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Review Article

Redox Modulation of Ca^{2+} Signaling in Human Endothelial and Smooth Muscle Cells in Pre-Eclampsia

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Abstract

Pre-eclampsia (PE) is a leading cause of maternal hypertension in pregnancy and is associated with fetal growth restriction, premature birth, and fetal and maternal mortality. Activation and dysfunction of the maternal and fetal endothelium in PE appears to be a consequence of increased oxidative stress, resulting from elevated levels of circulating lipid peroxides. Accumulating evidence implicates reactive oxygen species (ROS) in the pathogenesis of vascular dysfunction in PE, perhaps involving a disturbance in intracellular Ca^{2+} signaling. Several ion-transport pathways are highly sensitive to oxidative stress, and the resulting modulation of ion transport by ROS will affect intracellular Ca^{2+} homeostasis. We review the evidence that changes in ion transport induced by ROS may be linked with abnormalities in Ca^{2+}-mediated signal transduction, leading to endothelial and smooth muscle dysfunction in maternal and fetal circulations in PE. As dysregulation of Ca^{2+} signaling in fetal umbilical endothelial cells is maintained in culture and embryonic, fetal, and postnatal development is affected by the cellular redox state, we hypothesize that impaired redox signaling in PE may influence "programming" of the fetal cardiovascular system and endothelial function in adulthood. *Antioxid. Redox Signal.* 11, 1149–1163.

Introduction

Pre-eclampsia (PE) affects ~3% of pregnancies worldwide and is one of the leading causes of maternal and fetal morbidity and mortality worldwide (100, 112). The etiology of the disease is still unknown but most likely involves (a) a genetic predisposition, (b) immune maladaptation, (c) placental ischemia caused by hypoperfusion, and (d) lipid peroxidation products and cytokines released by the placenta. PE is generally accepted as a two-stage disorder, the first involving abnormalities in spiral artery formation, resulting in deficient blood supply to the placenta (Fig. 1). The second stage involves the effects of placental ischemia and hypoxia on the maternal and fetal vasculature (45, 100, 112). A general consensus exists that impaired development of the placenta in PE, caused by impaired cytotrophoblast invasion and remodeling of the spiral arteries (105) and subsequent ischemia, leads to fetoplacental hypoxia. Increased oxidative stress in the placenta of women with PE is well documented (87, 100, 112, 143, 148). The disturbance in the oxidant–antioxidant balance renders the tissue more vulnerable to injury by reactive oxygen species (ROS). ROS and the formation of lipid peroxides alter cell membranes by increasing the incorporation of cholesterol, oxidized free fatty acids (FFAs), and low-density lipoproteins (LDLs) (64, 65). Additional risk factors contributing to an increased incidence of PE include gestational diabetes (125), obesity (147), coronary artery disease (99), and chronic hypertension (114), and recent reviews have addressed these risk factors (100, 112).

ROS elicit further effects by influencing intracellular signaling pathways. In addition to modulating protein tyrosine kinases, protein phosphatases, mitogen-activated protein kinases, and transcription factors, ROS serve as key regulators of intracellular Ca^{2+} ($[Ca^{2+}]_i$) homeostasis and RhoA/Rho kinase signaling (57, 60, 123). ROS elevate $[Ca^{2+}]_i$ levels in vascular cells as a result of inositol trisphosphate–mediated Ca^{2+} mobilization, increased Ca^{2+} accumulation *via* modulation of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, and Ca^{2+} influx, all of which affect vascular contractility and tone (139).

In the vasculature, superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and reactive

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nitrogen species, such as nitric oxide (NO) and peroxynitrite ($ONOO^-$), are biologically important (15). Normally, generation of ROS in the vasculature is highly controlled, and at low concentrations, ROS function as signaling molecules regulating vascular smooth muscle cells (VSMCs) contraction–relaxation and proliferation. Under pathologic conditions, increased ROS production leads to endothelial dysfunction and increased contractility, VSMC proliferation, monocyte migration, lipid peroxidation, and inflammation, all of which contribute to vascular damage in cardiovascular disease (28).

Etiology of Pre-Eclampsia

PE is characterized by a pregnancy-related maternal hypertension and significant proteinuria. It is a multisystem disorder potentially involving hepatic, renal, and cerebral dysfunction and activation of the coagulation cascade. In PE, the maternal endothelium is an important target of placental factors released in response to ischemia/hypoxia (45, 83, 100, 106, 112). The strategic location of the endothelium enables it to respond to alterations in hemodynamics and humoral factors by synthesizing and releasing vasoactive substances, with the balance between endothelium-derived relaxing and contracting factors maintaining vascular homeostasis. When this delicate balance is disrupted, the vasculature is predisposed to vasoconstriction, leukocyte adherence, mitogenesis, vascular inflammation, and oxidative stress. Furthermore, markers of endothelial dysfunction may serve as predictors of the syndrome in women that develop PE, because many are often elevated weeks before the detection of clinical manifestations. As endothelial dysfunction in PE has been reviewed extensively in recent years (43, 45, 106), this review focuses principally on PE-induced changes in ROS and Ca^{2+} signaling.

Endothelial dysfunction in fetal and maternal circulations in PE may be associated with an abnormal regulation of intracellular Ca^{2+}. In this context, vascular endothelial growth factor (VEGF) expression and levels of placental growth factor (PlGF) are reduced during (24) or before the onset of PE (138). Moreover, VEGF is able to activate Ca^{2+} influx *via* TRP channels in human microvascular endothelial cells (22), suggesting that circulating angiogenic factors could be associated with endothelial dysfunction and the maternal syndrome in PE. Circulating levels of antiangiogenic proteins appear to be an additional factor contributing to PE, because soluble Fms-like tyrosine kinase 1 (sFlt-1) is elevated in PE, and levels of soluble endoglin (sEng) are elevated in maternal serum 2–3 months before onset of the disease (78). Increased circulating sEng in PE impairs binding of TGF-β1 to its receptors and downstream activation of eNOS (145).

A number of studies have shown that oxidative stress is increased in PE, with the ischemic placenta implicated as a source of ROS (65, 96, 100, 112). Vascular dysfunction may be a consequence of enhanced oxidative stress (87), as ROS can damage endothelial cells (ECs) (15) and impair glutathione synthesis (86). Under physiologic conditions, ROS production is balanced by the activities of pro- and antioxidants. Increased oxidative stress has been implicated in many disease processes in early life; however, maintaining an oxidized state is useful in many circumstances, and a balance of oxidizing and reducing reactions ensures normal fetal development and organogenesis (28). This balance is disturbed under conditions of ischemia because of an increased production of intracellular $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2, leading to disruption of cellular functions (55). This review focuses on the role of ROS generated in PE as modulators of ion-transport systems involved in Ca^{2+} regulation and the consequences of altered Ca^{2+} signaling for endothelial function.

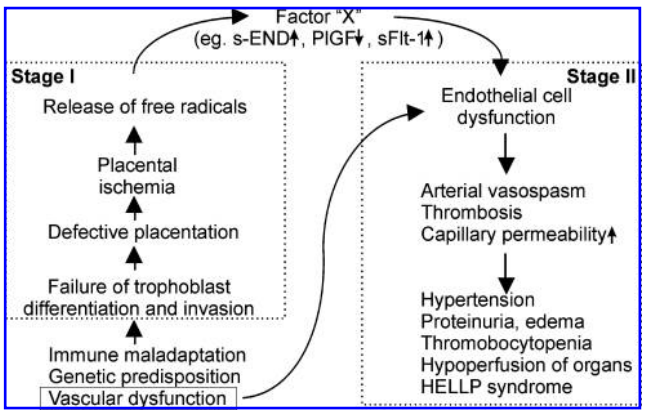

FIG. 1. The etiology of pre-eclampsia. A model of accepted pathways involved in the pathogenesis of PE. This two-stage disorder affects both fetal and maternal circulations, where predisposing genetic factors or an immune maladaptation or both cause an abnormal placentation. In many cases in PE, remodeling of the spiral arteries by cytotrophoblasts is impaired, leading to placental hypoxia. Under hypoxia, placental tissue is exposed to enhanced oxidative stress, leading to an enhanced release of placental factors into the maternal circulation that disrupt normal endothelial barrier function and permeability. Recently identified soluble endoglin (s-Eng) or the antiangiogenic soluble Fms-like tyrosine kinase 1 (sFlt-1) may modulate endothelial function. As a consequence, maternal VEGF and PlGF levels are low, aggravating maternal endothelial dysfunction and subsequently leading to hypertension and proteinuria. Other maternal syndromes such as thrombocytopenia or HELLP ensue in more-severe cases. PlGF, placental growth factor; VEGF, vascular endothelial growth factor; HELLP, hemolysis, elevated liver enzymes and low platelets.

Generation of Reactive Oxygen Species in Pre-Eclampsia

Superoxide generation in maternal tissue

One of the biomarkers of oxidative stress is oxidized low-density lipoproteins (oxLDLs), and women with elevated plasma oxLDLs are more likely to develop PE (108). Oxidative stress induced by homocysteine autooxidation and thiolactone formation may contribute further to vascular dysfunction in PE, as the disease is associated with hyperhomocysteinemia (13). In this context, elevated plasma levels of total homocysteine can lead to endothelial dysfunction associated with premature coronary artery disease, peripheral vascular disease, or recurrent venous thrombosis (6). The endoplasmic reticulum (ER) is a storage site for intracellular calcium and plays a key role in regulating Ca^{2+} signaling and homeostasis (30). Disruption of Ca^{2+} homeostasis induces ER stress through depletion of ER Ca^{2+}, although recent evidence suggests that homocysteine induces ER stress through mech-

anisms other than ER Ca^{2+} depletion (30). The ability of homocysteine to evoke cytosolic Ca^{2+} transients in endothelial cells (30) may be the consequence of redox changes induced by homocysteine. These documented changes in endothelial Ca^{2+} signaling highlight the importance of this risk factor for endothelial dysfunction in PE.

Notably, superoxide dismutase (SOD) activity is reduced in plasma (16) and erythrocytes (33) from women with PE, and lower SOD protein expression has been detected in subcutaneous fat vessels from women with PE (115). This is consistent with findings that SOD protein expression is increased in healthy pregnancies in response to enhanced oxidative stress but significantly reduced in pregnancies with first-trimester miscarriages (67).

When neutrophils are isolated from women with PE and stimulated with either PMA or *N*-formylmethionyl-leucyl-phenylalanine (fMLP), ROS production is significantly increased compared with that in normotensive controls without an increase in expression of NAD(P)H oxidase, suggesting augmented sensitivity to agonist stimulation (77). Furthermore, myeloperoxidase, a hemoprotein released from activated monocytes and neutrophils, is elevated in plasma and placental extracts in PE, suggesting that these leukocytes are in an activated state in PE (44). Several potential sources of oxidative stress are known in PE, including xanthine oxidase (XO), which metabolizes xanthine and hypoxanthine to uric acid with the production of $O_2^{\bullet-}$ and H_2O_2 (17). Usually this enzyme is present as the holoenzyme xanthine dehydrogenase/xanthine oxidase (XDH/XO). During hypoxia and in response to several cytokines, the activity of XDH/XO increases, leading to enhanced oxidative damage (135). Inadequate placental perfusion will reduce oxygen delivery, thereby enhancing XO activity in the placenta and cytotrophoblasts in PE (87).

Under conditions of limited substrate (L-arginine) or cofactor (tetrahydrobiopterin) supply, endothelial nitric oxide synthase (eNOS) becomes uncoupled and generates $O_2^{\bullet-}$ rather than NO (122). Notably, endothelial dysfunction in a rat model of pregnancy-induced hypertension has been attributed to $O_2^{\bullet-}$ (or ROS metabolites or both) produced after uncoupling of eNOS and activation of NAD(P)H oxidase (91). Angiotensin II receptor type 1 (AT_1) antibodies from women with PE have been implicated in abnormal Ca^{2+} homeostasis and shown to activate NAD(P)H oxidase (137). Moreover, increased expression of NAD(P)H oxidase in the placenta in PE suggests that the placenta may be an important source of $O_2^{\bullet-}$ (26), leading to subsequent delivery of ROS metabolites or peroxynitrite or bothinto the maternal and most likely fetal circulation. During the early stages of PE, it seems likely that ROS levels may also be elevated in the fetal circulation because of compromised antioxidant defenses.

Superoxide generation in fetal tissue

Additional evidence for enhanced NAD(P)H oxidase activity in PE is based on the finding that endothelin-1, known to be elevated in the disease, enhances expression of the oxidase and simultaneously reduces expression of eNOS and the plasmalemmal scaffold protein caveolin-1 in fetal umbilical vein ECs (HUVECs) (32). Enhanced lipid peroxide levels have been confirmed in placental homogenates and umbilical vein plasma (71). Increased oxidative stress in the fetal vasculature in PE is associated with a reduced expression of the chaperone protein Hsp90 (51), which plays an integral role in regulating activation of eNOS and NO production. We recently reported that HUVEC derived from PE pregnancies have a reduced capacity to generate $O_2^{\bullet-}$ as a consequence of diminished glucose-6-phosphate dehydrogenase (G6PD) activity (4), leading to decreased intracellular levels of NAD(P)H required as a substrate for NAD(P)H oxidase and a cofactor for eNOS, heme oxygenase-1, and glutathione recycling.

The activity of SOD is reduced in cytotrophoblasts, umbilical cord plasma, and erythrocytes in PE (16, 87,126), whereas Cu-Zn-SOD mRNA levels are decreased in PE placental tissue (143). The activities of other key enzymes with antioxidant properties, such as thioredoxin reductase or glutathione peroxidase, are also significantly reduced in placental tissue in PE (142, 143) or ECs treated with PE serum (157). The decrease in SOD expression and activity in PE highlights an important phenotype, because it is observed in both maternal and placental tissues. Consequently, the total antioxidant capacity may be compromised not only in women with PE but also in the developing fetus (71). It remains unclear whether ROS serve as primary or secondary mediators of the symptoms in this disease. Limited information is available on placental transfer and metabolism of lipid peroxides, and early reports suggest that the placenta is the main source of lipid peroxides in PE (96), secreting more lipid peroxides toward the maternal than the fetal circulation (148).

Nitric oxide

Diminished NO-dependent vasodilation in myometrial arteries (75) and excess ROS formation could aggravate the poor placental perfusion in PE, but the pathways involved remain to be elucidated. Several reports suggest that decreased bioavailability of NO (122) due to peroxynitrite ($ONOO^-$) formation *via* the reaction of NO with $O_2^{\bullet-}$ leads to nitration of tyrosine residues on proteins (115). This has been used as a marker of *in vivo* peroxynitrite levels, and increased immunoreactivity for nitrotyrosine has been detected in PE villous vascular endothelium, surrounding vascular smooth muscle and placental tissue (87). Roggensack *et al*. (115) showed that nitrotyrosine staining is significantly increased in maternal subcutaneous fat vessels from PE women, whereas the enhanced staining for eNOS may reflect a compensatory increase in NO production. Alternatively, enhanced endothelial NO synthesis may result from abnormally regulated intracellular Ca^{2+}, which we have reported in fetal endothelial cells isolated from PE pregnancies (130). However, elevated levels of cGMP (index of NO production) in the latter study may have been due to activation of guanylyl cyclase by lipid peroxidation products (92). Increased ROS-mediated $[Ca^{2+}]_i$ levels may activate calcium-dependent NO production in endothelial cells, leading to vascular relaxation, or, alternatively, quenching of NO by $O_2^{\bullet-}$ would generate the weak dilator $ONOO^-$ (107). Further support for abnormal NO production in PE comes from the findings that *S*-nitrosoalbumin and total *S*-nitrosothiol are elevated in maternal plasma, potentially accounting for reduced NO bioavailability (141).

Enhanced formation of $O_2^{\bullet-}$ in maternal and fetal tissues in PE supports the hypothesis that antioxidant defenses are compromised because of enzyme deficiencies and sustained

generation of ROS. Several groups have attributed the observed increase in ROS production to neutrophil activation by proinflammatory cytokines (140) released from the ischemic placenta. The resulting lipid peroxidation is a free radical chain reaction, involving oxidative conversion of polyunsaturated fatty acids such as arachidonic acid by •OH radicals.

Fatty acids

PE is further characterised by an imbalance in fatty acid metabolism (90) affecting triglyceride levels (111) and lipid peroxidation (150). Metabolism of the fatty acid arachidonic acid (AA) is altered in PE (144), and evidence strongly suggests that regulation of plasma lipids is abnormal in the disease (64,129). In this context, we reported that human fetal umbilical artery smooth muscle cells (HUASMCs) isolated from PE pregnancies exhibit augmented increases in intracellular Ca^{2+} in response to AA (129). The elevated $[Ca^{2+}]_i$ response to AA in PE smooth muscle cells was not observed in preterm HUASMCs obtained from normal pregnancies with a shorter gestation. As the increased Ca^{2+} sensitivity to AA was mimicked in HUASMCs from normotensive pregnancies after inhibition of cyclooxygenase or lipoxygenase or both (Figs. 2 and 3), we hypothesized that PE impairs metabolism of AA *via* these two pathways, with preferential metabolism of AA *via* the monooxygenase pathway, resulting in enhanced Ca^{2+} influx (129). Consistent with this hypothesis is our finding that cyclooxygenase-1 (COX-1) expression was reduced in HUASMCs in PE (data not shown). Various AA metabolites have been reported to stimulate Ca^{2+} influx, such epoxyeicosatrienoic acids (EETs), which have also been canvased as candidates for endothelium-derived hyperpolarizing factor. Moreover, blocking the metabolism of EETs by inhibiting their conversion by soluble epoxide hydrolase to the corresponding dihydroxy epoxyeicosatrienoic acids appears to lower blood pressure in hypertension.

Reduced levels of AA have been found in placental tissue of women with PE, suggesting an increased conversion of AA into the proinflammatory metabolites thromboxane and prostaglandins or leukotrienes (152). This may in part account for increased oxidative stress and thromboxane generation in placental trophoblasts (11). Thus, both dyslipidemia and elevated oxidative stress may act in concert to exacerbate endothelial or smooth muscle dysfunction (or both) in PE.

Although enhanced oxidative stress is accepted as a hallmark of PE (100, 112), reports exist to the contrary (12, 113). A comparison of these studies highlights that different techniques were used to define oxidative stress, with some limiting the detection of secondary lipid peroxidation to products such as aldehydes or other sources of oxidative stress, such as activated neutrophils. Furthermore, experiments have been performed with different tissues or cells of either fetal or maternal origin. Studies with tissues/cells from different ethnic groups (Afro-Caribbean *vs.* caucasian) and differences in the severity of the disease may in part explain reported differences in detection of biomarkers of oxidative stress. It seems most likely that an imbalance in the prooxidant/antioxidant ratio in PE (100,112) accounts for some of the reported alterations in Ca^{2+} homeostasis, because numerous reports highlight that ROS or lipid peroxidation products can interfere with Ca^{2+}-regulating systems such as ion channels, exchangers, and ion pumps (see Tables 1 and 2).

Effects of Oxidative Stress on Ca^{2+} Homeostasis

The majority of oxidative stress–related effects on ion-transport mechanisms are due to lipid peroxidation (57, 60, 159). ROS-induced lipid peroxidation could lead to nonspecific leak of ions through the lipid bilayer itself or modify the physical properties of phospholipids in such a way that channels, pumps, exchangers, associated proteins (or a combination of these) that regulate these transport pathways are altered (Fig. 4) (139). These altered lipid properties can then inactivate membrane-bound enzymes, leading to a disturbance in Ca^{2+} regulation (56). The main actions of ROS occur *via* (a) oxidation of sulfhydryl groups on ion-transport proteins, (b) peroxidation of membrane phospholipids, and (c) inhibition of membrane-bound regulatory enzymes and modification of the oxidative phosphorylation and ATP levels, although the exact mechanisms of action remain to be elucidated. The inhibition of sarco/endoplasmic Ca^{2+}-ATPase (SERCA) by ROS may involve peptide- and protein-derived

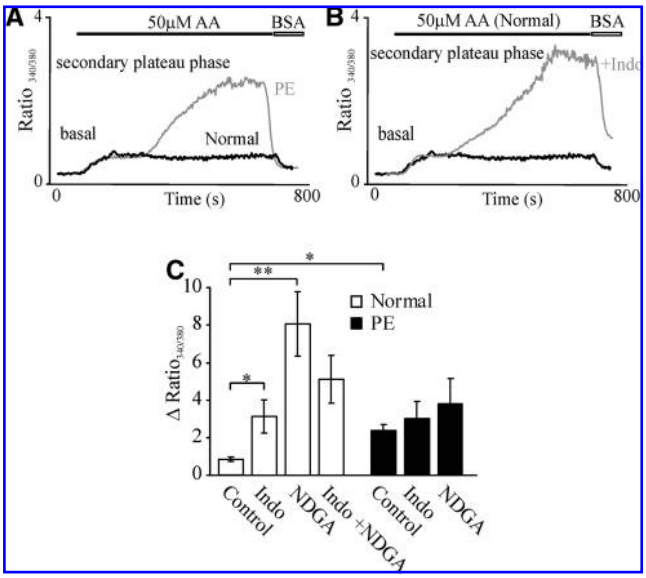

FIG. 2. Arachidonic acid–induced increases in $[Ca^{2+}]_i$ in normal and PE umbilical artery smooth muscle cells. (**A**) When umbilical artery smooth muscle cells (HUASMCs) were challenged with 50 μM arachidonic acid (AA) in the presence of extracellular Ca^{2+}, AA-evoked increases in $[Ca^{2+}]_i$ were significantly greater in PE cells (grey tone trace). (**B**) Normal HUASMCs were challenged with 50 μM AA in Ca^{2+}-containing solution in the absence (lower trace) or presence (grey tone upper trace) of 10 μM indomethacin (an inhibitor of cyclooxygenase). The presence of 10 μM indomethacin transformed the Ca^{2+} response of a normal cell to an AA-augmented response observed in a PE cell in the absence of indomethacin. (**C**) Summary of Ca^{2+} responses to AA in normal (open bars) or PE (solid bars) HUASMCs under control conditions or in the presence of 10 μM indomethacin (Indo) or 10 μM NDGA (lipoxygenase inhibitor). In normal HUASMCs, indomethacin potentiated the $[Ca^{2+}]_i$ response to AA, but in PE cells, the augmented response to AA was not elevated further. A combination of both inhibitors led to similar increases in AA-evoked $[Ca^{2+}]_i$ levels, as did application of either inhibitor alone. Data replotted from Figs. 1 and 2 in Steinert *et al.* (129) and denote mean ± SEM of three replicate measurements in four normal and eight PE cell cultures, $^*p < 0.04$, $^{**}p < 0.02$.

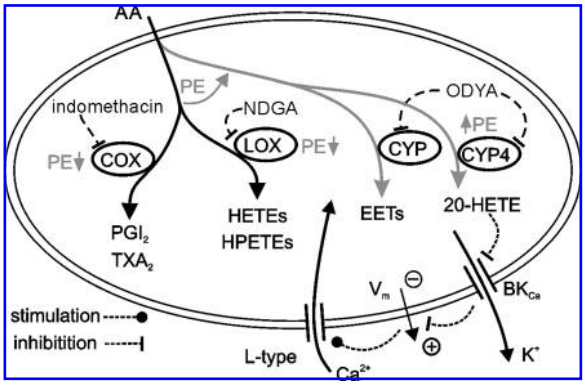

FIG. 3. Arachidonic acid metabolism in fetal vascular smooth muscle cells is altered in PE. Arachidonic acid is predominantly converted into prostaglandins, HETEs, and HPETEs *via* the actions of cyclooxygenase and lipoxygenase in normal HUASMCa. In PE, AA metabolism is diverted more toward cytochrome P-450, leading to the production of 20-HETE and EETs. 20-HETE has been reported to inhibit BK_{Ca} channels and hence depolarizes the plasma membrane. This depolarization favors the activation of voltage-gated Ca^{2+} channels, leading to enhanced cytosolic Ca^{2+} levels [see Steinert *et al*. (129)].

peroxides that selectively oxidize cysteine residues (35), and this impaired function can be reversed by the presence of the antioxidant *t*-butylhydroxytoluene (2). Furthermore, peroxynitrite has a dual effect on SERCA, stimulating its activity at lower but inhibiting it at higher concentrations. This stimulating effect, *via S*-glutathiolation, is blocked by oxidation of SERCA cysteine-674 by prolonged elevated levels of reactive oxygen and nitrogen species (3), implicating actions of ROS/RNS on intracellular Ca^{2+} signaling. At higher concentrations, peroxynitrite appears to inhibit SERCA Ca^{2+} uptake and therefore store Ca^{2+} release in endothelial cells, resulting in Ca^{2+} overload (29). Dysregulation of Ca^{2+} signaling associated with ER stress (29, 57) may contribute to endothelial dysfunction in PE.

Abnormalities of Ca^{2+} Homeostasis in Pre-Eclampsia

Cytosolic Ca^{2+} levels in maternal tissue

Intracellular Ca^{2+} stores. Ca^{2+} store release can be affected in a variety of ways by ROS. Biochemical evidence suggests that ROS modify the structure and function of the cardiac sarcoplasmic reticulum (SR) ryanodine-sensitive Ca^{2+} release channel (RyR), where the initial increase in the open probability (P_o) is followed by the irreversible loss of the channel function (59). In sheep cardiac SR, H_2O_2 directly modifies the gating properties of the RyR, causing an increase in P_o without affecting its modulation by ATP or caffeine (10). Furthermore, the IP_3-sensitive Ca^{2+} release is affected by oxidants, where $O_2^{\bullet-}$ stimulates IP_3-evoked Ca^{2+} release in vascular smooth muscle cells (132). Evidence suggests that IP_3-sensitive Ca^{2+} store release in ECs is induced by H_2O_2 (61), which could greatly contribute to elevated $[Ca^{2+}]_i$. The increases in $[Ca^{2+}]_i$ evoked by H_2O_2 are prevented by enhanced SOD and catalase activities, which scavenge $O_2^{\bullet-}$ or H_2O_2, respectively (34). These data indicate that $O_2^{\bullet-}$ might affect either phospholipase C activity or receptor, G-protein,

Table 1. Ca^{2+} Abnormalities in Vascular, Placental, and Circulating Blood Cells in Pre-eclampsia

Cell type	*Ca^{2+} changes*	*References*
Erythrocytes/red blood cell ghosts	Increased membranous Ca^{2+} content Diminished Ca^{2+} ATPase activity Increased basal $[Ca^{2+}]_i$ levels	74, 80, 88, 110, 134
Platelets	Increased basal $[Ca^{2+}]_i$ levels Increased sensitivity in $[Ca^{2+}]_i$ response to angiotensin IIIncreased $[Ca^{2+}]_i$ peak response to angiotensin II	1, 54
Maternal hand-vein endothelial cells	Increased $[Ca^{2+}]_i$ responses to ATP	85
HUVECs	Increased basal $[Ca^{2+}]_i$ levels Reduced histamine-evoked Ca^{2+} influx with negligible changes in store calcium release	130
HUASMCs	Increased Ca^{2+} sensitivity to arachidonic acid metabolites mediated *via* monooxygenase metabolism of arachidonic acid	129
Rat aortic smooth muscle cells and A-10 cell line	Attenuated $[Ca^{2+}]_i$ responses to angiotensin II when incubated with PE serum	47
Myometrial and placental trophoblasts	Increased basal $[Ca^{2+}]_i$ levels and reduced Ca^{2+} ATPase activity in freshly isolated trophoblastic cells Reduced Ca^{2+} ATPase activity in trophoblasts	19, 20
Lymphocytes	Increased basal $[Ca^{2+}]_i$ levels	58, 146

HUVECs, human umbilical vein endothelial cells; HUASMCs, human umbilical artery smooth muscle cells; PE, pre-eclampsia; AA, arachidonic acid; $[Ca^{2+}]_i$, intracellular calcium.

Table 2. Effects of Oxidative Stress on Ca^{2+} Homeostasis in Vascular and Other Cell Types

Cell type	*Ion-transport mechanism*	*Effects of oxidative stress*	*References*
Ca^{2+} channels			
Guinea-pig ventricular myocytes	L-type Ca^{2+} channel	Suppression of current by ROS generated by dihydroxyfumaric acid	52
Intracellular store channels			
Skeletal muscle	RyR Ca^{2+} release channel	Activation of current by increases in P_o induced by H_2O_2	10
Vascular smooth muscle cell	IP_3R Ca^{2+} release channel	Stimulation of Ca^{2+} release induced by HX + XO in the presence of catalase	132
ATPases			
Bovine aortic smooth muscle cell	SR Ca^{2+} ATPase	Inhibition of activity induced by HX + XO	131
Rat cardiac smooth muscle cell	SR Ca^{2+} ATPase	Inhibition of activity induced by X + XO and H_2O_2	31
Pig coronary artery smooth muscle cell	Plasma membrane Ca^{2+} ATPase	Inhibition of Ca^{2+} uptake induced by superoxide	49
Red blood cells	Na^+-K^+-ATPase	Inhibition of activity induced by *t*-butyl hydroperoxide	116
Na^+/Ca^{2+} exchanger			
Guinea-pig ventricular myocytes	Na^+/Ca^{2+} exchanger	Depression of Ni^{2+}-sensitive and Ca^{2+}-activated current induced by HX + XO	25
Bovine heart isolated sarcolemmal vesicles	Na^+/Ca^{2+} exchanger	Stimulation of activity by H_2O_2, $FeSO_4$ plus DTT, or HX + XO Inhibition of activity by HOCl	70
TRP channels			
Human syncytiotrophoblast	Polycystin-2 (PC2, TRPP2)	Inhibition of channel activity by H_2O_2	94

BAECs, bovine aortic endothelial cells; DTT, 2,4-dithiothreitol; IP_3R, inositol trisphosphate receptor; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; P_o, open probability; XO, xanthine oxidase; HX, hypoxanthine; HOCl, hypochlorous acid; X, xanthine.

phospholipase C coupling to produce higher levels of IP_3. ROS-mediated changes in G-protein activity/coupling have been reported in neurons and for G_i and G_s proteins in rat hearts. A report indicates that $O_2^{\bullet-}$ can directly affect the mechanism(s) responsible for CCE without altering Ca^{2+} store release (46). In contrast to ROS-mediated elevation of $[Ca^{2+}]_i$, oxidative stress can diminish Ca^{2+} signaling by direct effects on the influx pathway or by inhibition of the mechanism that links the internal Ca^{2+} store to plasmalemmal Ca^{2+} influx (37). Table 1 summarizes different effects of oxidative stress on several ion-transport systems involved in maintaining physiologic $[Ca^{2+}]_i$ levels.

ATPases. Studies investigating the effects of ROS on either of the Ca^{2+} sequestration pathways (SERCA, PMCA) revealed that both ATPases are highly sensitive to oxidative stress, with mostly inhibitory effects reported for sarcolemmal (69) and SR Ca^{2+} ATPase (50). However, it seems that the PMCA is less sensitive to ROS than is SERCA. $O_2^{\bullet-}$ uncouples the hydrolytic reaction of the PMCA and inhibits the hydrolytic reaction of the SERCA (76). A related study showed that H_2O_2 inhibits contractions of the pig aorta induced by angiotensin II, in which a diminished SERCA activity failed to refill the Ca^{2+} stores, thereby reducing agonist responses. In addition to the Ca^{2+}-extrusion pathways mentioned earlier, the Na^+/Ca^{2+} exchanger plays an important role, with high capacity for Ca^{2+} transport producing net Ca^{2+} fluxes both in and out of the cell, dependent on the Na^+/Ca^{2+} gradient and membrane potential. Data on the Na^+/Ca^{2+} exchanger in umbilical arteries isolated from PE pregnancies shows a reduced activity (119). This protein contains disulfide bonds, rendering it susceptible to modifications by oxidizing agents.

As Ca^{2+} regulation is also affected by membrane fluidity, the question arises whether ROS have the ability to alter this parameter *via* lipid peroxidation (42). Evidence suggests that ROS decrease membrane fluidity (66) leading to enhanced Ca^{2+} membrane permeability.

Abnormalities in Ca^{2+} Homeostasis in Pre-Eclampsia

Cytosolic Ca^{2+} levels in maternal tissue

Several groups have documented that PE is associated with changes in intracellular Ca^{2+} homeostasis (Table 1). Because Ca^{2+} levels are sensitively regulated, this equilibrium can readily be perturbed. Basal $[Ca^{2+}]_i$ is elevated in maternal lymphocytes (58), erythrocytes (110), and platelets (54) from women affected by PE, and membranous Ca^{2+} content is also increased in maternal erythrocytes (74). Strong indications exist that erythrocyte $[Ca^{2+}]_i$ is important for regulating their deformability and membrane fluidity, thereby affecting passage through the microcirculation (149). In arterial hypertension, erythrocyte Ca^{2+} levels are elevated, and deformability is reduced (23). In PE, the use of magnesium as a Ca^{2+} competitor has been shown to improve erythrocyte deformability and may therefore counteract reduced blood flow in PE (120). An endothelium-like functional NOS isoform has been described in erythrocytes (72), and elevated basal $[Ca^{2+}]_i$ would increase Ca^{2+}-dependent NO production, potentially contributing to abnormally high plasma NO or nitrite levels detected in PE (115). Moreover, leukocytes, granulocytes, and

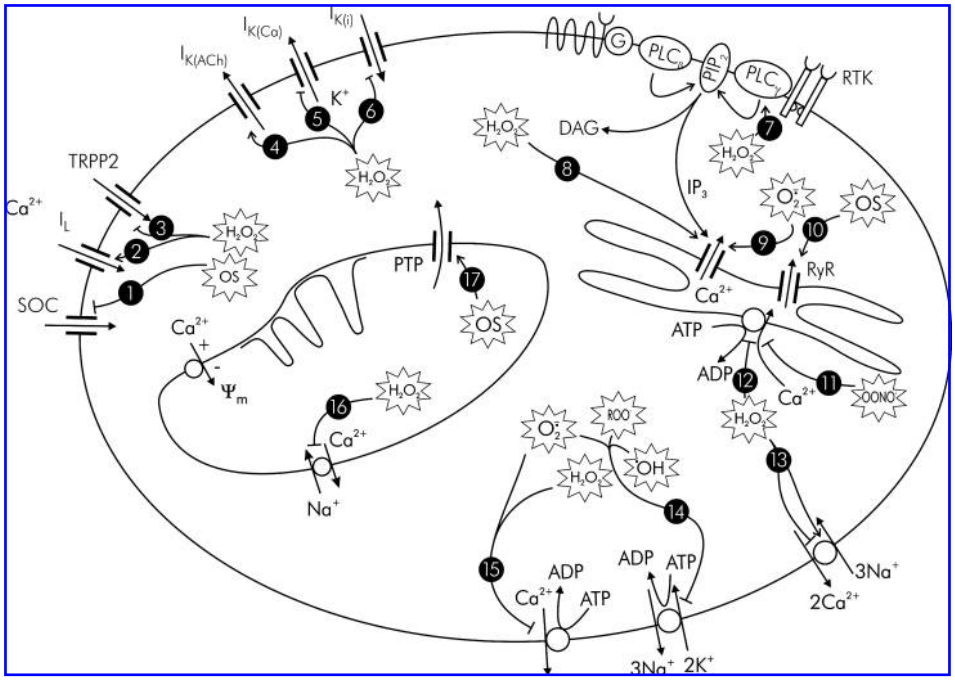

FIG. 4. Redox regulation of Ca^{2+} homeostasis in vascular cells. Numerous sources report that mainly H_2O_2 inhibits a variety of ion channels and ATPases. SOC is affected by ROS, as is the nonselective cationic leak (I_L), and TRP channels such as polycystin-2 channels (TRPP2) [see shaded numbers 1–3 (73, 94, 103)]. Potassium channels, which in turn affect the resting membrane potential (V_m), have been reported to be either inhibited (K_{Ca}, K_i) or activated (K_{ACh}) by ROS [shaded symbols 4–6 (79)]. Furthermore, indications exist that ROS can interact with the IP_3 pathway by affecting phospholipase C activity (PLC) [shaded symbol 7 (63, 132, 151)]. The IP_3- and ryanodine-sensitive (RyR) Ca^{2+}-release channels have been shown to be activated or sensitized by ROS [shaded symbols 8–10 (10, 62, 132, 158)]. Additional inhibitory actions of ROS or peroxynitrite have been reported for the Ca^{2+} uptake mediated by SERCA [shaded symbols 11, 12 (31, 35, 53, 131)]. Other Ca^{2+}-extrusion pathways, such as the Na^+-Ca^{2+} exchanger, Na^+-K^+ ATPase, or PMCA are affected by a variety of ROS with predominantly inhibitory actionsm resulting in compromised Ca^{2+} extrusion from the cytosol [shaded symbols 13–15 (25, 49, 70, 116, 159)]. An additional organelle involved in Ca^{2+} homeostasis is the mitochondrion, and ROS further diminish their ability to buffer cytosolic Ca^{2+} [shaded symbols 16, 17 (36, 68)]. ADP, adenosine-diphosphate; PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; SOC, store-operated Ca^{2+} entry; ROC, receptor-operated Ca^{2+} entry; I_L, leak current; G, G protein; TRPP2, polycystin-2; K_i, inward rectifying K^+ channel; K_{Ca}, Ca^{2+}-activated K^+ channel; K_{ACh}, acetylcholine-induced potassium current; $O_2^{\bullet-}$, superoxide anion; $^{\bullet}OH$, hydroxyl radical; $OONO^-$, peroxynitrite; PTP, permeability transition pore; ψ_m, mitochondrial membrane potential; OS, oxidative stress; GSSG, oxidized glutathione.

monocytes from women with PE exhibit elevated basal Ca^{2+} levels (146). Monocytes from PE pregnancies have greater Ca^{2+} peak responses to fMLP compared with normotensive controls (146), indicating that these cells may be activated in PE. Activation of circulating monocytes and neutrophils by elevated homocysteine and ROS levels in PE patients (104) may explain the immune maladaptation in PE pregnancies.

PE is further associated with thromboembolism (48), and elevated levels of fibrinogen and activities of factor VIII (154) can contribute to hypercoagulability. Cytosolic Ca^{2+} levels are increased in platelets from hypertensive patients and linked unexpectedly to a reduced ability of platelets to release NO (18), contributing to increased thromboembolism in PE. Although incubation of PE platelets with L-arginine fails to inhibit platelet aggregation (98), NO donors still have antithrombotic actions (98). These studies imply that despite enhanced cytosolic Ca^{2+}, a diminished Ca^{2+}-dependent NO release in PE platelets results in pathologic platelet aggregation.

One possible explanation for altered $[Ca^{2+}]_i$ regulation in PE is that angiotensin II receptor type I (AT_1)-activating antibodies from women with PE significantly increase cytosolic Ca^{2+} in Chinese hamster ovary cells (137). AT_1 antibodies may thus have the potential to alter Ca^{2+} homeostasis in a variety of cell types, and further studies comparing responses of fetal and maternal vascular cells are warranted.

Numerous vasoactive agonists stimulate endothelium-derived NO production *via* Ca^{2+}-dependent activation of eNOS (86, 93, 122). Augmented increases in $[Ca^{2+}]_i$ in maternal hand vein endothelial cells in response to ATP (85) may be a compensatory response in PE, although underlying mechanisms such as altered receptor expression or Ca^{2+} store release/influx pathways have not been characterized. In this context, Ca^{2+} responses to ATP or histamine are increased in cultured ECs pretreated with conjugated free fatty acids (39), establishing a link between abnormal Ca^{2+} regulation and dyslipidemia in PE. The enhanced Ca^{2+} response was associated with an increased generation of $O_2^{\bullet-}$, which could contribute to vascular dysfunction.

Cytosolic Ca^{2+} levels in fetal vascular tissue

Relatively few studies of intracellular Ca^{2+} in fetal tissue have been conducted, with abnormalities characterized by elevated basal or agonist-stimulated Ca^{2+} levels. Basal $[Ca^{2+}]_i$ is elevated in trophoblastic cells (20) and fetal umbilical vein ECs (HUVECs) (130). Figure 5 illustrates the differential Ca^{2+} responses to histamine in normal HUVECs and cells isolated from PE pregnancies. Whereas basal Ca^{2+} levels were elevated in HUVECs from PE pregnancies, histamine-stimulated Ca^{2+} entry was significantly inhibited. Paradoxically, NO production is increased in PE ECs (data not shown), which appears

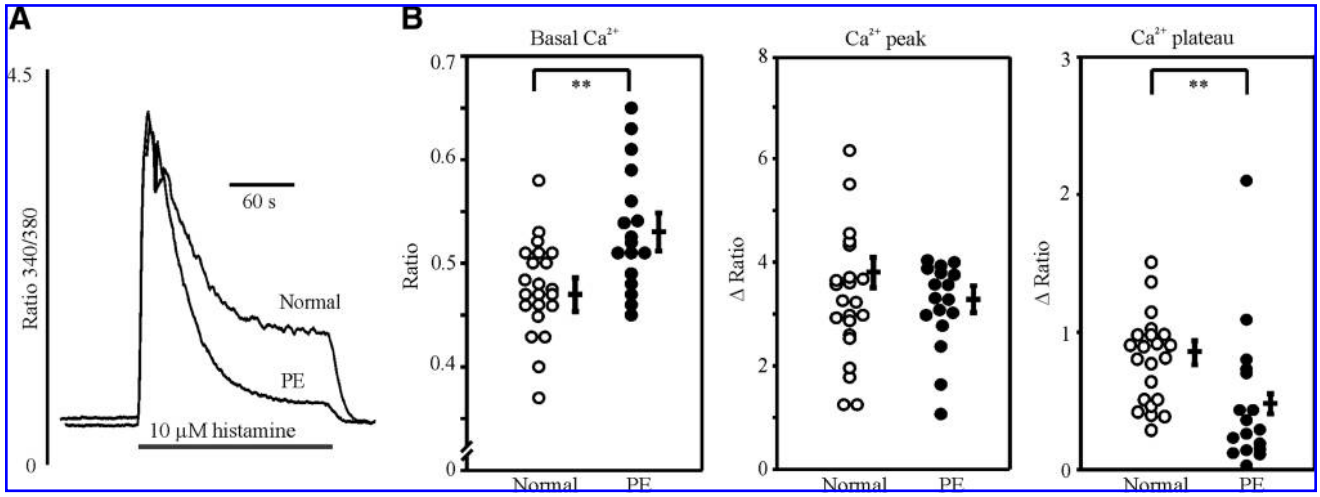

FIG. 5. Basal and histamine-stimulated $[Ca^{2+}]_i$ levels in fetal endothelial cells from normal and PE pregnancies. (**A**) Human umbilical vein ECs (HUVECs) were maintained in Ca^{2+}-containing solution, and basal and histamine-stimulated peak and plateau $[Ca^{2+}]_i$ levels monitored in single cells by using the 340/380nm fluorescence Fura 2 ratio. Basal, peak, and plateau $[Ca^{2+}]_i$ levels measured in cells from normal term (n = 22) and PE (n = 17) pregnancies are summarized in scatterplots shown in (**B**), where Δ ratio denotes the peak $[Ca^{2+}]_i$ or plateau $[Ca^{2+}]_i$ minus basal $[Ca^{2+}]_i$ level. Bars denote the mean ± SEM of measurements in 22 normal and 17 PE endothelial cell cultures. Significant differences between groups were assessed by using Mann–Whitney and Kruskal–Wallis nonparametric ANOVA tests; $^{**}p < 0.002$. Data re-plotted from Fig. 1 [Steinert *et al*. (130)].

inconsistent with the reduced Ca^{2+} influx detected in PE (Fig. 5B). A possible explanation for the decreased Ca^{2+} influx but increased NO release in PE ECs is that eNOS is located at the membrane associated with caveolae, but our Ca^{2+} measurements by using fura-2 monitor bulk cytosolic $[Ca^{2+}]$. We postulated that a distribution of the Ca^{2+} influx pathway, PMCA, and eNOS in PE cells may allow a greater fraction of the influx of Ca^{2+} to activate eNOS and then be pumped out without reaching the cytosol for detection by fura-2 (130). Alternatively, activation of soluble guanylyl cyclase by lipids peroxides rather than NO may have led to NO-independent increases in intracellular cGMP levels (92).

Figure 6 summarizes potential mechanisms by which ROS may modulate Ca^{2+} entry pathways in vascular cells in PE. In contrast to fetal umbilical artery smooth muscle cells (Fig. 3), basal levels of $[Ca^{2+}]_i$ were elevated in ECs isolated from PE pregnancies. Our findings further established that fetal ECs from PE pregnancies exhibited a decreased permeability to Ca^{2+} and Ba^{2+} but an increased permeability to Mn^{2+} and Gd^{3+}. We attributed this to a PE-induced phenotypic alteration of a single influx pathway or changes in the balance between different entry pathways with different relative permeabilities to Ca^{2+}, Ba^{2+}, Mn^{2+}, and Gd^{3+} (130). As Ca^{2+} entry in nonexcitable cells is mediated by a family of transient receptor potential (TRP) proteins, regulation of their expression in normal and PE endothelial cells certainly merits further investigation.

ATPases in maternal tissue

Some of the abnormally high cytosolic Ca^{2+} levels detected in PE may be due to a decrease in the expression or activity or both of membrane-bound enzymes, such as ATPases (134). In PE, Ca^{2+}-ATPase activity is reduced in maternal red blood cells (101). Diminished Ca^{2+}-ATPase activity was also observed in red blood cell ghosts or myometrial tissue isolated from women with PE (19, 80), consistent with the hypothesis that Ca^{2+}-regulating transporters are highly sensitive to lipid peroxidation products. Plasma membrane lipid peroxidation may inhibit membrane-bound enzymes such as Ca^{2+}-ATPases (88) and could account for altered $[Ca^{2+}]_i$ regulation in

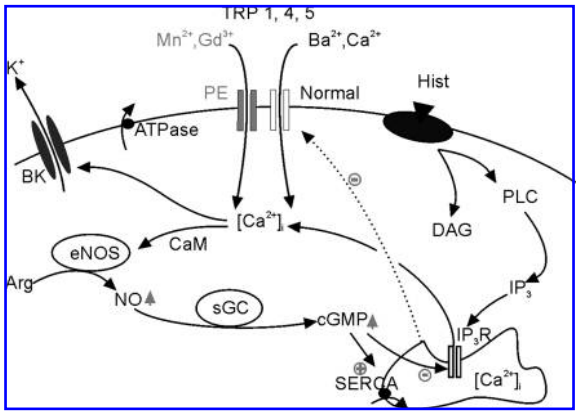

FIG. 6. Ca^{2+} influx and extrusion pathways in normal and PE endothelial cells. This schematic illustrates agonist (histamine, Hist)-induced activation of the PLC-IP_3 pathway. Release of IP_3 leads to activation of the IP3 receptor located at the endoplasmic reticulum and subsequent increases in cytosolic Ca^{2+}. Calcium in turn is extruded *via* the plasma membrane Ca^{2+} ATPase or pumped back into intracellular Ca^{2+} stores *via* SERCA. Cytosolic Ca^{2+} activates eNOS and Ca^{2+}-activated potassium channels (BK_{Ca}). The release of Ca^{2+} from intracellular stores leads a store-operated Ca^{2+} entry, which is reduced in PE. Our findings established that fetal endothelial cells from PE pregnancies exhibit a decreased permeability to Ca^{2+} and Ba^{2+} but an increased permeability to Mn^{2+} and Gd^{3+}, which we attributed to a PE-induced phenotypic alteration of a single influx pathway or alteration in the balance between different entry pathways that have different relative permeabilities to Ca^{2+}, Ba^{2+}, Mn^{2+}, and Gd^{3+} (130).

several cell types and tissues isolated from women with PE (1, 74).

ATPases in fetal tissue

In PE, Ca^{2+}-ATPase activity is reduced in placental trophoblasts (19). Furthermore, a lower expression of mRNA levels observed for the α_1 and α_3 isoforms of the Na^+-K^+-ATPase in the myometrium or the α_2 isoform in the placenta from patients with PE (89) may alter Na^+/K^+ with consequences for the membrane potential and $[Ca^{2+}]_i$.

Mitochondria

Another important aspect in the regulation of intracellular Ca^{2+} is its buffering by mitochondria. These organelles play a key role in $[Ca^{2+}]_i$ homeostasis, and mitochondrial Ca^{2+} transport is highly sensitive to lysophospholipids (117), known to be produced in much higher quantity by ECs exposed to PE serum (38). As PE is associated with functional damage of mitochondria in myometrial smooth muscle and interstitial cells, circulating leukocytes and placental trophoblasts (95, 124), this may in part explain disturbed $[Ca^{2+}]_i$ regulation in vascular cells. Mitochondria not only may be affected by oxidative stress in PE but also are a source of enhanced ROS generation (27). These organelles continuously produce ROS and, under conditions of ischemia, generate increased levels of $O_2^{\bullet-}$, which in turn dismutates into H_2O_2 and generates $^{\bullet}OH$ *via* the Fenton reaction. Obvious features of irreversible cell damage caused by oxidative stress are morphologic and functional changes within mitochondria (133), as reported in umbilical artery endothelial cells isolated from PE pregnancies (155). The increased oxidative stress generated by mitochondrial dysfunction may be correlated with the severity of PE (95). Further research is required to establish whether changes in $[Ca^{2+}]_i$ regulation are a consequence of PE-induced alterations of ion-channel activity caused by an imbalance between pro- and antioxidant processes favoring oxidants. The numerous studies documenting changes in $[Ca^{2+}]_i$ in PE tissues strongly support the hypothesis that alterations in Ca^{2+} signaling associated with this pregnancy-associated disease are related to enhanced oxidative stress. Elevated basal $[Ca^{2+}]_i$ or insufficient responses to agonists will have significant consequences for Ca^{2+}-dependent enzyme activities and could explain changes in intracellular signaling systems reported for other tissues in PE.

Pre-Eclampsia and In Utero Programing of Cardiovascular Disease

Epidemiologic studies suggest that restricted or imbalanced nutrition *in utero* affects programing of cardiovascular disease in adult life (7). Numerous experiments in rats and mice have established that changes in the maternal diet in the earliest stages of pregnancy can induce long-term programing of an increased susceptibility to disease in the offspring. As summarized in a recent editorial by Magness and Poston (84), a series of reviews and original articles highlight the consequences for offspring of pertubations in both the maternal and fetal environments during the early stages of pregnancy (5).

In the context of *in utero* programing and our findings of impaired Ca^{2+} signaling in umbilical vein ECs in PE, endothelial cells obtained from healthy newborns with a family history of type 2 diabetes exhibit a diminished capacity to generate ROS and NO. We previously reported that basal but not agonist-stimulated Ca^{2+} levels are elevated in HUVECs isolated from gestational diabetic pregnancies (127). As hypoxia due to repeated hypoperfusion and subsequent reperfusion of the placenta in PE results in oxidative stress at ~12–14 weeks of gestation, oxidative damage is propagated to the maternal and fetal vasculature *via* circulating lipid peroxides, H_2O_2, and toxic antigens. This may have implications for long-term programing of the fetal cardiovascular system, because men whose mothers had PE are at an increased risk of developing hypertension in adulthood (40). Fetal programing has also been implicated in a recent murine model of PE, in which male offspring showed elevated blood pressure and remained underweight until adulthood (81).

Animal Models, Therapy, and Future Research Directions

Treatment for pre-eclamptic patients has included dietary supplementation with antioxidants such as vitamin C, E (21, 109) or D (9), although with controversial outcome (107, 128). Vitamin intake during the peri-conceptional period has been found to reduce the risk of PE. Numerous studies have examined the health benefits of antioxidant supplementation in PE, yet no conclusive evidence suggests that prophylactic vitamin supplementation delays or ameliorates the onset of PE. The possibility that high plasma levels of vitamin C may be prooxidant (41) may account in some studies for the lack protection afforded by vitamin supplementation in PE (4). However, a positive outcome of vitamin E and C application on leukocyte–endothelium adhesion has been reported (14, 118).

More-effective strategies for the prevention and treatment of PE may potentially be forthcoming, with recent progress in the development of animal models. Overexpression of sFlt-1 in mice induces hypertension and fetal growth restriction (82). Inoculation of mice with activated immune Th1 cells leads to an altered balance between vasodilators and vasoconstrictors and subsequent hypertension, suggesting that activation of the immune system predisposes to the onset of PE (121, 156). In rats, volume expansion during pregnancy (0.9–1.8% NaCl in drinking water during the last week of gestation) increased oxidative stress and levels of 8-isoprostanes and thromboxane (8), and also increased placental production of $O_2^{\bullet-}$ and peroxynitrite (91). Moreover, numerous studies tried to identify unknown factor(s) in maternal plasma that may cause vascular dysfunction and hypertension (100), and studies in frog mesenteric vessels report that perfusion with PE plasma increases microvascular edema (97). It remains arguable whether animals model with an immune challenge, volume expansion, or PE plasma can accurately mimic the development of PE, because this human disease is not observed spontaneously in other species.

In summary, oxidative stress plays a major role in vascular diseases, with a clear link between disturbances of second-messenger systems and the occurrence of endothelial dysfunction (15). Whether oxidative stress affects ion channels, ATPases, or other ion-transport systems directly or diminishes other second-messenger systems such as NO-dependent vasodilation by generating peroxynitrite depends on a variety of conditions. Together with a reduced capacity of ROS scavenging systems in PE (4), substantial evidence indicates

that the disease is associated with enhanced oxidative stress. The endothelium is highly sensitive to oxidative stress, and reported abnormalities in Ca^{2+} homeostasis have been documented in numerous cell types (57, 60, 159), including endothelial cells (153). Detailed characterization of the mechanisms underlying ROS-induced changes in Ca^{2+} signaling and the consequences for endothelial function in PE will have important implications for therapeutic strategies to ameliorate oxidative stress, not only in pregnancy-associated diseases, but also in other cardiovascular complications associated with hypertension (136) and gestational diabetes.

Although low, physiologic concentrations of ROS regulate a variety of intracellular signaling pathways associated with cellular homeostasis (123), sustained oxidative stress and an overproduction of ROS in pregnancy-associated diseases may compromise vascular antioxidant defenses. This in turn most likely alters *in utero* programing (102) of redox-sensitive gene transcription and regulation of Ca^{2+} signaling in the fetal vasculature (129, 130), with potential consequences for vascular dysfunction in adulthood.

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Abbreviations

AA, Arachidonic acid; Ang II, angiotensin II; AT_1, angiotensin II receptor type 1; BAECs, bovine aortic endothelial cells; cGMP, cyclic guanosine monophosphate; COX-1, cyclooxygenase-1; DTT, 2,4-dithiothreitol; ECs, endothelial cells; EETs, epoxyeicosatrienoic acids; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ET-1, endothelin-1; ETYA, eicosatetraynoic acid; FFAs, free fatty acids; fMLP, formyl-methionine-leucyl-phenylalanine; G6PD, glucose-6-phosphate dehydrogenase; HELLP, hemolysis, elevated liver enzymes, and low platelets; HETEs, hydroxyeicosatetraenoic acids; Hist, histamine; HOCl, hypochlorous acid; HPETES, hydroperoxy-eicosatetraenoic acids; HUVECs, human umbilical vein endothelial cells; HUASMCs, human umbilical artery smooth muscle cells; HX, hypoxanthine; H_2O_2, hydrogen peroxide; Indo, indomethacin; IP_3R, inositol trisphosphate receptor; K_{ATP}, ATP-sensitive potassium channels; K_{ACh}, acetylcholine-induced potassium current; K_{Ca}, Ca^{2+}-activated potassium channels; LDL, low-density lipoprotein; LOX, lipoxygenase; MOX, mono-oxygenase; MPP, (2-methyl-1,2-di-3-pyridyl-1-propanone; NDGA, *nor*-dihydroguaiaretic acid; NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\bullet-}$, superoxide anion; 17-ODYA, 17-octadecynoic acid; ($^{\bullet}OH$, hydroxyl radical; $OONO^-$, peroxynitrite; oxLDL, oxidized low-density lipoprotein; PE, pre-eclampsia; PGI_2, prostacyclin; PGIS, prostaglandin I_2 synthase; PlGF, placental growth factor; PLA_2, phopholipase A_2; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; PMCA, plasma membrane Ca^{2+}-ATPase; P_o, open probability; ROC, receptor-operated channel; ROS, reactive oxygen species; RyR, ryanodine receptor; s-Eng, soluble endoglin; sFlt-1, Fms-like tyrosine kinase 1; SOD, superoxide dismutase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SOC, store-operated channel; SR, sarcoplasmic reticulum; TGFβ-1, tumor necrosis factor beta-1; TRP, transient receptor protein; TRPP2, polycystin-2, TRP-like Ca^{2+} selective channel; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; VGCC, voltage-gated Ca^{2+} channel; VSMC, vascular smooth muscle cell; V_m, membrane potential; X, xanthine; XDH, xanthine dehydrogenase; XO, xanthine oxidase; ψ_m, mitochondrial membrane potential.

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