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MINIREVIEW

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Nitric oxide and endothelium-dependent hyperpolarization mediated by hydrogen peroxide in health and disease

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Abstract

The endothelium plays crucial roles in modulating vascular tone by synthesizing and releasing endothelium-derived relaxing factors (EDRFs), including vasodilator prostaglandins, nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH) factors. Thus, endothelial dysfunction is the hallmark of atherosclerotic cardiovascular diseases. Importantly, the contribution of EDRFs to endothelium-dependent vasodilatation varies in a distinct vessel size-dependent manner; NO mainly mediates vasodilatation of relatively large, conduit vessels (eg epicardial coronary arteries), while EDH factors in small resistance vessels (eg coronary microvessels). Endothelium-derived hydrogen peroxide (H_2O_2) is a physiological signalling molecule serving as one of the major EDH factors especially in microcirculations and has gained increasing attention in view of its emerging relevance for cardiovascular diseases. In the clinical settings, therapeutic approaches targeting NO (eg NO donors) or non-specific elimination of reactive oxygen species (eg antioxidant supplements) are disappointingly ineffective for the treatment of various cardiovascular diseases, in which endothelial dysfunction and coronary microvascular dysfunction are substantially involved. These lines of evidence indicate the potential importance of the physiological balance between NO and H_2O_2/EDH factor. Further characterization and better understanding of endothelium-dependent vasodilatations are important to develop novel therapeutic strategies in cardiovascular medicine. In this MiniReview, we will briefly summarize the current knowledge on the emerging regulatory roles of endothelium-dependent vasodilatations in the cardiovascular system, with a special reference to the two major EDRFs, NO and H_2O_2/EDH factor, in health and disease.

KEYWORDS

endothelial function, endothelium, endothelium-dependent hyperpolarization, hydrogen peroxide, nitric oxide, nitric oxide synthase

1 | INTRODUCTION

The endothelium plays essential roles in modulating the tone of underlying vascular smooth muscle cells (VSMC) by synthesizing and releasing endothelium-derived relaxing factors (EDRFs), including vasodilator prostaglandins (eg prostacyclin), nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH) factors, as well as endothelium-derived contracting factors[1,2] (Figure 1). Since Feletou and Vanhoutte[3] and Chen et al[4] independently demonstrated the existence of putative EDH factors in 1988, several candidates have been identified as the nature of EDH factors, depending on the vascular bed, vessel size and species of interest. They include epoxyeicosatrienoic acids, metabolites of

arachidonic P450 epoxygenase pathway,[5,6] electrical communication through gap junctions,[7] K^+ ions[8] and as we demonstrated, endothelium-derived hydrogen peroxide (H_2O_2)[9,10] (Figure 1). Although, by definition, the contribution of EDH factors is determined only after the blockade of both vasodilator prostaglandins and NO, EDH-mediated responses are the major mechanism of endothelium-dependent vasodilatations in resistance arteries. The contribution of EDRFs to endothelium-dependent vasodilatations markedly varies as a function of vessel size; endothelium-derived NO mainly mediates vasodilatation of relatively large, conduit vessels, while EDH-mediated responses are the predominant mechanisms of endothelium-dependent vasodilatation of resistance arteries.[1,11] This vessel size-dependent contribution of NO and EDH factors is well preserved among species, from rodents to humans, to make a physiological balance between them.[1,2]

Endothelial dysfunction is characterized by impaired production and/or action of EDRFs and serves as the hallmark and potential predictor for atherosclerotic cardiovascular diseases, as well as metabolic disorders.[2] Various risk factors, such as smoking, diabetes mellitus, hypertension and hypercholesterolaemia, cause endothelial dysfunction, initiating the step towards atherosclerotic cardiovascular diseases.[2] Endothelium-derived H_2O_2 is one of the major EDH factors in various vascular beds in animals and humans and has gained increasing attention in view of its emerging relevance for cardiovascular disease.[1,2,9,10,12,13] In the clinical settings, it has been repeatedly reported that the effects of chronic nitrate therapy are neutral or even harmful in patients with cardiovascular diseases [14-18] and that antioxidant treatments are disappointingly ineffective to prevent cardiovascular events.[19] These lines of evidence suggest the importance of the physiological balance between NO and EDH factors in maintaining cardiovascular homeostasis and in curing diseases associated with endothelial dysfunction.

In this MiniReview, we will briefly summarize the current knowledge on the two endothelium-derived mediators, NO and EDH mediated by H_2O_2, with a special reference to their clinical implications and therapeutic approaches in cardiovascular diseases. An extensive review on the regulatory mechanisms of NO-mediated responses is available elsewhere.[20]

2 | ENDOTHELIAL MODULATION OF VASCULAR TONE: NO AND EDH

EDH factors cause hyperpolarization and subsequent relaxation of underlying VSMC with resultant vasodilatation of small resistance vessels and thus finely regulate blood pressure and tissue perfusion instantaneously in response to diverse physiological demands.[1,2] As mentioned above, endothelium-derived NO and EDH factors share the roles in modulating vascular tone in a distinct vessel size-dependent fashion.[1,11] Although prostaglandin E_2 and prostacyclin play important roles in the regulation of kidney functions,

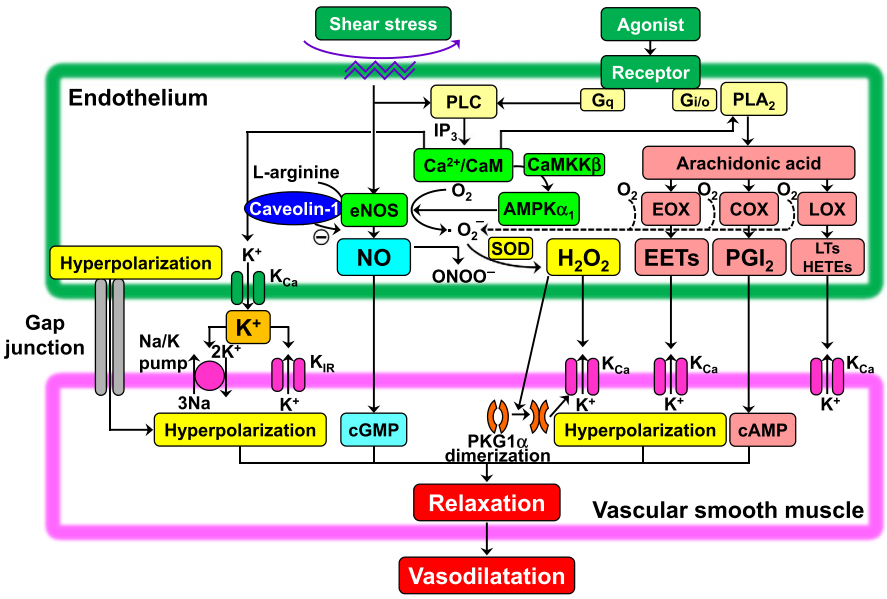

FIGURE 1 Endothelium-derived relaxing factors. AMPKα_1, α_1-subunit of AMP-activated protein kinase; CaM, calmodulin; CaMKKβ, Ca^{2+}/CaM-dependent protein kinase β; cAMP, cyclic AMP; cGMP, cyclic GMP; COX, cyclooxygenase; EETs, epoxyeicosatrienoic acids; eNOS, endothelial NO synthase; EOX, epoxygenase; H_2O_2, hydrogen peroxide; HETEs, hydroxyeicosatetraenoic acids; IP_3, inositol trisphosphate; K_{Ca}, calcium-activated potassium channel; K_{IR}, inwardly rectifying potassium channel; LOX, lipoxygenase; LTs, leukotrienes; NO, nitric oxide; $ONOO^-$, peroxynitrite; PGI_2, prostacyclin; PKG1α, 1α-subunit of protein kinase G; PLA_2, phospholipase A_2; PLC, phospholipase C; SOD, superoxide dismutase

such as glomerular filtration rate, renal blood flow and renal vascular tone,[21] vasodilator prostaglandins in general play a small but constant role, independent of vessel size. NO predominantly regulates the tonus of relatively large conduit vessels (eg aorta and epicardial coronary arteries), while the importance of EDH factors increases as vessel size decreases (eg small mesenteric arteries and coronary microvessels).[1,11] Thus, EDH-mediated vasodilatation is especially important in microcirculations, where blood pressure and tissue perfusion are critically determined. Moreover, such redundant mechanisms in endothelium-dependent vasodilatations are advantageous for ensuring proper maintenance of vascular tone under pathological conditions, where one of EDRFs-mediated responses is compromised favouring a vasoconstrictor, prothrombotic and proinflammatory state. Indeed, in various pathological conditions with atherosclerotic risk factors, NO-mediated relaxations are easily impaired, while EDH-mediated responses are fairly preserved or even enhanced to serve as a compensatory vasodilator system,[22-24] although there are exceptions where after acute or chronic exposure to hyperglycaemia, these compensatory roles of EDH-mediated mechanisms are disrupted.[25,26] Multiple mechanisms have been proposed for the enhanced EDH-mediated responses in small resistance vessels, including negative interactions between NO and several EDH factors, as discussed later. Although several EDH factors exist depending on the vascular bed, vessel size and species studied, endothelium-derived H_2O_2 at physiologically low concentrations is one of the major EDH factors in human [27] and mouse [9] small mesenteric arteries and human,[28] porcine [12] and canine [29-31] coronary arteries. Refer to an excellent textbook for more comprehensive information on other EDH factors.[32] Next, we will focus on endothelium-derived H_2O_2 as an EDH factor in detail.

3 | ENDOTHELIUM-DERIVED H_2O_2 AS AN EDH FACTOR

3.1 | Identification of H_2O_2/EDH factor

H_2O_2 was acknowledged as a physiological signalling molecule serving especially in microcirculation,[33] for blood pressure,[33,34] coronary microcirculation [29-31] and metabolic functions.[35,36] Reactive oxygen species (ROS) have been considered to be primarily harmful because of their highly damaging entity to cells and tissues and pathological implications in various cardiovascular diseases, including atherosclerosis, hypertension, heart failure, cardiomyopathy and coronary artery disease, where endothelial dysfunction is also substantially involved.[33,37] However, as exemplified by endothelium-derived H_2O_2/EDH factor, a growing body of evidence has demonstrated that physiological levels of ROS can serve as crucial signalling molecules in health and disease.[38] Following the original reports on the existence of EDH factors in 1988,[3,4] four sets of early observations and notions led us to hypothesize that a putative EDH factor might be a non-NO vasodilator substance (likely ROS) derived from endothelial NO synthases (NOSs) system. Firstly, both NO-mediated and EDH-mediated responses are susceptible to vascular injuries caused by various atherosclerotic factors, and conversely, the treatment of those risk factors can restore both responses.[1,10,22,39] Secondly, indeed, it was previously demonstrated that endothelium-derived free radicals exert vasoactive effects in endothelium-dependent vasodilatation and vasoconstriction in canine coronary arteries.[40] Thirdly, both endothelial NOS (eNOS)-derived NO generation and EDH-mediated responses are dependent on calcium/calmodulin.[41] Fourthly, a simple molecule (like NO) rather than complex substances may be favourable in modulating vascular tone instantaneously in response to physiological demands in the body. Thus, in 2000, we demonstrated for the first time that endothelium-derived H_2O_2 is an EDH factor in mouse mesenteric arteries; EDH-mediated hyperpolarizations and relaxations of underlying VSMC were inhibited by catalase, a specific H_2O_2 inhibitor, in small mesenteric arteries from wild-type mice and were significantly reduced in eNOS-knockout (KO) mice.[9] This was also the case in other blood vessels, including human mesenteric [27] and coronary [13] arteries, porcine [12] and canine [29-31] coronary arteries and piglet pial arterioles.[42] Several lines of evidence are available to support that H_2O_2 is a transferable factor derived from microvascular endothelium. For example, using a peroxide-sensitive fluorescence dye, we have previously demonstrated that an endothelium-dependent agonist acetylcholine induces endothelial production of H_2O_2, especially at the cell membrane, but not at the underlying vascular smooth muscle cell layer, in mouse small mesenteric arteries (approximately 200 μm in diameter) in the presence of a cyclooxygenase and a NO synthase inhibitors.[9,43] Moreover, our electron spin resonance methods revealed that endothelial cells produce and release a significant amount of H_2O_2/EDH factor in porcine coronary microvessels.[12] Furthermore, using a unique bioassay system, Liu et al provided direct evidence that endothelium-derived H_2O_2 is a transferrable EDH factor mediating flow-mediated dilation in a paracrine manner in human coronary arterioles.[13] Notably, the estimated concentrations of endothelium-derived H_2O_2/EDH factor were in micromolar order (<50 μmol/L),[12,30] which are much lower concentrations than those observed in various pathological conditions.[44] When applied exogenously in organ chamber experiments, approximately 10-100 μmol/L of H_2O_2 elicits vasodilatation of human coronary arterioles[13,45] and mouse small mesenteric arteries,[9,46,47] while higher concentrations of H_2O_2 rather induce vasoconstriction by releasing cyclooxygenase-derived thromboxane.[48] It is important to note that only 10%-15% of H_2O_2 applied exogenously reaches the

intracellular targets due to endogenous antioxidants and membrane impedance.[49]

3.2 | Source of H_2O_2/EDH factor

Endothelium-derived H_2O_2 is mainly generated by the dismutation of superoxide anions derived from various sources in the endothelium, including NADPH oxidase, mitochondrial electron transport chain, xanthine oxidase, lipoxygenase and NOSs (Figure 1).[44] Cu,Zn-superoxide dismutase (SOD) plays a key role in the synthesis of H_2O_2/EDH factor in the endothelium. eNOS produces superoxide anions under physiological conditions when synthesizing NO from L-arginine and oxygen, while Cu,Zn-SOD dismutates those superoxide anions into H_2O_2. Moreover, Cu,Zn-SOD-KO mice show markedly impaired EDH-mediated hyperpolarizations and relaxations in mesenteric arteries and coronary circulation without VSMC dysfunction.[50] Importantly, superoxide anions relevant to H_2O_2/EDH factor are not derived from pathologically uncoupled eNOS because H_2O_2-mediated EDH-type responses are not cancelled by NOS inhibitors and up-regulation of eNOS cofactor tetrahydrobiopterin has no effects on the responses.[51] It is widely accepted that NOS inhibitors (eg L-arginine analogues such as L-NNA and L-NAME) suppress superoxide generation from the oxygenase domain of eNOS or dysfunctional eNOS but not that from the reductase domain of eNOS, where superoxide generation is independent on the presence of Ca^{2+}/CaM. In addition, Stuehr et al demonstrated that eNOS can generate superoxide anions from its reductase domain under physiological conditions, where superoxide anions are converted to H_2O_2 to cause EDH-mediated responses, and that NOS inhibitors (ie L-arginine analogues) only suppress superoxide generation from oxidase domain of eNOS.[52] Previous studies by us and others have demonstrated that catalase significantly inhibits endothelium-dependent vasodilatation and hyperpolarization of small arteries in the absence of a NOS inhibitor.[9,34,53] These lines of evidence support our notion that eNOS is the main source of H_2O_2 as an EDH factor under physiological conditions, although other sources of superoxide anions in H_2O_2-mediated vasodilatation have been identified. For example, in human coronary arterioles, mitochondrial respiratory chain- and NADPH oxidase-derived H_2O_2 may be involved in flow-mediated dilatation and bradykinin-induced relaxation, respectively.[54,55]

3.3 | Regulatory mechanisms of physiologically relevant H_2O_2

Recent studies have provided potential regulatory mechanisms underlying the physiologically relevant H_2O_2 in the endothelium.[38] It is important to note that local subcellular concentrations at microdomains rather than net cellular concentrations may be critical to determine whether the effects of ROS can be detrimental or beneficial for cellular signalling and that co-localization of the source and target of H_2O_2 may help avoid non-specific harmful oxidations.[56,57] A good example of this concept is that only a minor increase in ROS caused by caveolar localization of NADPH oxidase-1 in hypertension is enough to interfere with NO-mediated signalling.[58] In addition, specific cysteine residues, such as peroxiredoxins, can function as a redox-dependent molecular switch to regulate ROS-mediated signalling.[44]

3.4 | Mode of action of H_2O_2/EDH factor

Oxidative modification of cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG) is a central mechanism by which H_2O_2 induces hyperpolarization and relaxation of underlying VSMC,[34,59] although other modes of action of H_2O_2/EDH factor have also been proposed.[10,60] Briefly, H_2O_2 induces dimerization of 1α-isoforms of PKG (PKG1α) through an interprotein disulphide bond formation between them to enhance the kinase activity through phosphorylation. The activated PKG1α subsequently stimulates K^+ channels with resultant hyperpolarization and vasodilatation in mouse mesenteric arteries[34] and human coronary arterioles.[13,45] H_2O_2 also promotes the translocation of PKG1α from cytoplasm to membrane in human[45] and porcine[61] coronary arteries. Such reversible post-translational modification, like phosphorylation, is favourable for the fine control of vascular tone in response to demand fluctuation in vivo.[20]

3.5 | Clinical significance of H_2O_2/EDH factor

The oxidant-mediated signalling by H_2O_2 is clinically important because it is associated with blood pressure control in vivo. Pharmacological inhibition of catalase, which decomposes H_2O_2 into oxygen and water, decreases arterial blood pressure associated with enhanced PKG1α dimerization in vivo.[61] Moreover, the "redox-dead" knock-in mice of Cys42Ser PKG1α, whose mutant PKG1α is unable to be activated by H_2O_2-induced dimerization because of the deletion in its redox-sensitive sulphur, exhibit markedly impaired EDH-mediated hyperpolarization and relaxation in resistance arteries ex vivo associated with systemic arterial hypertension in vivo.[34] Furthermore, H_2O_2 has potent vasodilator properties in coronary resistance vessels and plays important roles in coronary autoregulation,[29] cardioprotection against myocardial reperfusion injury[30] and tachycardia-induced metabolic coronary vasodilatations[31] in dogs in vivo. Given that coronary vascular resistance is

predominantly determined by the pre-arterioles and arterioles[62] where the effect of EDH-mediated responses on vascular tone outweighs that of NO-mediated relaxations, it is important to maintain the vessel size-dependent contribution of NO and EDH factors for the treatment of coronary artery disease (CAD). Taken together, endothelium-derived H_2O_2 functions as an important endogenous second messenger at its physiological low concentrations to elicit EDH-meditated vasodilatation and to maintain vascular homeostasis in the coronary circulation.[2,10,39]

4 | MECHANISMS OF ENHANCED EDH IN MICROCIRCULATION

4.1 | Diverse roles of endothelial NOSs system

The endothelium synthesizes and releases NO and H_2O_2/EDH factor to modulate vascular tone in a distinct vessel size-dependent manner through the diverse roles of endothelial NOSs system (Figure 2). In large conduit vessels, NOSs mainly serve as a NO-generating system to cause soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP)-mediated vasodilatation, whereas in small resistance vessels, they act as a superoxide-generating system to evoke H_2O_2/EDH factor-mediated responses.[43] Superoxide anions derived from reductase domain of NOSs under physiological conditions are converted to H_2O_2 to cause EDH-mediated responses.[52] Among three NOS isoforms (neural NOS [nNOS, NOS1], inducible NOS [iNOS, NOS2] and eNOS, NOS3) expressed in cardiovascular system, eNOS is the dominant isoform in blood vessels[63] and the most important isoform in generating H_2O_2/EDH factor in the endothelium.[52] As mentioned above, genetic ablation of eNOS in mice results in impaired EDH-mediated vasodilatation associated with systemic hypertension.[64] Using singly-eNOS-KO, doubly-n/eNOS-KO and triply-n/i/eNOS-KO mice, we have previously demonstrated that EDH-mediated relaxations are progressively reduced in accordance with the number of NOS genes ablated.[43] As compared with wild-type mice, H_2O_2-mediated EDH-type relaxations of small mesenteric arteries are reduced approximately by half in singly-eNOS-KO mice, further diminished in doubly-n/eNOS-KO mice, and are finally absent in triply-n/i/eNOS-KO mice without underlying VSMC dysfunction.[43] The remaining EDH-mediated relaxation of small mesenteric arteries in eNOS-KO mice is still sensitive to catalase.[9] Collectively, these results indicate that three NOSs isoforms compensate each other to maintain H_2O_2-mediated EDH-type relaxations (Figure 2).

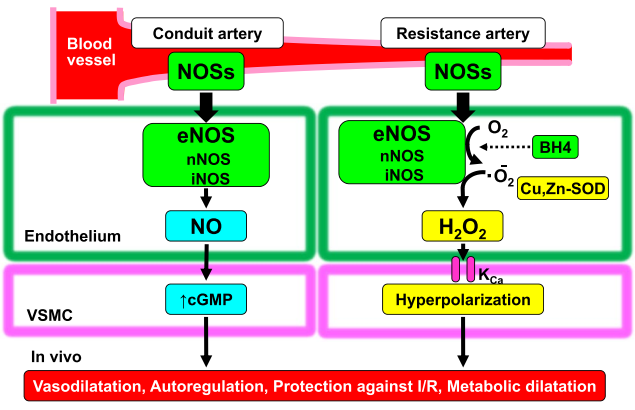

FIGURE 2 Vessel size-dependent roles of endothelial nitric oxide synthases system. BH4, tetrahydrobiopterin; cGMP, cyclic GMP; Cu,Zn-SOD, copper-zinc superoxide dismutase; eNOS, endothelial nitric oxide synthase; H_2O_2, hydrogen peroxide; I/R, ischaemia-reperfusion injury; iNOS, inducible NOS; K_{Ca}, calcium-activated potassium channel; nNOS, neural NOS; NO, nitric oxide; NOSs, nitric oxide synthases; VSMC, vascular smooth muscle cells

4.2 | Mechanisms for H_2O_2/EDH factor dominance in microcirculation

Accumulating evidence has provided mechanistic insights into vessel size-dependent contribution of NO and H_2O_2/EDH factor. Previous studies have shown that pre-treatment with NO donors attenuates EDH-mediated vasodilatation in porcine coronary arteries in vitro[65] and canine coronary microcirculation in vivo[66] and that NO exerts a negative-feedback effect on endothelium-dependent vasodilatation through cGMP-mediated desensitization in canine coronary arteries ex vivo.[67] Multiple mechanisms have been proposed for the negative interactions between NO and H_2O_2/EDH factor (Figure 3). Among them, cGMP-dependent activation of PKG desensitizes VSMC to H_2O_2 by inhibiting H_2O_2-induced PKG1α dimerization, a central mechanism of H_2O_2/EDH factor-mediated vasodilatation, and in turn, pharmacological inhibition of sGC sensitizes conduit vessels, but not resistance vessels, to H_2O_2-induced vasodilatation in mice.[68] In addition, mouse resistance vessels have less NO production and less antioxidant capacity, predisposing PKG1α to be more sensitive to H_2O_2-induced activation.[68] Other key players for enhanced H_2O_2/EDH factor-mediated vasodilatation in resistance vessels include endothelial caveolin-1 (a negative regulator of eNOS)[46,69] and α_1-subunit of endothelial AMP-activated protein kinase.[46,70] In contrast, phosphorylation at Try657 of eNOS in response to H_2O_2 leads to reduction in eNOS activity with resultant reduced NO production.[71] Taken together, these mechanisms are in line with the widely accepted view that EDH-mediated responses function as a compensatory vasodilator system when NO-mediated relaxations are compromised. It is important to maintain the vessel size-dependent contribution of NO and EDH factors because excessive endothelial NO production by either caveolin-1 deficiency or eNOS overexpression disrupts the physiological balance between NO and EDH factors

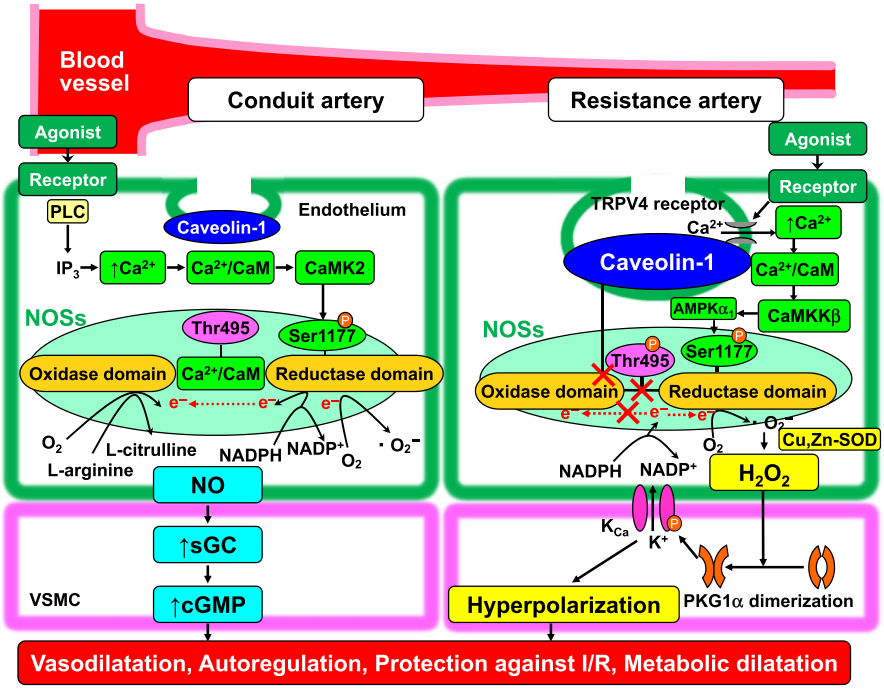

FIGURE 3 Molecular mechanisms of enhanced H_2O_2/EDH factor-mediated responses in microvessels. AMPKα_1, α_1-subunit of AMP-activated protein kinase; CaM, calmodulin; CaMK2, Ca^{2+}/CaM-dependent protein kinase II; CaMKKβ, Ca^{2+}/CaM-dependent protein kinase β; cGMP, cyclic GMP; Cu,Zn-SOD, copper-zinc superoxide dismutase; EDH, endothelium-dependent hyperpolarization; H_2O_2, hydrogen peroxide; I/R, ischaemia-reperfusion injury; IP_3, inositol trisphosphate; K_{Ca}, calcium-activated potassium channel; NO, nitric oxide; NOSs, NO synthases; P, phosphorylation; PKG1α, 1α -subunit of protein kinase G; PLC, phospholipase C; sGC, soluble guanylate cyclase; TRPV4, transient receptor potential vanilloid 4; VSMC, vascular smooth muscle cells

in endothelium-dependent vasodilatation, resulting in impaired cardiovascular homeostasis in mice in vivo.[47,69]

5 | CLINICAL IMPLICATIONS

5.1 | Importance of endothelial function tests

Assessment of endothelial functions has been acknowledged as an excellent surrogate marker of cardiovascular events in many clinical settings, although it is challenging to accurately assess EDH-mediated responses especially in humans in vivo because the contribution of EDH factors could be determined only after the blockade of both vasodilator prostaglandins and NO. EDH-mediated vasodilation can be enhanced to compensate for impaired NO-mediated responses in the early stage of atherosclerotic conditions.[10,23] However, after prolonged exposure to atherosclerotic risk factors, this compensatory role of EDH-mediated responses is eventually disrupted to cause metabolic disturbance.[25] Endothelial dysfunction, as evaluated by impaired flow-mediated dilation (FMD) of the brachial artery or digital reactive hyperaemia index (RHI) in peripheral arterial tonometry, is associated with future cardiovascular events in patients with coronary artery disease, and one standard deviation decrease in FMD or RHI is associated with doubling of cardiovascular event risk.[72]

5.2 | Role of H_2O_2/EDH factor in the pathophysiology of coronary artery disease

Obviously, previous studies focused structural and functional abnormalities of "epicardial" coronary arteries in patients with CAD because they are easily visible on coronary angiography and amenable to procedural intervention (eg percutaneous coronary intervention). However, those of coronary microvasculature, referred to as coronary microvascular dysfunction (CMD), have gained increasing attention in view of their unexpectedly high prevalence in and significant prognostic impact in this population.[73] The aetiologies of CMD still remain largely unknown and may be heterogeneous, for which several structural (eg vascular remodelling, vascular rarefaction and extramural compression) and functional abnormalities (eg endothelial dysfunction, VSMC dysfunction and microvascular spasm) have been proposed.[62] Given that H_2O_2 has potent vasodilator properties in coronary resistance vessels where EDH-mediated responses become relatively dominant to NO-mediated relaxations, it is highly possible that impaired H_2O_2/EDH factor-mediated vasodilatation is involved in the pathogenesis of CMD. Indeed, in eNOS-KO mice, CMD mediated

by H_2O_2/EDH factor is also present associated with cardiac diastolic dysfunction.[74] Thus, for the treatment of CAD, it is essential to maintain the physiological balance between NO and H_2O_2/EDH factor, which notion is supported by the fact that significant negative interactions exist between NO and several EDH factors[47,65-68] and that nitrates as NO donors are not beneficial for the treatment of CMD.[14,16]

More recently, it has been highlighted that endothelium-dependent CMD is associated with low endothelial shear stress, larger plaque burden and vulnerable plaque characteristic beyond conventional coronary risk factors in angina patients with angiographically normal coronary artery or non-obstructive CAD.[75,76] Altered oscillatory or low shear stress with disturbed flow on coronary artery wall is implicated in the local progression of atherosclerotic coronary plaque through endothelial and VSMC proliferation, inflammation, lipoprotein uptake and leucocyte adhesion.[75,76]

5.3 | Lessons from clinical trials targeting NO: too much of a good thing?

Although the role of CMD has been implicated in patients with obstructive CAD who underwent successful revascularization,[77] the effects of isosorbide-5-mononitrate were unexpectedly neutral in patients with microvascular ischaemia despite successful percutaneous coronary intervention.[18] Besides CAD, recent studies highlighted the high prevalence and pathophysiological relevance of CMD in patients with heart failure with preserved ejection fraction (HFpEF).[78-80] Contrary to the premise that enhancing NO-mediated vasodilatation could exert beneficial effects on patients with HFpEF, the results of systemic and long-term administrations of inorganic nitrite in those patients were disappointing or even harmful in randomized, clinical trials.[15,17] In a recent animal study, genetic ablation of endothelial arginase-1, an inhibitor of NO production, did not improve vasomotor function of resistance arteries in diabetic mice.[81] Similarly, antioxidant therapies for patients with cardiovascular diseases had no benefits.[19] These lines of evidence indicate that it is important to turn our attention to avoid excessive NO supplementation and to pay attention to the potential harm of non-specific elimination of ROS by antioxidants, although multiple mechanisms may be involved in the failure of antioxidant therapies, including inadequate dose, short treatment duration and pro-oxidant effects of antioxidants upon supplementation, and thus, so-called "antioxidant paradox" in clinical trials requires further investigations. An alternative explanation for such "paradox" of NO-targeted therapy may be nitrosative stress induced by an excessive amount of NO,[69,82] again suggesting the importance of physiological balance between NO and EDH factors in endothelium-dependent vasodilatation. Standard medications used for the treatment of cardiovascular diseases in the current era share the pleiotropic effects on endothelial function by enhancing NO-mediated vasodilatation with modest antioxidant capacities, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers and statins. Further research is warranted to address how to modulate CMD to improve clinical outcomes of patients with cardiovascular diseases.

6 | CONCLUSIONS

This MiniReview highlighted the potential importance of the physiological balance between NO and H_2O_2/EDH factor in a distinct vessel size-dependent manner through the diverse functions of endothelial NOSs system in maintaining cardiovascular homeostasis. It remains an open question how to improve endothelial functions without affecting the delicate balance between NO and EDH factors. Further characterization and better understanding of endothelium-dependent vasodilatations are indispensable to this end, which helps us develop novel therapeutic strategies in cardiovascular medicine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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