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Review article

Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in animals and humans

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Abstract

The endothelium plays an important role in maintaining vascular homeostasis by synthesizing and releasing several vasodilating substances, including vasodilator prostaglandins, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). Since the first report for the existence of EDHF, several substances/mechanisms have been proposed for the nature of EDHF, including epoxyeicosatrienoic acids (metabolites of arachidonic P450 epoxygenase pathway), K ions, and electrical communications through myoendothelial gap junctions. We have recently demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in mouse and human mesenteric arteries and in porcine coronary microvessels. For the synthesis of H_2O_2 as an EDHF, endothelial Cu,Zn-superoxide dismutase plays an important role in mesenteric arteries of mice and humans. We also have demonstrated that EDHF-mediated responses are attenuated by several arteriosclerotic risk factors, including diabetes mellitus and hyperlipidemia and their combination in particular. Recent studies have indicated that endothelium-derived H_2O_2 plays an important protective role in coronary autoregulation and myocardial ischemia/reperfusion injury in vivo. Indeed, our H_2O_2/EDHF theory demonstrates that endothelium-derived H_2O_2, another reactive oxygen species in addition to NO, plays an important role as a redox signaling molecule to cause vasodilatation as well as cardioprotection. In this review, we summarize our knowledge on H_2O_2/EDHF regarding its identification, mechanisms of synthesis, and clinical implications.

Keywords: Endothelium; Endothelium-derived hyperpolarizing factor; Membrane potential; Hydrogen peroxide

1. Introduction

The endothelium synthesizes and releases several vasodilator substances, including vasodilator prostaglandins, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) [1,2]. Although the nature of EDHF has not been fully elucidated, different EDHFs could exist depending on species, blood vessels, and the size of blood vessels with different hyperpolarizing mechanisms involved [1,2]. Since the first report for the existence of EDHF [3,4], several candidates have been proposed for the nature of EDHF. Currently, the major candidates for EDHF include epoxyeicosatrienoic acids (EETs), metabolites of arachidonic P450 epoxygenase pathway [5,6], K ions [7,8], and electrical communication through myoendothelial gap junctions [9,10] (Fig. 1). We have demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in mouse [11] and human [12] mesenteric arteries and in porcine [13] and canine [14] coronary microvessels (Fig. 1). Although not universally accepted, other investigators also have reported that H_2O_2 may be an EDHF in the human coronary microvessels [15] and piglet pial arterioles [16]. We also have recently demonstrated that endothelial Cu,Zn-superoxide dismutase (SOD) plays an important role in the synthesis of H_2O_2 as an EDHF synthase in mouse [17] and human [18] mesenteric arteries. In this review, we will summarize the latest knowledge on our H_2O_2/EDHF theory, in terms of the identification, mechanisms of synthesis and clinical implications.

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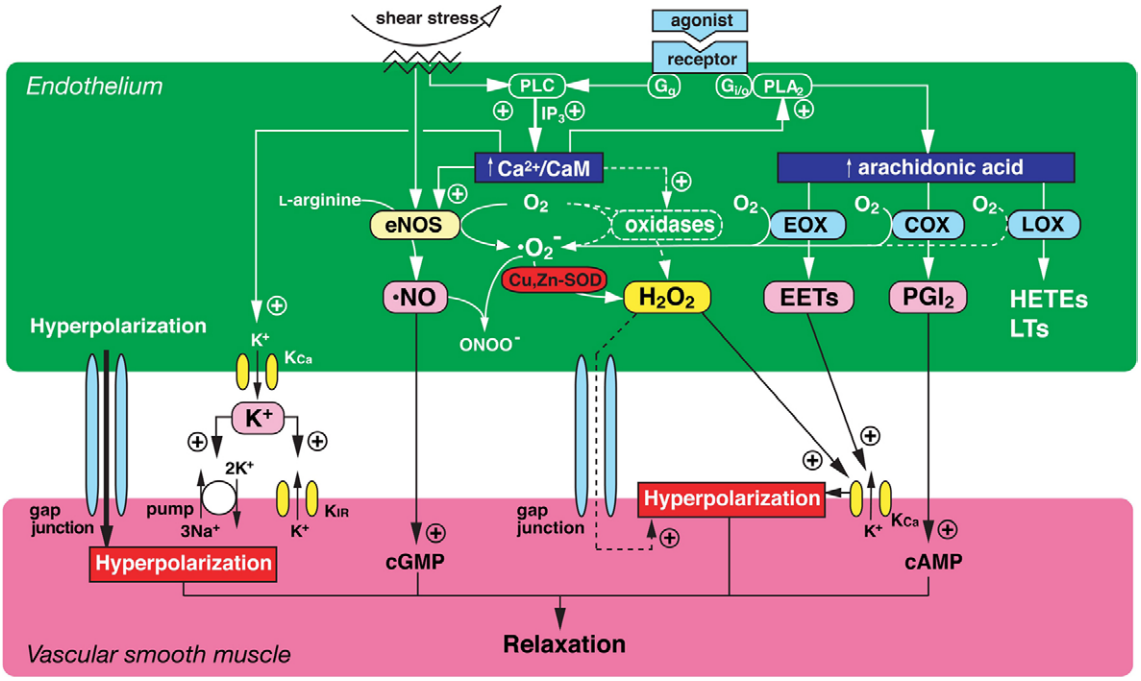

Fig. 1. Hypothesis on the nature of EDHF. Agonist stimulation and shear stress activate calcium–calmodulin complex and eNOS to produce NO, and also activate phospholipase A_2 to release arachidonic acid. NO activates soluble guanylate cyclase, produces cGMP, and relaxes vascular smooth muscle. Cyclooxygenase (COX) produces prostacyclin (PGI_2) from arachidonic acid and PGI_2 relaxes vascular smooth muscle in a cAMP-dependent manner. EDHF hyperpolarizes vascular smooth muscle by opening K channels and then elicits vasodilatation. Major candidates for the nature of EDHF include (1) epoxyeicosatrienoic acids (EETs), metabolites of arachidonic P450 epoxygenase pathway, (2) K ions released from the endothelium through endothelial K_{Ca} channels that activates Na,K-ATPase of vascular smooth muscle, and (3) electrical communication through myoendothelial gap junctions. We also have identified that (4) endothelium-derived H_2O_2 is an EDHF, for which eNOS is an important source.

2. History

It was known that acetylcholine induces hyperpolarization of vascular smooth muscle of rabbit mesenteric arteries [19] and that those hyperpolarizations are achieved in an endothelium-dependent manner [20]. In 1988, Feletou and Vanhoutte [3] and Chen et al. [4] independently demonstrated that a diffusible substance released by the endothelium causes hyperpolarization of underlying vascular smooth muscle, thus proposing the existence of EDHF [3,4].

2.1. Nature of EDHF

NO mediates vascular relaxation of relatively large, conduit arteries (i.e. aorta and epicardial coronary arteries), while EDHF plays an important role in modulating vascular tone in small, resistance arteries in vitro [21,22] and in human forearm microcirculation in vivo [23]. EDHF causes vascular relaxation by opening K channels and then hyperpolarizes membrane of vascular smooth muscle [1,2,22]. EDHF is synthesized not only upon stimulation by agonists but also by shear stress [24] and its synthesis and release are stimulated by increase in intracellular calcium in the endothelium [2,25], although calcium-independent endothelial cell hyperpolarization has also been reported [26]. Although NO and vasodilator prostaglandins elicit hyperpolarization of underlying vascular smooth muscle and NO may activate BK_{Ca} channels in some blood vessels [27], those responses to NO and vasodilator prostaglandins are largely inhibited by the inhibition of ATP-sensitive potassium (K_{ATP}) channels [2]. Importantly, substantial endothelium-dependent hyperpolarization exists even after the blockade of the synthesis of NO and vasodilator prostaglandins [2]. Thus, EDHF is apparently different from vasodilator prostaglandins or NO, and EDHF-mediated responses are classically defined as the endothelium-dependent responses (relaxations and hyperpolarizations) after the blockade of the synthesis of vasodilator prostaglandins and NO [2,25].

2.2. Vasodilating effect of reactive oxygen species (ROS)

Both NO- and EDHF-mediated responses are attenuated by various atherosclerotic risk factors [1,28], and the treatment of those risk factors improve both NO- and EDHF-mediated responses [1,29]. In various pathological situations, the production of ROS is increased while NO-mediated relaxations are attenuated. EDHF-mediated relaxations are temporarily enhanced to compensate the reduced NO-mediated relaxations, however, the EDHF-mediated responses also are subsequently reduced during the pathological process [1]. The endothelial synthesis of NO via eNOS activation is calcium/calmodulin-dependent and a similar requirement for calcium/calmodulin has been described for the EDHF-mediated response in the canine coronary artery [30]. These lines of evidence led us to hypothesize that EDHF is a non-NO vasodilator substance (possibly ROS) mainly derived from endothelial NO synthase (eNOS) [11].

The endothelium produces several kinds of ROS, including superoxide, hydrogen peroxide (H_2O_2), NO, peroxynitrite, and hydroxyl radicals [31,32]. ROS modulate vascular tone by several mechanisms, including alteration in K channels conductance [33]. Superoxide itself attenuates endothelium-dependent relaxations by scavenging NO [34], while it also causes relaxations of cat cerebral arteries [35]. Peroxynitrite, a potent ROS produced by the reaction of NO with superoxide, acts as a relaxing factor at its lower concentrations by activating sarco/endoplasmic reticulum Ca-ATPase (SERCA) in rabbit carotid arteries [36] and by activating cGMP pathway in canine coronary arteries [37]. Peroxynitrite also inhibits K_{Ca} channels activity of vascular smooth muscle [38]. H_2O_2 exerts a direct hyperpolarizing effect on vascular smooth muscle [39]. H_2O_2 elicits hyperpolarization of porcine coronary microvessels by opening large conductance K_{Ca} channels [40] and relaxes bovine pulmonary arteries by activating guanylate cyclase [41]. We examined each ROS as a possible candidate for EDHF and demonstrated that endothelium-derived H_2O_2 is an EDHF [11].

2.3. Vasodilator effect of H_2O_2

H_2O_2 has been reported to cause vasodilatation by several mechanisms, including cGMP in bovine pulmonary arteries [41], cyclooxygenase and cyclic AMP in canine cerebral arteries [42], and phospholipase A_2 in porcine coronary microvessels [43]. Exogenous H_2O_2 also causes vasodilatation by opening several K channels, including K_{ATP} channels in cat cerebral [35] and rabbit mesenteric arteries [44] and K_{Ca} channels in rat cerebral arteries [45]. It has been reported that H_2O_2 is a relaxing factor distinct from EDHF in rabbit femoral arteries [46] and that H_2O_2 stimulates the release of a chemically distinct EDHF in human submucosal intestinal microvessels [47]. H_2O_2 has also been reported to induce endothelium-dependent vasodilation through COX-1-mediated release of PGE_2 and to directly relax smooth muscle by hyperpolarization through K_{Ca} channel activation [48].

3. Identification of endothelium-derived H_2O_2 as an EDHF

We have demonstrated that EDHF-mediated relaxations and hyperpolarizations are inhibited by catalase, a specific inhibitor of H_2O_2, in mesenteric arteries of normal mice (Fig. 2) and are significantly reduced in eNOS$^{-/-}$ mice [11]. The specific inhibitory effect of catalase on H_2O_2 was confirmed as it lost its inhibitory effect when inactivated by aminotriazole [11]. We also have demonstrated that exogenous H_2O_2 elicits relaxations and hyperpolarizations of vascular smooth muscle of mouse mesenteric arteries by opening K_{Ca} channels and that acetylcholine causes endothelial H_2O_2 production in mouse mesenteric arteries [11]. Thus, we confirmed that H_2O_2 fulfills the criteria for an EDHF in mouse mesenteric arteries [11] (Fig. 2). We subsequently confirmed that H_2O_2 is an EDHF in human mesenteric arteries [12] and porcine [13] and canine [14] coronary microvessels (Fig. 2). We were able to directly demonstrate endothelial H_2O_2 production in porcine coronary microvessels by using electron spin resonance imaging [13]. The estimated concentration of endothelium-derived H_2O_2 is in micro molar order, which is consistent to its concentrations to cause EDHF-mediated responses [11,13]. Subsequently, other investigators reported that endothelium-derived H_2O_2 also is an EDHF in human coronary microvessels [15] and piglet pial arterioles [16], although other mechanisms for endothelium-dependent and endothelium-independent relaxation in response to H_2O_2 have been reported [46–48]. We also have recently demonstrated that endothelium-derived H_2O_2 plays an important cardioprotective role in coronary autoregulation [14] and myocardial ischemia/reperfusion injury in dogs in vivo [49]. Thus, we

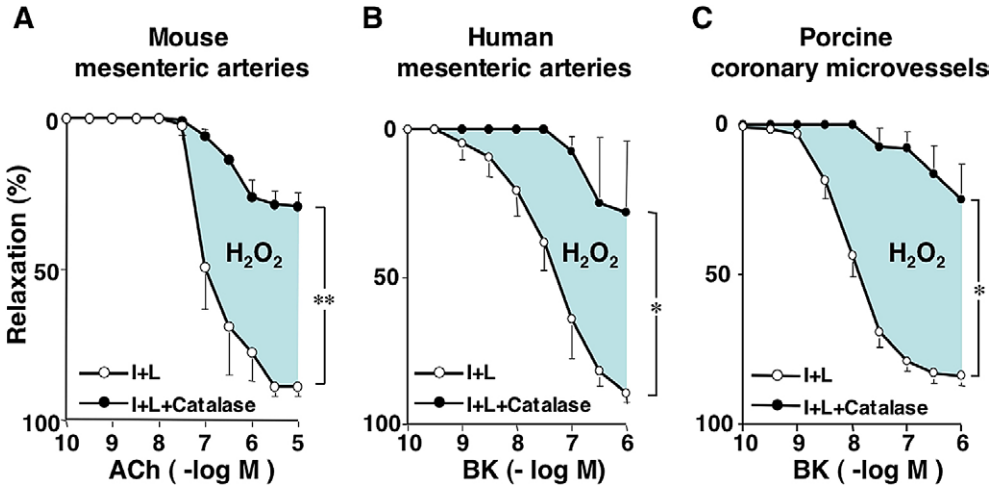

Fig. 2. Endothelium-derived H_2O_2 as an EDHF. EDHF-mediated relaxations in the presence of indomethacin and L-NNA are significantly attenuated by pretreatment with catalase, a specific scavenger of H_2O_2, in mouse (A, $N = 5$, Ref. [12]) and human (B, $N = 4$, Ref. [13]) mesenteric arteries and in porcine coronary microvessels (C, $N = 5$, Ref. [14]). I; indomethacin, L; L-NNA, ACh; acetylcholine, BK; bradykinin. * $P < 0.05$ ** $P < 0.01$ (Modified from Refs. [12–14] with permissions from American Society for Clinical Investigation (A), Elsevier (B) and American Heart Association, Inc. (C)).

have confirmed that endothelium-derived H_2O_2 acts as an EDHF, especially in microvessels [25], although the mechanisms for the endothelial production of H_2O_2/EDHF remains to be elucidated.

4. Mechanisms for endothelial synthesis of H_2O_2/EDHF

Several previous studies have addressed the mechanisms for the synthesis of EDHF (Fig. 1). In porcine epicardial coronary arteries, cytochrome P450 2C has been reported to act as an EDHF synthase to synthesize EETs as an EDHF [5]. cAMP is reported to enhance gap junctional electrical communication for EDHF-mediated responses [50]. It also is reported that agonist stimulation opens endothelial K_{Ca} channels with resultant release of K ion into myoendothelial space as an EDHF [8].

In the endothelium, H_2O_2 is synthesized by either spontaneous dismutation from superoxide or dismutation of superoxide by SOD [51]. In blood vessels, there are three SOD isoforms that dismutate superoxide into H_2O_2 [51] (Fig. 3). Cu,Zn-SOD (SOD1) is located mainly in cytosol, nucleus, and, to a lesser extent, in mitochondria [52], and dismutates superoxide derived from eNOS and prolongs the half life of NO [53] (Fig. 3). The Cu,Zn-SOD activity approximately accounts for 50–80% of all SOD activities in vascular wall [54]. Mn-SOD (SOD2) is located in mitochondria and dismutates superoxide derived from respiratory chains [51] (Fig. 3). Extracellular-SOD (ecSOD, SOD3) is located extracellularly and dismutates extracellular superoxide to protect the diffusion of NO [55] (Fig. 3). As mentioned above, we have demonstrated that eNOS is one of the major contributor for the synthesis of H_2O_2 as an EDHF [11]. eNOS produces superoxide when it synthesizes NO from L-arginine, while Cu,Zn-SOD dismutates those superoxide anions into H_2O_2 [56]. Since heparin, which inhibits ecSOD activities, had no effect on EDHF-mediate response, we excluded ecSOD as a source of EDHF [17]. Mn-SOD is located in mitochondria and does not seem to be involved in EDHF synthesis because in mouse mesenteric arteries, endothelium-derived H_2O_2 is mainly derived from membrane, where Cu,Zn-SOD is located [17] (Fig. 3). Therefore, we hypothesized that endothelial Cu,Zn-SOD plays an important role for the synthesis of H_2O_2 as an EDHF synthase [17].

In Cu,Zn-SOD$^{-/-}$ mice, EDHF-mediated relaxations and hyperpolarizations are markedly attenuated in mesenteric arteries and coronary microvessels compared with control mice without alteration in vasodilator properties of vascular smooth muscle [17]. In addition, supplement of SOD mimetics, Tempol [57], restores EDHF-mediated responses [17]. These results may reflect the restoration of contribution of H_2O_2 as an EDHF and/or improved myoendothelial communication as a result of a reduction in ROS generation [58]. Similarly, in human mesenteric arteries, supplement of another SOD mimetics, Tiron [59], enhances EDHF-mediated relaxations and hyperpolarizations [18]. H_2O_2 also is an EDHF in porcine coronary microvessels, where the EDHF-mediated relaxations are enhanced by the pretreatment with Tiron [13]. In human isolated coronary arterioles, it was suggested that H_2O_2 derived from mitochondria is involved in flow-mediated dilatation [60].

Abnormalities of Cu,Zn-SOD are known to be involved in various pathological conditions. In Cu,Zn-SOD$^{-/-}$ mice, endothelium-dependent relaxations of carotid artery is attenuated [54]. In those mice, the size of myocardial infarction after coronary ligation is larger than in normal mice [61] and cerebral injury after transient global ischemia is enhanced [62]. When maintained on Cu-deficient diet, rats have reduced Cu,Zn-SOD activity and NO-mediated relaxations [63]. By contrast, overexpression of Cu,Zn-SOD protects cerebral

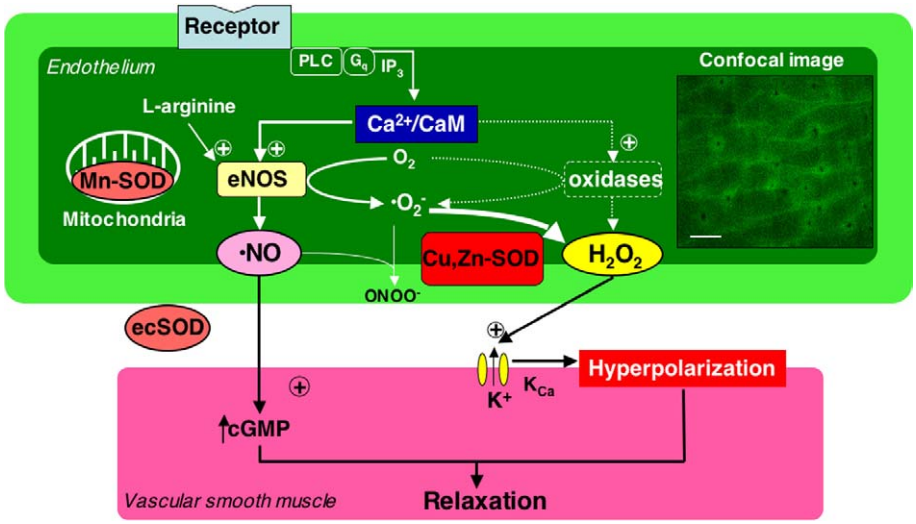

Fig. 3. SOD isoforms in the endothelium. In the endothelium, three SOD isoforms are known to be present, including Cu,Zn-SOD, Mn-SOD and ecSOD. Cu,Zn-SOD is located mainly in cytosol and membrane, and is known to dismutate superoxide derived from eNOS and other oxidases and prolongs the half life of NO. Mn-SOD is located in mitochondria and dismutates superoxide derived from respiratory chains. ecSOD is located extracellularly. H_2O_2, which is dismutated from superoxide anions by Cu,Zn-SOD, acts as an EDHF.

injury after transient ischemia [64]. Cu,Zn-SOD mutation is observed in patients with familial amyotrophic lateral sclerosis and Parkinson's disease [65]. Mn-SOD$^{-/-}$ mice are embryo-lethal, and develop dilated cardiomyopathy and neurodegeneration [66,67]. ecSOD is upregulated by exercise training [68], and gene transfer of ecSOD reduces cerebral vasospasm after subarachnoid hemorrhage [69] and decreases arterial blood pressure in spontaneously hypertensive rats (SHR) [70]. Thus, each SOD isoform has its important physiological role. Our H_2O_2/EDHF theory has uncovered the new and important role of endothelial Cu,Zn-SOD as an EDHF synthase, in addition to its classical role to scavenge superoxide to prolong the half life of NO [17].

5. Clinical implications

5.1. Influence of atherosclerotic risk factors on EDHF-mediated responses

Atherosclerotic risk factors, such as hypertension, diabetes mellitus, hyperlipidemia, smoking, and aging, cause endothelial dysfunction [1]. Especially, NO-mediated relaxations are prone to be attenuated by various risk factors [1]. Importantly, EDHF-mediated relaxations also are attenuated by various risk factors [28,29]. Indeed, in mesenteric arteries of SHR [71] and those of streptopzotocin-induced diabetic rats, EDHF-mediated responses are attenuated [29,72]. EDHF-mediated responses also are attenuated with aging and hyperlipidemia in human mesenteric arteries [28]. Acetylcholine-induced relaxations and hyperpolarizations are attenuated in mesenteric arteries of ovariectomized female rats [73]. On the other hand, EDHF-mediated relaxations were enhanced in cerebral circulation of ovariectomized animals [74,75]. In apolipoprotein E-deficient (ApoE$^{-/-}$) mice, EDHF-mediated responses of mesenteric arteries are fairly preserved, whereas in streptozotocin-induced diabetic mice, the responses are significantly attenuated [76]. In streptozotocin-induced diabetic ApoE$^{-/-}$ mice, EDHF-mediated responses are markedly reduced while NO-mediated relaxations are rather enhanced [76]. Thus, the combination of diabetes mellitus and hyperlipidemia significantly attenuates EDHF-mediated responses [76] (Fig. 4). The detailed mechanisms for the reduced EDHF-mediated responses, including eNOS and Cu,Zn-SOD function, remain to be examined in future studies.

5.2. Treatment of impaired EDHF-mediated responses

Several treatments are known to improve endothelial dysfunction, including impaired EDHF-mediated responses [1,25,29]. Estrogen replacement therapy significantly improves EDHF-mediated responses in ovariectomized female rats [77]. Estrogen also has an acute enhancing effect on both NO-mediated and EDHF-mediated responses of forearm circulation of postmenopausal women [78]. In mesenteric arteries of SHR rats, reduced EDHF-mediated responses are significantly ameliorated by long-term administration of ACE inhibitors [71]. Recently, we have demonstrated that this enhancing effect of ACE inhibitors on EDHF-mediated responses are indeed mediated by endothelium-derived H_2O_2 as an EDHF [79]. In diabetic rats, insulin therapy significantly ameliorates EDHF-mediated responses [72]. Long-term administration of eicosapentaenoic acid (EPA), a major component of fish oil, improves both NO-mediated and EDHF-mediated relaxations in humans [80,81]. Nifedipine, a Ca^{2+} channel blocker, improves EDHF-mediated responses

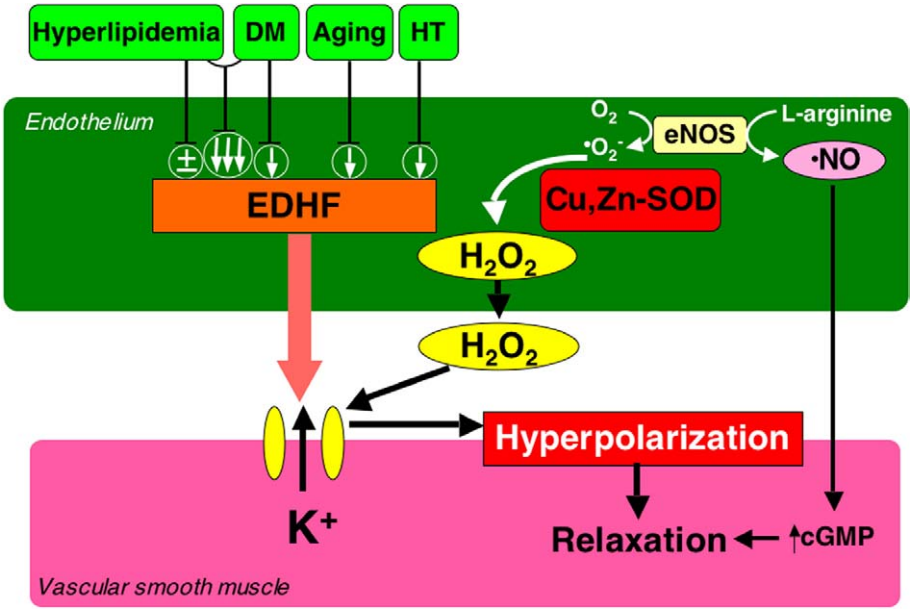

Fig. 4. Identification, mechanisms of synthesis, and influence of risk factors of EDHF. Endothelium-derived H_2O_2 dismutated from superoxide by Cu,Zn-SOD acts as an EDHF in animals and humans. Atherosclerotic risk factors significantly attenuate EDHF-mediated responses. The mechanisms involved in the reduced EDHF-mediated responses remain to be examined in detail in future studies.

in porcine coronary arteries [82]. Exercise training also significantly enhances both NO-mediated and EDHF-mediated responses [83,84]. Thus, several drugs and life-style modification (e.g. dietary EPA, exercise) improve EDHF-mediated responses, contributing to the maintenance of vascular homeostasis.

6. Conclusion

Endothelium-derived H_2O_2 is an EDHF in animals and humans, and endothelial Cu,Zn-SOD plays an important role for the synthesis of H_2O_2 as an EDHF (Fig. 4). Several risk factors significantly attenuate EDHF-mediated responses, while their combination markedly attenuate the responses (Fig. 4). Several drugs (e.g. ACE inhibitors and estrogen) and life-style modification (e.g. dietary EPA and exercise) effectively improve EDHF-mediated responses (Fig. 4).

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