The following sections describe the evidence for ER stress in different diseases, where ET-1 levels are also increased, and attempt to highlight the likely link between the two, in the context of the recent findings concerning the induction of placental ER stress by ET-1 in pregnancy disorders.

Neurodegenerative Diseases

Unfolded or misfolded proteins that accumulate during conditions of ER stress can form aggregates in the ER, as well as the cytosol. These aggregates are highly toxic, as they impair the ubiquitin proteasome pathway (Bence et al., 2001), and excessive accumulation of unfolded proteins triggers apoptotic pathways. Neurons are thought to be sensitive to protein aggregates, and there are numerous studies that have reported ER stress is involved in neurodegenerative diseases (Althausen et al., 2001; Milhavet et al., 2002; Hayashi et al., 2005; Hoozemans et al., 2005; Unterberger et al., 2006; Lin and Popko, 2009; Deslauriers et al., 2011). ET-1 has been linked to the generation of oxidative damage in neurodegenerative diseases, but there has been no assessment of the role of ET-1–induced ER stress in these conditions.

Multiple Sclerosis

Multiple sclerosis (MS) is characterized by inflammatory demyelination and ensuing neurodegeneration. ER stress has been proposed to be one mediator of this inflammation, and numerous groups have implicated ER stress in MS-related disease mechanisms (Lin and Popko, 2009; Deslauriers et al., 2011). The study by Deslauriers et al. (2011) found increased levels of inflammatory cytokines, as well as increased splicing of XBP-1 and higher levels of GRP78 in the white matter of MS patients compared with healthy control subjects. ER stress and inflammation have also been found in the MS model, experimental autoimmune encephalomyelitis (EAE). EAE is characterized by T-cell activation and entry into the central nervous system with neuroinflammation, recapitulating many of the events that occur in MS (Kap et al., 2010). Several markers of ER stress, including GRP78, CHOP, and splicing of XBP-1, were all increased in EAE compared with control subjects (Deslauriers et al., 2011). The study by Yung et al. (2008) showed that these same markers of ER stress are also elevated in pregnancy disorders, and the later study by Jain et al. (2012) identified ET-1 as a key stimulus of this stress.

ET-1 is widely distributed in the body, including the neurons and glia in the central nervous system (Filipovich and Fleisher-Berkovich, 2008). Furthermore, ET-1 levels are increased in several neurodegenerative diseases, and thus numerous studies have investigated the role of ET-1 in these conditions. Speciale et al. (2000) found increased levels of ET-1 and nitric oxide (NO) in the cerebrospinal fluid of patients with MS (Speciale et al., 2000). Other studies have also demonstrated increased ET-1 plasma levels in MS patients compared with sex- and age-matched healthy control subjects (Haufschild et al., 2001; Pache et al., 2003).

Activation of glial cells in the brain is believed to contribute to the pathogenesis of multiple sclerosis (Matsumoto et al., 1992). Activation of glia leads to production of proinflammatory
and cytotoxic factors, such as prostaglandins and NO that have been connected to increased neurotoxicity in in vitro neuron-glia cultures. ET-1 has been proposed to significantly enhance the synthesis of prostaglandin E\(_2\) and NO in glial cells (Filipovich and Fleisher-Berkovich, 2008). Although the exact mechanism by which ET-1 stimulates glial prostaglandin E\(_2\) synthesis is not clear, one possibility is activation of PLC, which participates in a secondary pathway of arachidonic acid release, the first step in prostaglandin E\(_2\) synthesis. The regulation of NO synthesis by ET-1 is also thought to occur via the stimulation of PLC. ET-1-mediated activation of PLC leads to the formation of IP\(_3\), which stimulates Ca\(_{\text{2+}}\) release from the ER. The resulting increase in cellular Ca\(_{\text{2+}}\) can induce the production of NO (Filipovich and Fleisher-Berkovich, 2008). The study by Jain et al. (2012) demonstrated that ET-1 induces ER stress via activation of the PLC/IP\(_3\) pathway to induce Ca\(_{\text{2+}}\) release from the ER, which disrupts ER Ca\(_{\text{2+}}\) homeostasis and hence stimulates ER stress. As the upregulation of proinflammatory factors (prostaglandins and NO) is considered to be mediated via PLC activation, there is considerable overlap with the signaling pathways implicated in the ET-1-induced ER stress response. Therefore, it is likely that, in addition to the effects described earlier, the elevated levels of ET-1 in multiple sclerosis also contribute to inflammatory demyelination via induction of ER stress.

Activation of glial cells in the brain is also thought to contribute to the pathogenesis of Alzheimer’s disease (Rogers et al., 1988).

**Alzheimer’s Disease**

Alzheimer’s disease is a progressive neurodegenerative condition characterized by extracellular lesions in the cerebral cortex composed largely of aggregates of amyloid-\(\beta\) peptide (Gething, 2000). Mutations in the presenilin-1 and presenilin-2 proteins have been proposed to cause early-onset Alzheimer’s disease by increasing the generation and secretion of amyloid-\(\beta\) peptides (\(\alpha\beta\)) in the brain. \(\alpha\beta\) has been reported to induce ER stress leading to neuronal cell death (Costa et al., 2012). Recent studies have found that 4-phenylbutyrate attenuates the activation of ER stress and associated neuronal cell death by activating the HRD1 ubiquitin ligase that promotes \(\alpha\beta\) clearance (Kaneko, 2012; Mimori et al., 2012). In addition to their effects on \(\alpha\beta\) generation, mutant versions of presenilin-1 have been found to induce altered ER Ca\(_{\text{2+}}\) homeostasis and render cultured neurons more susceptible to cell death induced by ER stress (Terro et al., 2002). Indeed, a study by Milhavet et al. (2002) found that brains of mice harboring Alzheimer’s disease–associated mutants of presenilin-1 have increased levels of CHOP, a marker of ER stress–induced cell death.

Autopsy studies have also shown that the PERK-eIF2\(\alpha\) pathway is hyperactive in the brains of patients with Alzheimer’s disease (Unterberger et al., 2006), further implicating ER stress in the pathogenesis of Alzheimer’s. In addition, a study by Hoozemans et al. (2005) found increased expression of the ER chaperone GRP78 (also indicative of UPR activation) in cases of Alzheimer’s disease compared with healthy control subjects. ET-1 has previously been shown to activate the PERK-eIF2\(\alpha\) pathway and induce GRP78 levels in pregnancy disorders (Jain et al., 2012). Given that ET-1 levels are elevated in Alzheimer’s disease (Palmer et al., 2009, 2012), ET-1 might compound the effect of presenilin-1 on disrupting ER Ca\(_{\text{2+}}\) homeostasis and rendering neurons more susceptible to cell death induced by ER stress. Previous research has looked at the vasoactive function of ET-1 as a pathologic factor responsible for the decrease in cerebral blood flow in...
Alzheimer’s disease (Palmer et al., 2009). Based on the evidence at hand, further research is required to investigate the role of ET-1 in inducing ER stress in Alzheimer’s disease.

**Acute Neurodegeneration**

Apart from the more chronic neurodegenerative diseases, ER stress is also shown to be present in acute brain disorders, such as ischemia. Focal cerebral ischemia in mice has been shown to upregulate phospho-eIF2α levels (Althausen et al., 2001), whereas global ischemia in mice has been found to induce CHOP and ATF4 (Hayashi et al., 2005). It has been reported that hippocampal neurons from CHOP-deficient mice are more resistant to cell death induced by ischemia/reperfusion compared with normoxic controls (Tajiri et al., 2004). Fewer neurons had degenerated in the CHOP−/− mice after ischemia, suggesting an important role for ER stress in ischemia/stroke. Ischemia/reperfusion has been demonstrated to also be a potent stimulus for induction of ET-1 levels (Jain et al., 2012), and given the prevalence of elevated ET-1 levels in neurodegenerative diseases and the concomitant induction of ER stress, ET-1 is likely one causative factor of this stress.

**Diabetes**

Diabetes mellitus is a metabolic disorder characterized by varying or persistent hyperglycemia due to reduced insulin action. Type 1 diabetes is characterized by a severe lack of insulin production due to specific destruction of the pancreatic β cells that typically develops over several years (Eizirik et al., 2001). Type 2 diabetes results from β-cell dysfunction with a reduced ability of the pancreatic β cells to secrete enough insulin to stimulate glucose utilization by peripheral tissues (Mathis et al., 2001).

Accumulating evidence suggests that reduction in β-cell mass, due to increased β-cell apoptosis and defective β-cell regeneration, is a key component in both types of diabetes (Mathis et al., 2001). Studies indicate a role of the ER in the sensing and transduction of apoptotic signals. One of the first evidences of the involvement of ER stress in diabetes came from studies on the Akita mouse, which is a spontaneously diabetic model characterized by progressive hyperglycemia with reduced β-cell mass. Genetic analyses revealed that a mutation in the insulin 2 gene (C96Y) is responsible for the diabetic phenotype in this mouse (Wang et al., 1999). Further investigations have revealed that progressive hyperglycemia in the mouse was accompanied by elevated levels of ER stress markers, such as GRP78 and CHOP. Since proinsulin is a major ER client protein in pancreatic β cells, it was suggested that its maldoping is the cause of this ER stress (Sundar Rajan et al., 2007).

Further evidence implicating ER stress in the pathophysiology of diabetes came from studies of Wolcott-Rallison syndrome (WRS) (Delepine et al., 2000). WRS is a rare, autosomal recessive disorder characterized by early infancy-onset diabetes mellitus. Mutations in the EIF2AK3 gene, which codes for pancreatic PERK, were found to be the underlying cause of this disorder. In addition, Harding et al. (2000) found that Perk−/− mice developed a clinical syndrome similar to that seen in WRS patients. Perk−/− cells were unable to phosphorylate eIF2α and attenuate translation in response to ER stress. The absence of PERK rendered these cultured cells prone to apoptosis, which led to progressive destruction of β cells (Harding et al., 2000).

Nitric oxide, one of the important effectors of β-cell death in type 1 diabetes and vascular complications in type 2 diabetes, has been shown to exert its effects by triggering ER stress (Oyadomari et al., 2001). Oyadomari et al. (2001) have shown that NO depletes ER Ca²⁺, which induces ER stress and leads to apoptosis. NO depletion of ER Ca²⁺ is thought to occur either by inhibition of Ca²⁺ uptake from cytosol through sarco/endoplasmic reticulum ATPase or by activation of Ca²⁺ release to the cytosol through the ryanodine receptor. ET-1 is a potent stimulus of NO (Stricklett et al., 2006), and given the recent evidence implicating ET-1 in the induction of ER stress by stimulating Ca²⁺ release through the IP3 receptor (Jain et al., 2012), ET-1 could be a potent stimulus of ER stress in diabetes through this dual action culminating in disruption of ER Ca²⁺ homeostasis. This is consistent with the finding by several groups that ET-1 levels are increased in patients with diabetes compared with control subjects (Seligman et al., 2000; Schneider et al., 2002). Many of the markers of ER stress implicated in diabetes that were discussed earlier have also been shown to be activated by ET-1. The study by Jain et al. (2012) demonstrated that ET-1 induces increased levels of the ER chaperone protein GRP78 and directly affected the PERK pathway. It was also proposed that, in vivo, ET-1 may act in synergy with other pathologic factors (NO in diabetes?) to induce more potent ER stress, with the activation of additional proinflammatory pathways and induction of apoptosis. The sustained elevated plasma levels of ET-1 found in diabetes could act continuously on pancreatic β cells to further exacerbate this effect.

**Cardiovascular Disease**

ER stress is also implicated in cardiovascular diseases such as cardiac hypertrophy, heart failure, atherosclerosis, and ischemic heart disease.

**Cardiac Hypertrophy and Heart Failure**

Cardiac hypertrophy is an adaptive response of the heart to hemodynamic overload, during which terminally differentiated cardiomyocytes increase in size without undergoing cell division. Although initially this hypertrophic response may serve to compensate cardiac function, prolonged hypertrophy can become detrimental, resulting in cardiac dysfunction and heart failure (Wang et al., 2003).

Morphologic analysis of degenerated cardiac muscle cells in patients with cardiac hypertrophy revealed dilated ER cisternae, which is an indicator of a stressed ER (Maron et al., 1975). Yung et al. (2008) also demonstrated this morphologic change in the ER cisternae that accompanied elevated levels of ER stress markers in pregnancy disorders, such as pre-eclampsia and IUGR. The later study by Jain et al. (2012) then demonstrated ET-1 as an important stimulus of this ER stress. Plasma ET-1 levels are found to be elevated in cases of heart failure (von Lueder et al., 2004).

Several studies have found a marked increase in GRP78 expression in hypertrophic and failing hearts, suggesting that activation of the UPR is associated with pathophysiology of
heart failure in humans (Okada et al., 2004; Dally et al., 2009). Further studies in a mouse model demonstrated that prolonged ER stress induced CHOP-mediated apoptosis in heart failure but not hypertrophic hearts, and it was suggested that the CHOP-dependent cell death pathway may be involved in the transition from cardiac hypertrophy to heart failure (Minamino and Kitakaze, 2010). The role of ET-1–induced ER stress in cardiomyopathy is supported by a recent study that found quercetin treatment caused a decrease in myocardial levels of ER stress, accompanied by a significant reduction in ET-1 levels (Arumugam et al., 2012).

**Atherosclerosis**

Atherosclerosis is a disease wherein arteries harden and narrow because of the accumulation of plaque (made up of fatty substances, cholesterol, calcium, and cellular waste) in the arterial inner lining, leading to heart attack or stroke (Yoshida, 2007). Accumulation of homocysteine has been found to be a risk factor for atherosclerosis (Lawrence de Koning et al., 2003). Homocysteine was found to induce ER stress as evinced by increased expression of GRP78, GRP94, and CHOP (Werstuck et al., 2001). The induction of CHOP promotes macrophage apoptosis, which leads to an accumulation of macrophage debris that form plaques in blood vessels. These then contribute to atherosclerosis observed in hyperhomocysteinemia (Yoshida, 2007). Consistent with this, CHOP−/− macrophages are less sensitive to apoptosis induced by homocysteine. These findings strongly support a role for ER stress–induced apoptosis in the development of atherosclerosis. However, there has been no investigation into the mechanism of induction of this ER stress by homocysteine. Interestingly, homocysteine has been found to induce ET-1 levels in bovine aortic endothelial cells (Sethi et al., 2006). Indeed, ET-1 levels are elevated in atherosclerotic plaques from human tissue (Zeihler et al., 1995), and these elevated levels of ET-1 could be one source of ER stress observed in atherosclerosis.

**Ischemic Heart Disease**

Atherosclerosis is causally involved in ischemic diseases, such as myocardial infarction and cerebral infarction (Hansson, 2005; Myoishi et al., 2007). Ischemic heart disease is a condition characterized by reduced blood supply to the heart. In conditions of cerebral infarction and acute myocardial infarction, the most effective therapy is early and successful reperfusion. However, recanalization of an occluded artery can cause damage through ischemia/reperfusion injury (Miyazaki et al., 2011). A natural cause of reperfusion injury is coronary artery vasospasm followed by coronary artery dilation (Hoffman et al., 2004). Ischemia/reperfusion–induced generation of ROS and depletion of ATP reduce Ca2+-storage within the ER, as ROS can damage the endoplasmic reticulum Ca2+-uptake system through oxidation of essential thiol groups on the transmembrane channels, whereas low ATP results in inhibition of ATP-dependent sarcoplasmic/endoplasmic reticulum Ca2+-ATPases (Yung et al., 2008). The resulting loss of Ca2+ homeostasis, compounded by the low ATP concentrations, leads to suppression of ATP and Ca2+-dependent post-translational modifications (Brostrom and Brostrom, 2003). As a result, misfolded proteins accumulate that then provoke ER stress response pathways. Azfer et al. (2006) reported that ER stress–associated genes, such as CHOP, are induced in the heart of an ischemic heart disease mouse model. Other studies also found increased ER stress markers in cardiomyocytes from near the site of myocardial infarction in mice as well as humans (Thuerauf et al., 2006; Severino et al., 2007).

As discussed earlier, the study by Jain et al. (2012) demonstrated that ischemia/reperfusion is also a potent stimulus of ET-1. These findings were consistent with previous work that found ET-1 levels are stimulated under oxidative stress; ROS that have been implicated in the pathophysiology of renal ischemia/reperfusion injury stimulate ET-1 production (Hughes et al., 1996). Incubation of human mesangial cells with ROS donors, such as xanthine/xanthine oxidase and hydrogen peroxide, caused a dose-dependent increase in ET-1 levels. Therefore, ET-1 levels stimulated under ischemia/reperfusion may compound the effect of ROS and low ATP levels during ischemia/reperfusion on induction of ER stress.

Collectively, ET-1–induced ER stress is likely an important pathologic factor in the diseases discussed. The following sections describe potential strategies for therapeutic intervention to block pathology associated with potential ET-1–induced ER stress in disease.

**Receptor Antagonists as Potential Therapeutic Tools against ET-1–Induced ER Stress**

The study by Jain et al. (2012) demonstrated that ET-1 induces ER stress via the ETB receptor–mediated activation of the PLC/IP3 pathway that leads to a disruption in ER Ca2+-homeostasis. The action of ET-1 via the ETB receptor was confirmed using an ETB receptor antagonist, BQ788 (N-cis-2,6-dimethylpiperidinocarbonyl-1-methyleucyl-n-1-methoxy-carbonyltryptophanyl-n-norleucine), which is a potent and selective inhibitor of ET-1 binding to the ETB receptor. This compound therefore provides a useful tool to block ET-1–induced ER stress via the ETB receptor.

As explained earlier, release of inflammatory mediators by glial cells is thought to play an important role in neuro-inflammation that contributes to the pathogenesis of neurodegenerative diseases, such as MS and Alzheimer’s disease (Rogers et al., 1988; Filipovich and Fleisher-Berkovich, 2008). BQ788 has been found to inhibit the production of both NO and prostaglandin E2 in glial cell cultures (Filipovich and Fleisher-Berkovich, 2008). Accordingly, the study by Filipovich and Fleisher-Berkovich (2008) proposes that BQ788 may have therapeutic potential in pathologic conditions of the brain.

ET receptor antagonists have also been investigated in a variety of cardiovascular conditions, including hypertension, atherosclerosis, and heart failure. In animal models of chronic heart failure, prolonged ETA receptor blockade improves cardiac function and prolongs survival (Luscher and Barton, 2000). Treatment of heart failure patients with the dual ET receptor antagonist bosentan has also been found to help lower pulmonary artery pressure (Sutsch et al., 1998). Dual ETA/ETB receptor blockade by bosentan also yields improvement in certain patients with coronary atherosclerosis.
(Wenzel et al., 1998). This enhanced effect of dual antagonism of the ET receptors indicates that, in addition to blocking the vasoconstrictive effects of ET-1 via the ETA receptor, blocking the ETB receptor may have the additional advantage of blocking the proinflammatory effects of ET-1, including the potential induction of ER stress. Further studies will indicate the precise contribution of ETB receptor blockade to ER stress–induced pathology.

Dual ET receptor antagonists have also been applied in diabetes. A study by Hocher et al. (2001) found that dual ETA/ETB receptor antagonists reduce proteinuria and normalize renal matrix protein expression in hyperglycemic rats with streptozotocin-induced diabetes. Blockade of the ETB receptor was proposed to reduce glomerular NO synthesis. As discussed earlier, NO is an important effector of β-cell death in type 1 diabetes and vascular complications in type 2 diabetes, through its effects on ER stress (Oyadomari et al., 2001). Therefore, ETB receptor antagonism might be useful for the dual blockade of ET-1–induced NO, as well as the direct induction of ER stress by ET-1.

Although the use of ET receptor antagonists has been useful in ameliorating disease pathology, there are certain drawbacks to consider. The use of ET antagonists has previously been associated with side effects, such as an increase in heart rate, facial flush, and/or facial edema (Fleisch et al., 2000). In addition, certain ET antagonists may interfere with anticoagulants, such as warfarin, and therefore pose a risk of thrombosis (Weber et al., 1999). These studies indicate the importance of only regulated antagonism of the ET receptor system as a therapeutic tool. An alternative strategy for prevention of ET-1–induced ER stress would be to target the actual elevated levels of ET-1 in pathologic conditions.

**Targeting the Elevated Levels of ET-1 in Pathologic Conditions**

ET-1 is derived from a larger precursor peptide (pre-proET-1; 212 amino acids) that is first cleaved at specific basic residues to give rise to bigET-1 (38 amino acids) and subsequently cleaved to generate mature ET-1 (Struck et al., 2005). The latter is achieved by the action of the endothelin-converting enzyme (ECE), which cleaves at the motif Trp73↓Val74.

Given the role of ECE-1 in ET-1 production, inhibition of this enzyme might provide a useful strategy to lower the pathologic increase in ET-1 levels in disease. Accordingly, a study by Minamino et al. (1997) found that ECE-1 may contribute to the process of injury-induced neointimal formation and atherosclerosis through increased production of ET-1, which could be blocked using the ECE-1 inhibitor phosphoramidon. Likewise, blocking the elevated ECE-1 expression levels in failing human hearts helps reduce elevated plasma ET-1 levels to normal values, which results in additional arterial vasodilatation, improved cardiac index, and decreased systemic and pulmonary vascular resistance (Galatius-Jensen et al., 1996).

Although there are numerous studies on ECE regulation of the ET system in cardiovascular diseases, and indeed investigation into inhibitors for therapeutic intervention, there are only very limited data on ECE antagonists in neurodegenerative diseases. However, previous research has found increased ECE activity in neurodegenerative conditions. It is known from animal models that matrix metalloprotease-2 (MMP-2) is identical to ECE in that it cleaves bigET-1 to the mature active ET-1. MMP-2 is expressed in and around MS plaques (Leppert et al., 2001), and it has been suggested that MMP-2 is at least one cause of the elevated levels of ET-1 in the cerebrospinal fluid and plasma of patients with MS (Speciale et al., 2000; Pache et al., 2003). Another study found increased levels of ECE-2 in Alzheimer's disease (Palmer et al., 2009). Neural tissues are abundant in ECE-2, which is an isoform of ECE-1 with 59% sequence homology and similar catalytic activity (Palmer et al., 2009). Palmer et al. (2009) propose that increased ECE-2 levels in Alzheimer’s disease are a source of the increased ET-1 and reduced cerebral blood flow prevalent in Alzheimer’s disease. Given the evidence of increased ECE activity in neurodegenerative diseases, further research is warranted to investigate the precise therapeutic value of ECE antagonism.

Studies have also found increased ECE-1 expression and activity in diabetes (Anstadt et al., 2002). Dual inhibition of neutral endopeptidase and ECE by SLV306 (2-[3(S)-1-[(2/R)-(ethoxy(carbonyl)-4-phenylbutyl)cyclopentan-1-y]amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl]acetic acid) reduces proteinuria and urinary albumin excretion (improves kidney function) in diabetic rats (Thone-Reinke et al., 2004).

However, although inhibition of ECE blocks the production of ET-1, recent identification of ECE-independent pathways contributing to ET-1 formation, such as non-ECE metalloproteases (in multiple sclerosis) (Leppert et al., 2001) and the renin-angiotensin system (in cardiovascular diseases) (d’Uscio et al., 1998), might limit the effectiveness of these drugs. The author would like to propose an alternate, novel strategy for targeting the elevated ET-1 levels in pathologic conditions as a means of blocking the ET-1–induced ER stress: using ET-1 traps.

Previous research has investigated the use of cytokine traps to target the elevated levels of specific cytokines in disease states as a means of blocking their pathologic activity. Economides et al. (2003) constructed cytokine traps by engineering inline fusions of the extracellular domains of target cytokine receptors [e.g., interleukin-6Ra (IL-6Ra) and glycoprotein (gp) 130]. These were fused to the Fc portion of human IgG1, which directs formation of disulfide-linked dimers, so that transfection of expression constructs into mammalian cells results in secretion of the desired dimeric inline cytokine trap (Fig. 3). Such soluble receptors bind cytokines with high affinity and were found to be potent cytokine blockers. The use of these traps was verified in both in vitro and in vivo models, where they were found to potently block target cytokine action and displayed good bioavailability in vivo. The same strategy was applied for other cytokines, including IL-1 and IL-4.

Given that the structure of human ET-1 has been elucidated, including characterization of the receptor binding site, development of ET-1 traps might provide a useful strategy for therapeutic intervention in the diseases described earlier. The three-dimensional structure of ET-1 has been determined using X-ray crystallography (Janes et al., 1994). The overall structure of the molecule consists of an N-terminal–extended β strand with a bulge between residues 5 and 7, followed by a central region that consists of a hydrogen-bonded loop between residues 7 and 11. The C terminus is a single, long, somewhat irregular helix that extends to the end of the molecule. The overall tertiary structure of the (N-terminal) head group region, as well as the nature of the side chain at position 2, appears to be important for specificity of binding of the ETA receptor, whereas the ETB receptor binding pocket primarily recognizes determinants that lie in the helical (C-terminal) tail of ET. The crystal structure of ET-1 would be useful for aiding our understanding of the nature of the receptor/ligand binding sites and for providing a basis for the design of ET-1 traps. Key differences in the structure of ET-1 distinguish it from its analogs and must be considered in the design of such traps, which would need to be specific to ET-1. For example, a Ser/Thr replacement at position 2 in ET-3 results in a considerable drop in binding affinity for the ETA receptor. The longer Thr side chain is found to not fit well into a complementary binding pocket of the ETA receptor.

As described earlier for cytokines, ET-1 traps can be designed that bind the ET-1 receptor–binding domain and hence render the targeted ET-1 inactive. Following clinical assessment of the extent of elevated ET-1 plasma levels in a patient, one could modulate the dose of ET-1 traps to be administered to target only the “excess” levels of ET-1. In a way, the ET-1 traps would “sponge” up the extra, pathologic levels of ET-1 without disrupting the physiologic activities of ET-1. Further investigation into the design and engineering of such traps would allow an evaluation of the potential application of ET-1 traps for therapeutic intervention.

Conclusions

The ubiquitous distribution of ET-1 and its receptors implicates its involvement in a wide variety of physiologic and pathologic processes in the body. This review highlights the likely link between increased ET-1 levels in various pathologic conditions and the prevalence of ER stress in these same diseases. Indeed, both ET-1 and ER stress are also implicated in several other pathologic conditions not addressed in this review, including different cancers and human immunodeficiency virus/AIDS. It is hoped that the links outlined in this review will prompt future research into the precise mechanism of action of ET-1 in inducing ER stress in each individual disease and subsequent investigation into potential therapeutic strategies for circumventing this stress, including those outlined in this review. A fuller investigation into this may lead to the development of therapies for one or more of the devastating diseases implicated.

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