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Endoplasmic Reticulum Stress, a Driver or an Innocent Bystander in Endothelial Dysfunction Associated with Hypertension?

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Abstract
Purpose of Review Hypertension (htn) is a polygenic disorder that effects up to one third of the US population. The endoplasmic reticulum (ER) stress response is a homeostatic pathway that regulates membrane structure, protein folding, and secretory function. Emerging evidence suggests that ER stress may induce endothelial dysfunction; however, it is unclear whether ER stress-associated endothelial dysfunction modulates htn.
Recent Findings Exogenous and endogenous molecules activate ER stress in the endothelium, and ER stress mediates some forms of neurogenic htn, such as angiotensin II-dependent htn. Human studies suggest that ER stress induces endothelial dysfunction, though direct evidence that ER stress augments blood pressure in humans is lacking. However, animal and cellular models demonstrate direct evidence that ER stress influences htn.
Summary ER stress is likely one of many players in a complex interplay among molecular pathways that influence the expression of htn. Targeted activation of specific ER stress pathways may provide novel therapeutic opportunities.

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Introduction

Hypertension (HTN) is a polygenic disorder associated with activation of the sympathetic nervous system, upregulation of the renin-angiotensin-aldosterone system (RAS), altered G-protein coupled receptors, epigenetics, and inflammation. Vascular endothelial cells line the blood vessels and serve as a barrier and signal transducer between the blood and interstitium. Endothelial dysfunction is related to disruption of normal vascular endothelial homeostasis, due in part to imbalances between vasodilators and vasoconstrictors, growth factors and inhibitors, and inflammatory and anti-inflammatory molecules. Endothelial dysfunction plays a key role in hypertension (htn), atherosclerosis, peripheral arterial disease, and thombotic conditions and is associated with reductions in nitric oxide (NO), impaired endothelium-dependent dilation (EDD), platelet aggregation, reduction in anti-oxidants, increased pro-inflammatory cytokines, leukocyte adhesion, and fibrinolysis (rev in [1]). In this review, we will predominately focus on endothelial dysfunction associated with hypertension.

Background on Endoplasmic Reticulum Stress

The endoplasmic reticulum (ER) folds, modifies, degrades, and transports proteins, and plays a key role in calcium storage, lipid biosynthesis, and numerous metabolic processes. Pathophysiological stress, including nutrient deprivation or excess, altered protein glycosylation, oxidative stress, reducing agents, lipids, changes in ER calcium content, microRNAs

(miRNAs), infections, and TLR signaling, can interfere with normal three-dimensional protein folding in the ER, causing the accumulation of toxic misfolded proteins. Thus, cells have evolved a complex intracellular signaling pathway, the unfolded protein response (UPR) [2, 3], which represses protein synthesis and increases ER chaperone content to restore normal ER function. The UPR promotes adaptive responses to rapidly changing cellular conditions in a dynamic and coordinated manner; however, when these pathways are overwhelmed by sustained ER stress, the UPR initiates pro-apoptotic pathways (rev. in [4–6]) and autophagy [7]. Indeed, it has been posited that the UPR transitions to ER stress when the ER functions in an environment that lies outside of its normal physiological range [4].

The UPR plays its most vital role in secretory cells including endothelial cells, which secrete factors that regulate vascular homeostasis (rev. in [8, 9]). In mammalian cells, there are three major arms of the UPR: (1) inositol requiring protein-1α/X box binding protein-1 (IRE1α/XBP-1), (2) protein kinase RNA (PKR)-like ER kinase (PERK), and the (3) activating transcription factor-6 (ATF6) pathways. Seventy-eight kilodaltons glucose-regulated protein/immunoglobulin binding protein (GRP78/BiP) is an ER chaperone that senses and activates the UPR. In unstressed cells, GRP78/BiP binds to the ER luminal domains of IRE1α, PERK, and ATF-6 and maintains them in a dormant state. During ER stress, GRP78/BiP binds to the misfolded proteins and dissociates from and activates the transmembrane sensors (IRE1α, PERK, and ATF6). After GRP78/BiP dissociation, full activation of the UPR may require binding of unfolded proteins to the luminal domains of IRE1α, PERK, and ATF-6 [10, 11]. Once activated, the PERK pathway rapidly attenuates protein translation, whereas the ATF6 and the IRE1α/XBP-1 cascades transcriptionally upregulate ER chaperone genes to promote efficient folding and degradation of proteins, facilitating efficient ER function.

PERK

PERK is an ER transmembrane protein that is activated during ER stress and phosphorylates eukaryotic translation initiation factor 2α (eIF2α) which suppresses translation of 90% of cellular mRNAs [5, 12, 13]. A subset of genes including activating transcription factor-4 (ATF4) [14] are preferentially translated by phosphorylated eIF2α (p-eIF2α). ATF4 binds to promoter/enhancer regions and transcriptionally augments expression of UPR target genes, which include C/EBP homologous protein (CHOP, GADD153) [15], GADD34 [16], vascular endothelial growth factor (VEGF) A [17], TRB3 [18], E-selectin, and genes important in amino acid metabolism [19, 20]. PERK also phosphorylates and activates NRF2 [21].

IRE1α/XBP-1 Pathway

IRE1α is a membrane-bound serine/threonine kinase with endonuclease activity [3, 22] that splices a 26 bp intron from XBP-1 during ER stress. XBP-1 splicing induces a translational frame-shift that generates a transcription factor, which transcribes genes involved in ER maintenance, expansion, and ER-associated degradation (ERAD) [23, 24]. IRE1α also activates apoptosis signal-regulating kinase (ASK1), c-Jun *N*-terminal kinase (JNK), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB, rev. in [25]), which are involved in apoptotic, autophagy, and inflammatory pathways [26–30]. XBP-1 preserves cell survival during the UPR; however, after prolonged stress, the IRE1α/XBP-1 arm of the UPR is attenuated, sensitizing the cells to apoptosis mediated by the PERK/CHOP pathway [31, 32]. XBP-1 regulates VEGFA expression [33] and VEGFA rapidly activates all three ER stress sensors (IRE1α, PERK, and ATF6) and promotes endothelial survival [34].

IRE1α also directly cleaves mRNAs in a process described as regulated IRE1-dependent decay (RIDD) [35, 36]. RIDD assists the PERK arm of the UPR in reducing ER accumulation of misfolded proteins. RIDD activity may also induce the rapid clearance of microRNAs [37] and activate the Nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome to promote inflammation and programmed cell death [38]. However, the relevance of RIDD activity in the endothelium is unknown.

ATF6

ATF6 is the third ER stress sensor that is bound as an inactive precursor in the ER membrane. During ER stress, ATF6 is transported to the Golgi and cleaved to release its cytoplasmic bZIP domain [39], which translocates to the nucleus and activates the transcription of target genes which include GRP78/BiP, XBP-1, GRP94, oxygen-regulated protein 150 (ORP150), ER oxidoreductin 1β (ERO1β), $p58^{IPK}$, and ER degradation-enhancing α-mannosidase-like protein (EDEM) (rev. in [40]).

Central/Neurogenic Hypertension and ER Stress

Seminal studies by Young and colleagues revealed that ER stress in the brain mediates angiotensin (Ang) II-dependent htn. Intraventricular injection of ER stress inducers (thapsigargin (TG) and intracerebral tunicamycin (TM)) increases mean arterial pressure (MAP) in mice [41••, 42]. Low-dose Ang II increases expression of ER stress markers in the brain subfornical region (SFO) and distension of the ER cisternae and reduces ribosome density. This is associated

with increases in reactive oxygen species (ROS) and NFκB expression. Additionally, treatment with an ER stress inhibitor (tauroursodeoxycholic acid, TUDCA) and overexpression of GRP78/BiP (an ER chaperone) in the SFO prevent Ang II-induced htn [41••]. Interestingly, Ang II-mediated ER and oxidant stress does not increase apoptotic cell death in the SFO [43]. In a similar manner in spontaneously hypertensive rats (SHR), Chao and colleagues observed increased expression of ER stress markers and evidence of autophagy in the rostral ventrolateral medulla (RVLM) prior to the development of htn. Intracisternal treatment with salubrinal (a molecule that reduces dephosphorylation of phosphorylated-eIF2α) and ROS scavengers stabilize ER stress and reduce BP [42].

In contrast, in a murine model of deoxycorticosterone acetate (DOCA)-salt-induced htn, intra-ventricular administration of TUDCA and adenoviral expression of GRP78/BiP does not reduce BP [44] but attenuates an 80 beat per minute reduction in heart rate and reduces saline intake (and urine output) [45]. DOCA-salt intake also increases CHOP (an ER stress-induced transcription factor) expression and induces ultrastructural ER changes in the SFO and supraoptic nuclei. In this study, DOCA-treated CHOP-deficient mice had modest reductions in saline intake compared with their wild-type controls, suggesting that CHOP may modulate saline intake. Again, there were no differences in BPs, suggesting that DOCA-salt causes ER stress in the brain and it is "mechanistically linked" with changes in saline intake, but not hypertension [42, 45]. These studies suggest that ER stress likely contributes to the pathogenesis of some forms of neurogenic htn, likely independently of endothelial dysfunction.

Human Studies

Many ER stress studies utilize ER chaperones such as TUDCA and 4-phenylbutyrate (PBA) to abrogate ER stress. In a clinical study, Walsh and colleagues demonstrated that oral TUDCA mitigates post-prandial hyperglycemia-induced endothelial dysfunction (brachial artery flow-mediated dilatation, FMD) independently of changes in blood glucose. However, the investigators did not confirm that TUDCA reduces endothelial expression of ER stress markers [46••]. Additionally, Kaplon and colleagues used fluorescent microscopy and showed enhanced UPR activation in human endothelial cells obtained from non-diabetic obese patients compared with controls [47]. Admittedly, confirmatory Western blotting or mRNA evaluation would have strengthened their observations. In endothelial cells and peripheral blood mononuclear cells (PBMC) derived from healthy individuals, Intralipid® infusions (IV dietary fat emulsions) activate the UPR [48]. Thus in humans, obesity, hyperglycemia and hyperlipidemia can activate the endothelial UPR.

Studies have shown that in scleroderma patients (systemic sclerosis), presence of the HLA-B35 allele confers a higher risk of pulmonary htn. Lenna and colleagues demonstrated in cultured endothelial cells that HLA-B35 upregulates endothelin-1 (ET-1) and reduces eNOS expression, and this is associated with activation of the UPR [49•, 50]. Additionally, in PBMCs isolated from patients with limited cutaneous systemic sclerosis, presence of the HLA-B35 allele correlates with elevated GRP78/BiP and DNAJ homolog subfamily B member 1 (ER stress markers), inflammation (IL-6), and proliferation [51]. Interestingly, dasatinib, an oral tyrosine kinase inhibitor used to treat chronic myelogenous leukemia, can cause pulmonary htn. Parallel human and rat studies suggest that pulmonary htn associated with dasatinib is related to activation of ER stress in the pulmonary endothelium [52]. As a whole, these studies provide very weak evidence that activation of the UPR induces endothelial dysfunction; however, direct evidence that ER stress augments blood pressure in humans is lacking.

Animal Models

Rodent models have provided more convincing evidence regarding the pathophysiological connections between ER stress and hypertension. In a murine model of Ang II-induced Htn (2 week infusion in mice), Ang II increases expression of ER stress markers (ATF4, CHOP mRNA, and p-eIF2α) and this is associated with reduced phosphorylation of eNOS and EDD in the aorta and mesenteric resistance arteries (MRA). ER chaperones (PBA and TUDCA) significantly reduce systolic blood pressures (SBP, by >30 mmHg) and augment eNOS phosphorylation and EDD [53••, 54, 55]. Kassan's studies suggest that ER stress impairs macrovascular endothelial function in a TGFβ1-dependent manner and microvascular endothelial function via an oxidative stress-dependent mechanism [53••].

In normotensive Sprague-Dawley rats, TM (10 μg/kg/day, subcutaneous osmotic pump for 28 days) increases SBP by over 30 mmHg and this is associated with increased aortic vascular smooth muscle fibrosis and apoptosis [54]. TM (10 pg/g × 7 days) also increases SBP and DBP in C57BL/6 mice [55]. In SHR, suppression of ER stress reduces BPs and endothelium-dependent contractions (EDC) in aortae and this is associated with reduced endothelial expression of cyclooxygenase-1 (COX-1), H_2O_2, cytosolic phospholipase A_2 ($cPLA_2$) activity, and pro-apoptotic marker expression [56•, 57]. Additionally, in rat-isolated aortic rings, TM causes insulin-stimulated vasoconstriction (impaired vasorelaxation) and this is likely related to ER stress-associated expression of ET-1 [58]. TUDCA treatment also reduces endothelial dysfunction in diabetic mice (db/db) [59]. Indeed, studies suggest that in endothelial cells, CHOP directly inhibits transcriptional

activation of the eNOS promoter [60]. Thus, these studies clearly show in rodents that activation of ER stress by exogenous agents induces endothelial dysfunction and hypertension.

However, in mice, ER stress induction with intra-peritoneal TM (1 mg/kg of two injections/week × 2 weeks) increases aortic and MRA expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) activity in the absence of significant changes in SBP, suggesting that a threshold level of ER stress must be overcome to increase BP [53••, 61]. Notably, a single dose of TM (1 μg/g bw) induces apoptosis of renal tubular cells and acute tubular necrosis, and it is well-known that renal dysfunction impacts BP [62, 63]. Therefore, renal pathophysiological changes should be thoroughly evaluated when investigating ER stress and its links with endothelial dysfunction and htn.

The Western diet is high in saturated fats and has been associated with insulin resistance, vascular dysfunction, and inflammation [64]. Cheang and colleagues demonstrated that high fat diet (HFD)-induced endothelial dysfunction and aortic ER stress is reversed by TUDCA [65•]. HFD feeding of rats causes endothelial apoptosis, swollen mitochondria, and extended ERs in the thoracic aorta [66]. Similarly, fenofibrate (a PPARα agonist) reduces impaired EDD and ER stress in HFD-fed rats. However, it is not clear whether fenofibrate's effects are related to lipid-lowering or activation of PPARα. In *db/db* mice and HFD-fed mice, exercise (4 weeks running) reduces ER (and oxidative) stress and restores aortic EDD and insulin-induced dilation of mesenteric arteries. These findings are attenuated in PPARδ-deficient mice, suggesting that PPARδ inhibits ER stress and plays a key role in exercise-induced improvements in diabetic endothelial dysfunction [67•].

Additionally, acetylcholine (ACH)-mediated vasodilation is impaired in chronically HFD-fed rats [68]. In rat aortae, TUDCA and an AMPK activator (aminoimidazole-4-carboxamide ribonucleotide, AICAR) restore impaired palmitate-induced EDD and eNOS phosphorylation [69]. Kim and colleagues also demonstrated that insulin-stimulated vasodilatation of mesenteric arterioles is impaired in HFD-fed mice and this effect is dependent on TLR4 [70]. Thus, studies in HFD-fed rodents have revealed clear links between ER stress and endothelial dysfunction and identified key players including PPARδ and TLR4.

Cellular Models of ER Stress and Endothelial Dysfunction

At the cellular level, palmitate, the most abundant circulating FFA, induces endothelial dysfunction. Physiological concentrations of palmitate activate ER stress in rodent endothelial cells and increase oxidative stress, inflammation (increased interleukin (IL)-1β, IL-6, and vascular cell adhesion protein (VCAM)) and impair EDD (decrease eNOS phosphorylation and NO). ER stress inhibitors (TUDCA, PBA) and AMPK activators (AICAR and salicylate) attenuate these effects [66, 68, 69, 71]. In human aortic endothelial cells (HAEC), palmitate increases expression of E-selectin, tumor necrosis factor-α (TNF-α), IL-1β, IL-6, spliced XBP-1, and phosphorylated eIF2α in a TLR4-dependent manner [70].

During ER stress, higher ROS production is related to reductions in anti-oxidants and increased protein folding in the ER. In coronary endothelial cells, ER stress upregulates NADPH oxidase (Nox2 and Nox4 mRNA) and p38 mitogen-activated protein kinase and this is associated with a reduction in eNOS activity and impaired vascular function [61] (rev in [72]). Thioredoxin-interacting protein (TXNIP) binds to and negatively regulates thioredoxin's anti-oxidant function (scavenges ROS). During ER stress, the IRE1α and PERK pathways activate the NLRP3 inflammasome via induction of TXNIP [38, 73, 74]. In rat aortic endothelial cells (RAEC), palmitate activates ER stress, augments TXNIP expression, and activates the inflammasome. AICAR attenuates TXNIP induction, NLRP3 inflammasome activation, and subsequent endothelial expression of IL-1β, IL-6, and VCAM, again suggesting that AMPK plays a key role in inhibition of ER stress. In this study, the authors hypothesized that AMPK phosphorylates dynamin-related protein-1 (Drp-1) and inhibits mitochondrial fission thereby inhibiting ROS expression and ER stress [69]. In human umbilical vein endothelial cells (HUVEC) and murine endothelial cells sirtuin, a class III histone deacetylase prevents ER stress and microRNA-204 expression and regulates caveolin 1 (Cav1) function and endothelial vasorelaxation [75]. Oxidized phospholipids also induce ER stress in aortic endothelial cells, and this is associated with inflammatory gene expression in an ATF4- and XBP-1-dependent manner. Indeed, in human atherosclerotic lesions, there is evidence of UPR activation in the regions containing oxidized phospholipids [76•]. Thus, at the cellular level, activation of ER stress by endogenous and exogenous mediators induces a plethora of inflammatory mediators, which negatively modulate endothelial function and likely impact blood pressure.

CKD

Chronic Kidney Disease (CKD) is associated with endothelial dysfunction and htn. In primary HAEC, incubation with urea (20 mM for 48 h) increases ER stress markers, ROS and NOX activity, and these changes are abrogated by over-expression of uncoupling protein 1 (UCP-1) and reductions in mitochondrial ROS. However, the investigators did not use ER stress inhibitors to demonstrate direct links between induction of ER stress and their endothelial model of CKD [77]. In HUVECs, incubation of uremic sera induces ER stress, impairs proliferation, increases expression of monocyte chemotactic protein-1

(MCP-1), VEGF, and NFκB [78], and reduces insulin-stimulated NO release and eNOS phosphorylation [79]. Pretreatment with ER stress inhibitors (PBA or TUDCA), anti-oxidants, and overexpression of GRP78/BiP inhibit uremic serum-induced ER stress and insulin resistance [78, 79]. In a similar manner, in aortae isolated from rats with 5/6 nephrectomy, CKD is associated with activation of ER stress and insulin-resistance [79]. In Dahl salt-sensitive (SS) hypertensive rats, PBA reduces salt-induced htn, albuminuria, and preserves the glomerular filtration barrier [80]. Interestingly, reduction of BP with anti-hypertensive agents does not improve renal pathology suggesting that ER stress inhibition limits the progression of CKD in a hypertensive rat model, independent of its effects on BP. Accordingly, in a model of diabetes and htn, Wang and colleagues demonstrated that activation of ER stress likely explains the pathophysiological synergy between htn and hyperglycemia promoting renal injury [81•]. Moreover, in ischemia-reperfusion injury, ischemic preconditioning of the renal arteries may be protective by modulating ER stress and augmenting NO release in the kidney [82]. These studies suggest that ER stress is activated in the renal endothelium and likely plays a key role in endothelial dysfunction associated with CKD and potentially systemic htn.

Hyperhomocysteinemia

Homocysteine (HC) is an amino acid derived from the metabolism of methionine, and hyperhomocysteinemia is associated with endothelial dysfunction and higher risks of coronary artery, cerebrovascular, peripheral arterial disease, and venous thrombosis. HC may activate ER stress by disrupting disulfide bond formation and increasing the unfolded protein load [83]. HC impairs EDD (rev. in [84]) by activation of ER and oxidative stress via the PERK and IRE-1 pathways [85–87] (rev. in [84]). In human vascular endothelial cells and HUVECs, HC induces endothelial apoptosis by activation of activating transcription factor 3 (ATF3) and JNK in a CHOP- and IRE-1α-dependent manner [86–89]. HC-induced ER stress also causes endothelial detachment-mediated apoptosis associated with expression of T cell death-associated gene 51 (TDAG51) [90]. Additionally in porcine coronary endothelial cells, HC impairs endothelial vasorelaxation by reducing currents and cell surface expression of Ca^{2+}-activated K^+ channels that regulate vascular relaxation. These effects of HC are restored by ER stress inhibition [91]. Thus, HC activates ER stress in endothelial cells and negatively regulates endothelial viability and vasorelaxation.

Novel Modulators of ER Stress

Recent studies have identified pharmacological agents that inhibit ER stress and endothelial dysfunction and could potentially be used as anti-hypertensive agents. These novel molecules are depicted in Table 1 and include vitamin D, which in humans weakly modulates hypertension [92]. Mice with diet-induced vitamin D deficiency have elevated BPs, high plasma renin, and reduced urinary sodium excretion [93]. Activated vitamin D (1, 25-$(OH)_2$ vitamin D) reduces TM and high glucose-induced ER stress in HUVECs. However, in this study, the investigators did not determine whether vitamin D directly alters endothelial function [94]. A number of compounds including Salidroside (herb used to treat high altitude-sickness), Piceatannol (an analog of reservatrol), L-serine and glycine, and black tea extracts improve HC-induced ER stress, apoptosis, ROS generation, and EDD in endothelial cells [95–98]. Additionally, berberine, a component of Chinese herbal remedies, reduces endothelial-dependent contractions in carotid arteries of SHR, in a COX-2-dependent manner. Berberine also increases AMPK phosphorylation and inhibits ER stress in carotid arteries [99]. Another component of Chinese herbal remedies Ilexgenin A, attenuates ER stress, ROS, and NLRP3 inflammasome activation, increases LKB1 phosphorylation, eNOS phosphorylation, and improves EDD in HFD-fed rats [71]. A number of other compounds including mangiferin, curcumin, hydrogen sulfide, paeonol, and angiotensin 1–7 also abrogate endothelial ER stress [100–104]. In HUVECs, induction of heme oxygenase-1 (HO-1) by cobalt (III) protoporphyrin IX chloride (CoPP) prevents ER stress and reduces high-glucose induced oxidative stress, inflammation, and apoptosis, and improves high-glucose induced NO release, angiogenic capacity, and VEGFA expression [105]. In a similar manner, in HUVECs, carbon monoxide (CO) induces Nrf2-dependent HO-1 expression in a PERK-dependent manner and prevents apoptosis triggered by ER stress (via CHOP suppression) [106]. However, the direct effects of CO on endothelial function were not evaluated. Thus, a number of pharmacologically active compounds can alter endothelial ER stress, positively modulate endothelial function, and potentially serve as novel anti-hypertensive agents.

Common Pitfalls in Evaluation of ER Stress

As a whole, these studies support the concept that ER stress induces endothelial dysfunction and htn, and they are based on the assumption that ER stress is maladaptive. However, ER stress facilitates cellular adaptation to aberrant cellular conditions; thus, it ultimately benefits organismal survival. It remains unclear and should be an active area of research to determine whether all three arms of the UPR are simultaneously or sequentially activated, and whether certain physiological stimuli preferentially activate specific arms of the ER stress response. There are a few reports of selective activation of ER stress pathways in endothelial cells; CO increases phosphorylation of PERK and eIF2α without activating IRE1α or ATF6 [106]. Similarly, in HAEC, palmitate activates XBP-1

Table 1 Novel inhibitors of endothelial endoplasmic reticulum stress

Agent	Concentration	Model	Cells	Effect	Mechanism	Ref.
1,25 $(OH)_2$ Vitamin D_3	10–1000 nM	TM and high glucose	HUVECs	Inhibits ER stress Reduces high-glucose induced cell death	Dependent on expression of vitamin D receptor	[93, 94]
Piceatannol	10 μM	HC-induced ER stress	HUVECs	Inhibits ER stress Reduces apoptosis Reduces ROS	↑ HO-1/CO ↑ NRF2 binding to HO-1 gene	[96]
Salidroside	300 μmol/L	HC-induced ER stress	HUVECs	Inhibits ER stress Improves EC viability	↓ BiP, CHOP, *p*-IRE1α, and *p*-PERK	[95]
Glycine, L-serine	10 mM	HC-induced ER stress	EA.hy926 human EC	Improves EC viability Reduces apoptosis	L-Serine decreases HC uptake; glycine reduces intracellular HC levels	[98]
Black tea extracts Theaflavin-3,3′-digallate	0.03–0.5 μg/ml	HC-induced ER stress in rat aortae Ang II-dept htn	Rat aortic EC	Inhibits ER stress Improves ACH-induced EDD Improves htn	↓ plasma HC levels and HC metabolic enzymes. In Ang II model ↓ ROS	[97]
Metformin GW1516	100 mg/kg/day 100 μmol/L 100 nmol/L	HFD-induced ER stress TM-induced ER stress	Mice aortae Mouse aortic endothelial cells	Reverses impaired EDD in HFD mice Reverses TM-induced ER/oxidative stress Improves NO	Dependent on expression of PPARδ Metformin ↑ phosphorylation AMPKα and PPARδ	[65•]
Ilexgenin (natural triterpenoid)	0.1–10 μM	Palmitate and TG-induced ER stress HFD-fed mice	EA.hy926 human EC Rat vascular EC Mice aortae	Inhibits ER stress ↓ ROS ↓ TXNIP induction Reduces apoptosis Restored NO in aortae	↓ Inflammasome activation ↑ LKB1 and AMPK phosphorylation ↓ Free fatty acids	[71]
Berberine	1 μmol/L	Reduces endothelial-dept. contractions	Carotid arteries of SHR rats	Inhibits ER stress ↓ ROS	↑ p-AMPK-Thr^{172} ↓ COX-2	[99]
Curcumin	10 μM	Palmitate-induced insulin resistance	HUVECs	Inhibits ER stress Reduces insulin-resistance Inhibits ubiquitin-proteasome system Enhances autophagy	↓ p-IRS-$1Ser^{307}$ ↓ JNK-Thr^{183} Inhibition of autophagy abolishes curcurmin's effects	[101]
Cobalt (III) protoporphyrin IX chloride (CoPP, HO-1 inducer)	100 μM	High-glucose-induced ER stress	HUVECs	Inhibits ER stress Reduces ROS Reduces inflammation, apoptosis	↑ NO release ↓ VEGFA expression	[105]
Paeonol 2′-Hydroxy-4′-methoxyacetophenone	0.1 μM	TM-induced ER stress	C57BL/6 aortic rings HUVECs	Reverses impaired EDD Inhibits ER stress Reduces ROS Enhances NO release	↑ p-eNOS-Ser^{1177} ↑ p-AMPK-Thr^{172} ↑ PPARδ Dependent on PPARδ	[103]
Angiotensin 1–7	1 μM	Ang II-impaired EDD TM-induced ER stress	C57BL/6 aortic rings HUVECs	Reverses impaired EDD in aortae Inhibits ER stress Increases NO	↑ p-eNOS-Ser^{1177} Effect dept. on G-protein coupled Mas receptor	[104]
Mangiferin	10 μM/L	High glucose	EA.hy926 human EC	Reduces ROS Reduces inflammation (NLRP3 inflammasome) and apoptosis	↑ p-AMPK-Thr^{172} ↓ p-IRE1α, TXNIP ↓ ET-1 ↑ NO production	[100]
Sodium hydrogen sulfide		Ang II stimulation	HUVECs	Inhibits ER stress ↓ ROS, apoptosis	↓ CHOP and GRP78/BiP	[102]

and phosphorylated eIF2α without upregulation of GRP78/BiP and CHOP [70]. However, XBP-1 splicing and eIF2α phosphorylation are early UPR activation events, and it is possible that GRP78/BiP and CHOP may be augmented at later time points (unpublished observations). Additionally, many of the experimental observations in endothelial cells have been made in a static manner, neglecting natural oscillations and temporal patterns. It will be important to develop more precise tools, such as fluorescent markers to facilitate investigation of these natural fluctuations.

Organismal aging is associated with the accumulation of misfolded proteins in the ER [107, 108], and htn manifests as humans age. None of these studies have accounted for the ramifications of cellular aging in the endothelium, and alternative ER stress-associated effects on the endothelium may be operative. It is also likely that circulating inflammatory cells exposed to ER stress may secrete factors that negatively impact endothelial function and this hypothesis has not yet been directly tested. Moreover, recent work has suggested that neurons undergoing a stress response may signal to non-neuronal cells (in a cell-nonautonomous manner) to influence proteostasis (protein homeostasis) in other tissues and cells (rev. in [108, 109]). These studies call into question the original assumption that neurogenic htn does not directly influence peripheral endothelial dysfunction.

Future Directions and Therapeutic Considerations

Ultimately, the purpose of these studies is to obtain a greater understanding of endothelial pathophysiology to develop targeted treatment strategies for htn. It will be important to evaluate the influences of other highly conserved proteostasis networks, including the heat shock response, mitochondrial UPR, ubiquitin-proteosome pathway, autophagy, and the integrated stress response [107]. It is likely that there is significant cross-talk among these pathways adding to the complexity of observations and conclusions. A closer evaluation of genomics and metabolomics may clarify cross-talk of these networks. Selective activation of ER stress pathways may also provide therapeutic opportunities. Selective activators of PERK [110], IRE1α, and ATF6 are being developed (rev. in [108]) and will need to be tested in relevant hypertension models. Finally, it will be important to perform detailed and robust human studies to verify findings derived from animal and cellular model systems.

Conclusions

These studies provide evidence in human, animal, and cellular models that ER stress is activated in the endothelium and can alter endothelium-dependent relaxation and contraction factors that ultimately influence expression of hypertension. ER stress is likely one of many players in a complex interplay among molecular pathways that influence the expression of htn.

Compliance with Ethical Standards

Conflict of Interest The author declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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