Chapter 6
Pathophysiology of Coronary Microvascular Dysfunction

Shigeo Godo and Hiroaki Shimokawa

Abstract Coronary microvascular dysfunction (CMD) has been implicated in a wide spectrum of cardiovascular disease. The underlying mechanisms of CMD appear to be heterogeneous, including several structural and functional alterations. Among them, central to coronary vasomotion abnormalities are threefold: enhanced coronary vasoconstrictive reactivity (i.e. coronary spasm) at epicardial and microvascular levels, reduced endothelium-dependent and -independent coronary vasodilator capacity, and increased coronary microvascular resistance, all of which can cause myocardial ischemia due to CMD and often coexist in various combinations even in the absence of obstructive coronary artery disease. The endothelium plays essential roles in modulating vascular tone by synthesizing and releasing endothelium-derived relaxing factors, including vasodilator prostaglandins, nitric oxide (NO), and endothelium-dependent hyperpolarization (EDH) factors in a distinct vessel size–dependent manner; NO mainly mediates vasodilatation of relatively large, conduit vessels (e.g. epicardial coronary arteries), while EDH factors in small resistance vessels (e.g. coronary microvessels). Endothelium-derived hydrogen peroxide (H_2O_2) is a physiological signaling molecule serving as one of the major EDH factors especially in coronary microcirculation and has gained increasing attention in view of its emerging relevance for cardiovascular disease. In this chapter, we will briefly summarize the latest knowledge on the pathophysiology of CMD with a special reference to endothelial modulation of vascular tone mediated by H_2O_2/EDH factor and coronary microvascular spasm, in addition to discussing clinical implications of and therapeutic approaches to CMD in cardiovascular disease.

Keywords Coronary microvascular dysfunction · Endothelial function · Endothelium-dependent hyperpolarization · Nitric oxide · Hydrogen peroxide

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Abbreviations

CAD	Coronary artery disease
cGMP	Cyclic guanosine monophosphate
CMD	Coronary microvascular dysfunction
EDH	Endothelium-dependent hyperpolarization
EDRF(s)	Endothelium-derived relaxing factor(s)
EETs	Epoxyeicosatrienoic acids
eNOS	Endothelial nitric oxide synthase
H_2O_2	Hydrogen peroxide
HFpEF	Heart failure with preserved ejection fraction
IHD	Ischemic heart disease
INOCA	Ischemia and no obstructive coronary artery disease
NO	Nitric oxide
NOS	Nitric oxide synthase
PGs	Prostaglandins
PKG	cGMP-dependent protein kinase
ROS	Reactive oxygen species
sGC	Soluble guanylate cyclase
SOD	Superoxide dismutase
VSMC	Vascular smooth muscle cells

6.1 Introduction

A growing body of evidence has demonstrated that coronary microvascular dysfunction (CMD) plays important roles in the pathophysiology of cardiac ischemia in patients with a wide spectrum of cardiovascular disorders, including ischemic heart disease (IHD) [1, 2], aortic stenosis [3], and heart failure with preserved ejection fraction (HFpEF) [4–6]. More than 50% of patients undergoing invasive coronary angiography for the evaluation of suspected obstructive coronary artery disease (CAD) have no significant coronary artery stenosis [7], where the role of CMD has been recognized as an alternative mechanism for symptoms and signs of myocardial ischemia. Indeed, recent studies using comprehensive assessment of coronary physiology by multimodality protocols have unveiled that a substantial proportion of patients with ischemia and no obstructive coronary artery disease (INOCA) differ in the underlying coronary microvascular physiology [8–11]. Mechanistically, structural and functional abnormalities of "epicardial" coronary arteries in patients with IHD are the focus of previous studies; however, those of coronary microvasculature, referred to as CMD, have attracted much attention in view of their unexpectedly high prevalence in and significant prognostic impact on this population in many clinical settings [12–14]. The etiologies of CMD

appear to be heterogeneous; several structural (e.g. luminal obstruction, vascular remodeling, vascular rarefaction, and extramural compression) and functional alterations (e.g. endothelial dysfunction, vascular smooth muscle cells [VSMC] dysfunction, and microvascular spasm) have been proposed for the pathophysiological mechanisms of CMD [15–19]. Among them, central to coronary vasomotion abnormalities [1, 20] are enhanced coronary vasoconstrictive reactivity (i.e. coronary spasm) not only at epicardial but also at microvascular levels, reduced endothelium-dependent and -independent coronary vasodilator capacity (e.g. coronary flow reserve [CFR] <2.0), and increased coronary microvascular resistance (e.g. index of microvascular resistance [IMR] >25), all of which can cause myocardial ischemia due to CMD even in the absence of obstructive CAD and often coexist in various combinations in patients with angina and non-obstructive CAD [8, 10, 11].

In this chapter, we will briefly summarize the current knowledge on the pathophysiology of CMD with a special reference to endothelial modulation of vascular tone and coronary microvascular spasm, in addition to briefly discussing clinical implications of and therapeutic approaches to CMD in cardiovascular disease. Further discussions on the coronary microcirculation physiology are available elsewhere [15–19, 21, 22].

6.2 Endothelial Modulation of Vascular Tone: NO and EDH Factors

6.2.1 Vessel Size–Dependent Contribution of Endothelium-Derived Relaxing Factors

The endothelium plays pivotal roles in modulating the tone of underlying VSMC by synthesizing and releasing endothelium-derived relaxing factors (EDRFs) in an autocrine and paracrine manner, including vasodilator prostaglandins (PGs) (e.g. prostacyclin), nitric oxide (NO), and endothelium-dependent hyperpolarization (EDH) factors, as well as endothelium-derived contracting factors [1, 23, 24] (Fig. 6.1). Endothelial dysfunction is characterized by reduced production and/or action of EDRFs, serving as the hallmark of atherosclerotic cardiovascular diseases as well as one of the major pathogenetic mechanisms of CMD [15–18]. Of note is that these EDRFs regulate vascular tone in a distinct vessel size–dependent fashion [25, 26] (Fig. 6.1); endothelium-derived NO mainly mediates vasodilatation of relatively large, conduit vessels (e.g. epicardial coronary arteries), while EDH factors-mediated responses are the predominant mechanisms of endothelium-dependent vasodilatation of resistance arteries (e.g. coronary microvessels). By contrast, vasodilator PGs play a small but constant role in general, independent of vessel size. This vessel-size-dependent contribution of NO and EDH factors in endothelium-dependent vasodilatation is well preserved from
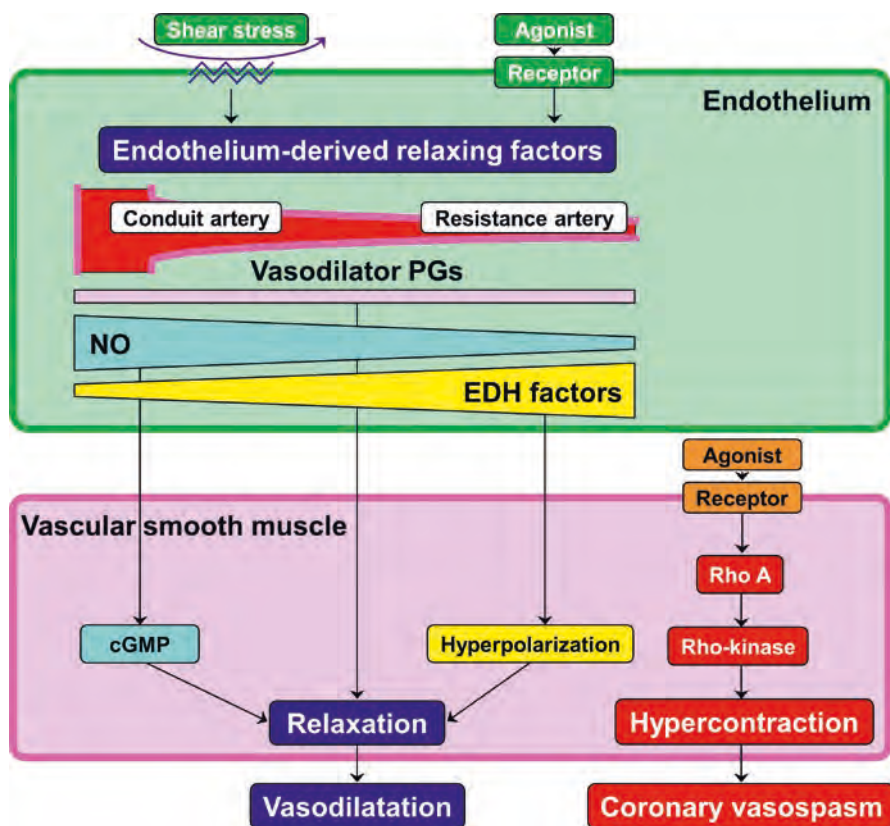
Fig. 6.1 Vessel size–dependent contribution of endothelium-derived relaxing factors and Rho-kinase-mediated vascular smooth muscle hypercontraction. *cGMP* cyclic guanosine monophosphate, *EDH* endothelium-dependent hyperpolarization, *NO* nitric oxide, *PGs* prostaglandins. (Reproduced from Shimokawa and Godo [24])

rodents to humans, shaping a physiological balance between them [1, 23]. Thus, EDH factors-mediated vasodilatation is a vital mechanism especially in microcirculations, where blood pressure and organ 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 impaired, favoring a vasoconstrictor and proinflammatory state. Indeed, in various pathological conditions with atherosclerotic risk factors, NO-mediated relaxations are easily compromised, while EDH factors-mediated responses are fairly preserved or even enhanced to serve as a compensatory vasodilator system [26, 27]. Multiple mechanisms are involved in the enhanced EDH factors-mediated responses in small resistance vessels, including negative interactions between NO and several EDH factors, as discussed later. The regulatory mechanisms of NO-mediated responses are extensively reviewed elsewhere [28–30].

6.2.2 EDH Factors: The Predominant Mechanism of Vasodilatation in Small Arteries

In 1998, Feletou and Vanhoutte [31] and Chen et al. [32] independently demonstrated the existence of endothelium-derived non-NO, non-prostanoid relaxing factors, unforeseen EDH factors. EDH factors-mediated responses are the major mechanism of endothelium-dependent vasodilatations in resistance arteries, although, by definition, the contribution of EDH factors is determined only after the blockade of both vasodilator PGs and NO. EDH factors cause hyperpolarization and subsequent relaxation of underlying VSMC with resultant vasodilatation of small resistance vessels and thus finely regulate blood pressure and organ perfusion instantaneously in response to diverse physiological demands [23, 33]. The nature of EDH factors varies depending on the vascular bed, vessel size, and species of interest, including epoxyeicosatrienoic acids (EETs), metabolites of arachidonic P450 epoxygenase pathway [34, 35], electrical communication through gap junctions [36], K^+ ions [37], and as we demonstrated, endothelium-derived hydrogen peroxide (H_2O_2) [24, 38] (Fig. 6.2). EETs
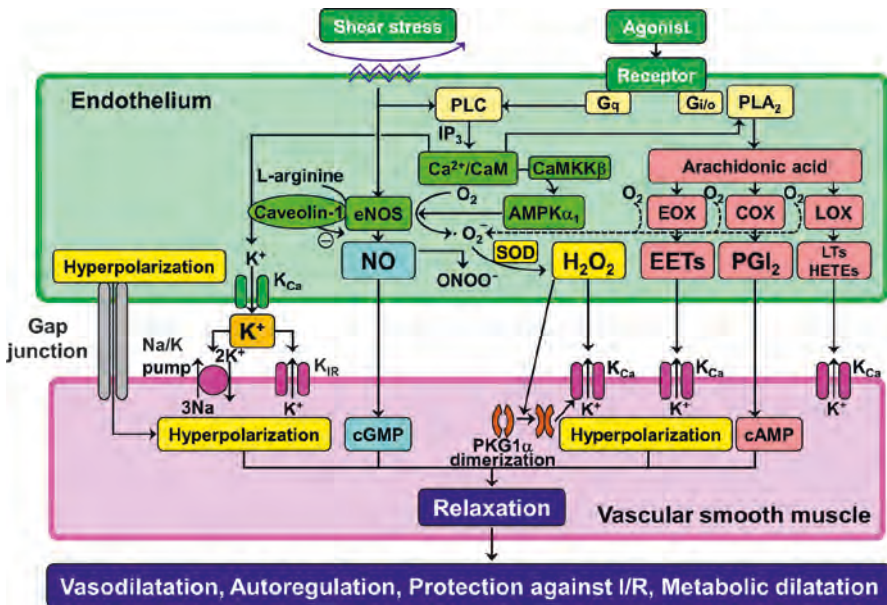

Fig. 6.2 Molecular mechanisms of endothelial modulation of vascular tone. *AMPKα1* α1-subunit of AMP-activated protein kinase, *CaM* calmodulin, *CaMKKβ* Ca^{2+}/CaM-dependent protein kinase β, *cAMP* cyclic AMP, *cGMP* cyclic GMP, *COX* cyclooxygenase, *EETs* epoxyeicosatrienoic acids, *eNOS* endothelial NO synthase, *EOX* epoxygenase, *HETEs* hydroxyeicosatetraenoic acids, *H_2O_2* hydrogen peroxide, *IP_3* inositol trisphosphate, *I/R* ischemia-reperfusion injury, *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. (Reproduced from Shimokawa and Godo [24])

mainly participate in EDH-mediated relaxations in bovine [34], porcine [35], and human coronary arteries [39]; K$^+$ ions in rat hepatic and mesenteric arteries [37, 40], porcine [41] and bovine [42] coronary arteries, and human kidney interlobar arteries [43]; and H$_2$O$_2$, at physiologically low concentrations, in human [44], porcine [45], and canine coronary arteries [46–48].

Coronary vascular resistance is predominantly determined by the pre-arterioles (>100 μm in diameter) and arterioles (<100 μm) where EDH factors-mediated responses become more prominent than NO-mediated relaxations. Given that H$_2$O$_2$ has potent vasodilator properties in coronary resistance vessels, impaired H$_2$O$_2$-mediated vasodilatation may lead to CMD. In the next section, we will focus on endothelium-derived H$_2$O$_2$ as an EDH factor in detail. Readers are encouraged to refer to an excellent textbook for more comprehensive information on the role of other EDH factors [49].

6.3 Endothelium-Derived H$_2$O$_2$ as an EDH Factor

6.3.1 Identification of H$_2$O$_2$/EDH Factor

Reactive oxygen species (ROS) have been considered to be primarily harmful because of their detrimental property to cells and tissues and pathological implications in various cardiovascular diseases including CMD [50]. However, as exemplified by endothelium-derived H$_2$O$_2$/EDH factor, many studies have demonstrated that physiological levels of ROS can serve as crucial signaling molecules in health and disease [51] and have acknowledged H$_2$O$_2$ as a physiological signaling molecule, regulating blood pressure [52], metabolic functions [53, 54], and coronary microcirculation [46–48].

Following the original reports on the existence of EDH factors in 1988 [31, 32], we hypothesized that a putative EDH factor might be a non-NO vasodilator substance (likely ROS) derived from endothelial NO synthases (NOSs) system, based on a hint from several early observations and notions. First, both NO-mediated and EDH-mediated responses are susceptible to vascular injuries caused by atherosclerotic risk factors, and inversely, the treatment of those risk factors can restore both responses [1, 26]. Second, it had been previously demonstrated that endothelium-derived free radicals exert endothelium-dependent vasodilator and vasoconstrictor effects in canine coronary arteries [55]. Third, both endothelial NOS (eNOS)-derived NO generation and EDH-mediated responses are dependent on calcium/calmodulin [56]. Fourth, a simple molecule (like NO), rather than complex substances, may be opportune for modulating vascular tone instantaneously in response to various physiological demands in the body. On the basis of these notions, in 2000, we demonstrated for the first time that endothelium-derived H$_2$O$_2$ is an EDH factor in mouse mesenteric arteries; EDH-mediated hyperpolarizations and relaxations of underlying VSMC were inhibited by catalase, a specific H$_2$O$_2$ inhibitor, in

small mesenteric arteries from wild-type mice and were significantly reduced in eNOS-knockout (KO) mice [38]. This was also true for other vascular beds, including human mesenteric [57] and coronary [58] arteries, porcine [45] and canine [46–48] coronary arteries, and piglet pial arterioles [59]. Notably, the estimated concentrations of endothelium-derived H_2O_2/EDH factor are in micromolar order (<50 μmol/L) [45, 47], which are much lower concentrations than those observed in various pathological conditions [60]. When applied in organ chamber experiments, approximately 10–100 μmol/L of exogenous H_2O_2 elicits vasodilatation of human coronary arterioles [58, 61] and mouse small mesenteric arteries [38, 62, 63], while higher concentrations of H_2O_2 rather induce vasoconstriction by releasing cyclooxygenase-derived thromboxane [64]. Here, only 10–15% of H_2O_2 applied exogenously reaches the intracellular targets due to endogenous antioxidants and membrane impedance [65].

6.3.2   Source of H_2O_2/EDH Factor

Endothelium-derived H_2O_2 is mainly produced 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 (Fig. 6.2) [60]. 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 (i.e. L-arginine analogs) and upregulation of eNOS co-factor tetrahydrobiopterin has no effects on the responses [66]. 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. Cu,Zn-SOD-KO mice show markedly impaired EDH-mediated hyperpolarizations and relaxations in mesenteric arteries and coronary circulation without VSMC dysfunction [67]. Other sources of superoxide anions in H_2O_2-mediated vasodilatation have been identified in human coronary arterioles, including mitochondrial respiratory chain in flow-mediated dilatation [68] and NADPH oxidase in bradykinin-induced relaxation [69].

6.3.3   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 [51]. 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 signaling and that co-localization of the source and target of H_2O_2

may help to avoid non-specific harmful oxidations [70, 71]. In addition, specific cysteine residues, such as peroxiredoxins, can function as a redox-dependent molecular switch to regulate ROS-mediated signaling [60]. Moreover, a novel mechanism of CMD in human CAD has been proposed [22, 72, 73]. As mentioned above, healthy human coronary circulation is regulated by NO and low physiological levels of H_2O_2/EDH factor. However, various atherosclerotic risk factors (e.g. aging, hypertension, obesity, and smoking) can cause a switch from NO to H_2O_2 in the mediator of endothelium-dependent vasodilatation in human coronary arteries. The resultant impaired production of NO and pathologically elevated levels of H_2O_2 manifest as CMD that favors a vasoconstrictor and pro-inflammatory state, leading to the development of coronary atherosclerosis [22, 72, 73].

6.3.4 Mode of Action of H_2O_2/EDH Factor

Among several modes of action of H_2O_2/EDH factor [74, 75], oxidative modification of cGMP-dependent protein kinase (PKG) plays a central role in H_2O_2-induced hyperpolarization and relaxation of underlying VSMC [52, 76] (Fig. 6.2). Briefly, H_2O_2 induces dimerization of 1α-isoforms of PKG (PKG1α) through an interprotein disulfide 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 [52] and human coronary arterioles [58, 61]. H_2O_2 also promotes the translocation of PKG1α from cytoplasm to membrane in human [61] and porcine [77] coronary arteries. Such reversible post-translational modification, like phosphorylation, is advantageous for the fine control of vascular tone in response to various demand fluctuation in vivo [30].

6.3.5 Clinical Significance of H_2O_2/EDH Factor

The oxidant-mediated signaling by H_2O_2 is of clinical importance because it is associated with blood pressure control in vivo [52]. Pharmacological inhibition of catalase decreases arterial blood pressure in association with enhanced PKG1α dimerization in vivo [77]. 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 sulfur, exhibit markedly impaired EDH-mediated hyperpolarization and relaxation in resistance arteries ex vivo associated with systemic arterial hypertension [52]. Furthermore, physiological levels of H_2O_2 have potent vasodilator properties in coronary resistance vessels, contributing to coronary autoregulation [46], cardioprotection against myocardial ischemia-reperfusion injury [47], and tachycardia-induced metabolic coronary vasodilatations [48] in dogs in vivo. Given that coronary vascular

resistance is predominantly determined by the prearterioles and arterioles [17] where the effect of EDH-mediated responses on vascular tone is superior to that of NO-mediated relaxations, it is important to maintain the vessel size–dependent contribution of NO and EDH factors for the treatment of CMD. 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 [23, 74]. 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 [78–82] and that antioxidant treatments are disappointingly ineffective to prevent cardiovascular events [83]. These lines of evidence suggest the importance of the physiological balance between NO and H_2O_2/EDH factor in maintaining cardiovascular homeostasis and in curing diseases associated with endothelial dysfunction.

6.4 Mechanisms of Enhanced H_2O_2/EDH Factor in Microcirculation

6.4.1 Diverse Roles of Endothelial NOSs System

Endothelium-derived NO and EDH factors share the roles in modulating vascular tone in a distinct vessel size–dependent manner through the diverse roles of endothelial NOSs system (Fig. 6.2). In large conduit vessels, NOSs mainly act as a NO-generating system to cause soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP)-mediated vasodilatation, whereas in small resistance vessels, they serve as a superoxide-generating system to evoke H_2O_2/EDH factor-mediated responses [84]. Among three NOS isoforms (neural NOS [nNOS, NOS1], inducible NOS [iNOS, NOS2], and eNOS, NOS3) expressed in the cardiovascular system, eNOS is the dominant isoform in blood vessels [85] and the most important isoform in generating H_2O_2/EDH factor in the endothelium [86]. As mentioned above, genetic ablation of eNOS in mice results in impaired EDH-mediated vasodilatation associated with systemic hypertension [87]. 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 [84]. 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 [84]. The remaining EDH-mediated relaxation of small mesenteric arteries in eNOS-KO mice is still sensitive to catalase [38]. Collectively, these results indicate that three NOSs isoforms compensate each other to maintain H_2O_2-mediated EDH-type relaxations.

6.4.2   Mechanisms for H₂O₂/EDH Factor Dominance in Coronary Microcirculation

Accumulating evidence has provided mechanistic insights into vessel size–dependent contribution of NO and H_2O_2/EDH factor in coronary microcirculation. Pretreatment with NO donors attenuates EDH-mediated vasodilatation in porcine coronary arteries in vitro [88] and canine coronary microcirculation in vivo [89] and NO exerts a negative-feedback effect on endothelium-dependent vasodilatation through cGMP-mediated desensitization in canine coronary arteries ex vivo [90]. Mechanistically, 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 conversely, pharmacological inhibition of sGC sensitizes conduit vessels, but not resistance vessels, to H_2O_2-induced vasodilatation in mice [91]. 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 [91]. Other key players for enhanced H_2O_2/EDH factor-mediated vasodilatation in coronary microcirculation include endothelial caveolin-1, a negative regulator of eNOS [62, 92], and α1-subunit of endothelial AMP-activated protein kinase [93]. Taken together, these mechanisms are compatible with the widely held 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 in endothelium-dependent vasodilatation, compromising coronary flow reserve in mice in vivo [63, 92].

6.5   Coronary Microvascular Spasm

Besides endothelial dysfunction, CMD can be caused by endothelium-independent mechanisms in general, which encompass impaired coronary microvascular dilatation and enhanced coronary microvascular constriction. Coronary artery spasms at both epicardial and microvascular levels have been implicated in a wide variety of IHD [1]. Mechanistically, Rho-kinase-induced myosin light chain phosphorylation with resultant VSMC hypercontraction is the central mechanism in the pathogenesis of coronary artery spasm at epicardial [94, 95] as well as at microvascular [96] levels, whereas the role of endothelial dysfunction may be minimal (Fig. 6.1) [1, 20]. Intracoronary administration of a Rho-kinase inhibitor, fasudil, is effective not only for relieving refractory coronary spasm resistant to nitrates or calcium-channel blockers but also for suppressing coronary microvascular spasm in most patients with the disorder [97]. In addition, enhanced epicardial and coronary microvascular

spasms are associated with increased production of other vasoconstrictive mediators, such as endothelin [98] and serotonin [99] in patients with CMD.

Intracoronary acetylcholine (ACh) provocation test is useful in inducing coronary artery spasm with high sensitivity and specificity in the cardiac catheter laboratory [100]. A high prevalence of ACh-induced coronary microvascular spasm has been reported in one-third of patients with stable chest pain and non-obstructive CAD [101, 102]. The Coronary Vasomotion Disorders International Study (COVADIS) Group proposed a consensus set of standardized diagnostic criteria for microvascular angina attributable to CMD, including ACh-induced coronary microvascular spasm [103]. The diagnostic value of these criteria has been demonstrated by a recent randomized clinical trial [104]. More recently, we have demonstrated that increased coronary microvascular resistance as evaluated by IMR is associated with Rho-kinase activation in the pathogenesis of coronary functional abnormalities [11]. Considering that patients with coronary artery spasm are not necessarily associated with conventional coronary risk factors and positive results of non-invasive functional stress tests, comprehensive assessment of coronary physiology using multimodality protocol is of diagnostic value to identify coronary vasomotion abnormalities and to avoid false reassurance in patients with INOCA [10, 11, 100].

6.6 Clinical Implications

6.6.1 Importance of Endothelial Function Tests

Assessment of endothelial function has been acknowledged as an excellent surrogate marker of future cardiovascular events in many clinical settings [105], although it is challenging to specifically assess EDH factors-mediated responses in humans in vivo. The reason for this difficulty is at least twofold: (1) the contribution of EDH factors could be determined only after the blockade of both vasodilator PGs and NO and (2) coronary resistance arteries are not visible on coronary angiography. Endothelial dysfunction is manifested as impaired production and/or action of EDRFs. EDH factors-mediated vasodilation can be temporarily enhanced to compensate for impaired NO-mediated responses in the early stage of atherosclerotic conditions [33, 74]. However, after prolonged exposure to atherosclerotic risk factors, this compensatory role of EDH factors-mediated responses is finally disrupted to cause metabolic disturbance [106]. Endothelial dysfunction, as evaluated by impaired flow-mediated dilation (FMD) of the brachial artery or digital reactive hyperemia index (RHI) in peripheral arterial tonometry, is associated with future cardiovascular events in patients with CAD and one standard deviation decrease in FMD or RHI is associated with doubling of cardiovascular event risk [105]. More recently, peripheral endothelial dysfunction has been shown to be common in patients with coronary vasomotion abnormalities [107, 108].

6.6.2 Role of H₂O₂/EDH Factor in the Pathophysiology of Coronary Artery Disease

Previous studies focused on 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 (e.g. percutaneous coronary intervention). However, those of coronary microvasculature, referred to as CMD, have gained increasing attention as a novel research target in this population [12–14]. It is conceivable that impaired H_2O_2/EDH factor-mediated vasodilatation is involved in the pathogenesis of CMD in light of its potent vasodilator properties in coronary resistance vessels where EDH factors-mediated responses become relatively dominant to NO-mediated relaxations. A good example of this is that CMD caused by impaired H_2O_2/EDH factor is also associated with cardiac diastolic dysfunction in eNOS-KO mice [109]. Thus, it is essential to maintain the physiological balance between NO and H_2O_2/EDH factor for the treatment of CAD, which notion is supported by the fact that significant negative interactions exist between NO and several EDH factors [63, 88–91] and that nitrates as NO donors are not beneficial for the treatment of CMD [78, 80]. 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 INOCA [110, 111]. Shear stress is one of the important physiological cues that make endothelial cells synthesize and release EDRFs to maintain vascular homeostasis, while 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 leukocyte adhesion [110, 111]. Indeed, altered shear stress on the coronary artery wall has been implicated in the local progression of atherosclerotic coronary plaque [112].

6.6.3 Lessons from Clinical Trials Targeting NO: Less Is More?

Although the role of CMD has been implicated in patients with obstructive CAD who underwent successful revascularization [113], the effects of isosorbide-5-mononitrate were unexpectedly neutral in patients with residual microvascular ischemia despite successful percutaneous coronary intervention [82]. Moreover, recent studies highlighted the high prevalence and pathophysiological relevance of CMD in patients with HFpEF [4–6]. 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 neutral or even harmful in randomized clinical trials [79, 81]. Similarly, antioxidant therapies for patients with cardiovascular diseases had no

benefits [83], although multiple mechanisms may be involved in so-called "antioxidant paradox" in clinical trials, including inadequate dose, short treatment duration, and pro-oxidant effects of antioxidants upon supplementation. 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. An alternative explanation for such "paradox" of NO-targeted therapy may be nitrosative stress induced by an excessive amount of supplemental NO [92, 114], again suggesting the importance of physiological balance between NO and EDH factors in endothelium-dependent vasodilatation. Although standard medications used for the treatment of cardiovascular diseases 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.

6.6.4 CMD as Systemic Vascular Dysfunction beyond the Heart

Recent studies have highlighted the importance of CMD with major clinical implications. First, if complicated with CMD, even angina patients who have angiographically normal coronary arteries or non-obstructive CAD are associated with increased future cardiac events, including myocardial infarction, percutaneous or surgical revascularization, cardiac death, and hospitalization for unstable angina [11, 12, 115, 116]. Moreover, the prevalence of CMD in this clinical entity is not negligible [8–11]. Although contemporary non-invasive stress tests have limited diagnostic accuracy for detecting CMD in patients with chest pain and non-obstructive CAD [9, 117], comprehensive invasive assessment of coronary vasomotor reactivity using intracoronary ACh, adenosine, and other vasoactive agents is safe, feasible, and of diagnostic value to identify patients with CMD [8, 9, 100, 104, 118]. Second, CMD is a cardiac manifestation of the systemic small artery disease [107], which supports the novel concept of "primary coronary microcirculatory dysfunction" [119]. Despite the high prevalence of CMD in patients with INOCA, they are often underestimated and offered no specific treatment or follow-up under the umbrella of "normal" coronary arteries. On the contrary to this otherwise common practice, patients with CMD are predisposed to future coronary events and associated worse outcomes [12, 115, 116]. Furthermore, CMD may be attributable to residual cardiac ischemia even after successful revascularization of significant epicardial coronary stenosis [113]. Identifying CMD in patients with stable IHD may provide physicians with useful information for decision making and risk stratification beyond conventional coronary risk factors.

6.7 Conclusions

This chapter highlighted the pathophysiology of CMD with emphasis placed on endothelial modulation of vascular tone mediated by H_2O_2/EDH factor and coronary microvascular spasm. It remains an open question for future research how to improve CMD without affecting the delicate balance between NO and EDH factors. Further characterization and better understanding of CMD are indispensable to this end, which helps us develop novel therapeutic strategies in patients with the disease.

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