Vascular Pharmacology 86 (2016) 31–40

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Review

# The factor in EDHF: Cytochrome P450 derived lipid mediators and vascular signaling

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ARTICLE INFO

*Article history:*
Received 13 November 2015
Received in revised form 20 January 2016
Accepted 6 March 2016
Available online 11 March 2016

*Keywords:*
Arachidonic acid
Epoxyeicosatrienoic acid
Soluble epoxide hydrolase
Hypertension
Angiogenesis

ABSTRACT

Cytochrome P450 (CYP) epoxygenases metabolize arachidonic acid to generate epoxyeicosatrienoic acids (EETs). The latter are biologically active and reported to act as an endothelium-derived hyperpolarizing factor (EDHF) as well as to affect angiogenic and inflammatory signaling pathways. In addition to arachidonic acid, the CYP epoxygenases also metabolize the Ω-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid and docosahexaenoic acid, to generate bioactive lipid epoxide mediators. The latter can be more potent than the EETs but their actions are under investigated. The Ω3-epoxides, like the EETs, are metabolized by the soluble epoxide hydrolase to corresponding diols and epoxide hydrolase inhibition increases epoxide levels and demonstrates anti-hypertensive as well as anti-inflammatory effects. It seems that the overall consequences of CYP epoxygenase activation largely depend on enzyme substrate preference and the endogenous Ω-3/Ω-6 PUFA ratio. This review outlines the evidence for a physiological role for EETs in the regulation of vascular homeostasis.


**Contents**



*Abbreviations:* $BK_{Ca}$, large conductance $K_{Ca}$ channels; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; DHDP, dihydroxydocosapentaenoic acids; DHET, dihydroxyeicosatrienoic acids; DiHOME, dihydroxyoctadecenoic acid; EDHF, endothelium-derived hyperpolarizing factor; EpOME, epoxyoctadecamonoenic acid; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; G protein, guanine nucleotide binding protein; HETE, hydroxyeicosatetraenoic acid; IκB, inhibitor of κB; $IK_{Ca}$, intermediate conductance $K_{Ca}$ channels; $K_{ATP}$ channels, ATP-sensitive potassium channels; $K_{Ca}$, calcium activated potassium channels; LDL, low density lipoprotein; LOX, lipoxygenase; MAP kinase, mitogen activated protein kinase; NFκB, nuclear factor κB; NO, nitric oxide; PG, prostaglandin; $PGI_2$, prostacyclin; PK, protein kinase; $PLA_2$, phospholipase $A_2$; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; sEH, soluble epoxide hydrolase; TRP channels, transient receptor potential channels.

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http://dx.doi.org/10.1016/j.vph.2016.03.001
1537-1891/

## 1. Introduction

Local vascular tone is determined by a variety of factors such as neurotransmitters released from autonomic nerves, circulating vasoactive compounds, tissue metabolites and endothelium-derived autacoids. The best characterized vasodilator autacoids are nitric oxide (NO) and prostacyclin ($PGI_2$), but a substantial component of the vasodilator response observed in response to receptor-dependent agonists or increases in flow is insensitive to inhibitors of NO synthases or cyclooxygenases (COXs). The existence of a NO/$PGI_2$-independent component of endothelium-dependent relaxation is particularly prominent in the microcirculation as well as in coronary, mesenteric and renal arteries. Since the NO/$PGI_2$-independent vasodilatation originally described was co-incident with vascular smooth muscle hyperpolarization, and was abolished by depolarizing concentrations of potassium, it was proposed to be mediated by an endothelium-derived hyperpolarizing factor or "EDHF" [1,2].

### 1.1. A bit of history: comparing the concepts for EDHF

As the name implies an endothelium-derived hyperpolarizing factor would be expected to be a substance generated in and released from endothelial cells that is able to stimulate the hyperpolarization of underlying vascular smooth muscle cells. Such a factor would not be expected to be generated in large amounts under resting conditions but its production should be stimulated by hemodynamic stimuli such as cyclic stretch, fluid shear stress and increases in pressure (myogenic response), as well as in response to classical vasodilating agonists. Characterizing the nature of the proposed factor was partially a case for "fridge pharmacology" i.e. the testing of a number of enzymatic inhibitors and ion channel blockers on agonist induced hyperpolarization responses. Relatively early on the field split into two camps: those studying arteries in which the EDHF response was blocked by iberiotoxin and implicating large conductance calcium activated potassium ($BK_{Ca}$) channels [3–5], and those studying arteries in which the response was abrogated by the combination of charybdotoxin and apamin implying small and intermediate conductance $K_{Ca}$ ($SK_{Ca}$ and $IK_{Ca}$) channels [6–9]. While the former responses were largely also sensitive to substances such as the cytochrome P450 (CYP) inhibitors, clotrimazole, miconazole and 17-octadecynoic acid, arteries in which EDHF responses were linked to $SK_{Ca}$ and $IK_{Ca}$ channel activation were generally not. For quite a while the field failed to advance significantly with an "it is" versus "it is not" type of discussion dominating the literature. The realization that myo-endothelial gap junctional communication may also represent a means of transferring hyperpolarization between endothelial cells and smooth muscle cells without the need for a diffusible factor per se [10–12] prompted a review of the available evidence and the realization that at least three different mechanisms may underlie the agonist induced NO- and $PGI_2$-independent hyperpolarization of smooth muscle cells from different vascular beds [13]. Thus, the original EDHF type responses can now be attributed to (i) the activation of endothelial cell $SK_{Ca}$ and $IK_{Ca}$ channel activation and the induction of $K^+$ ion-induced vascular smooth muscle cell hyperpolarization, (ii) myo-endothelial gap junctional transfer, and (iii) the generation of CYP-derived products of arachidonic acid [14,15]. There may also be an additional role for endothelial cell derived hydrogen peroxide [16,17]. Which of these mechanisms dominates in which vascular bed is determined by a number of factors, including the architecture of the vasculature as well as by tissue specific differences in gene expression.

### 1.2. Cytochrome P450-derived lipid mediators

The fact that arachidonic acid was able to induce vascular smooth muscle cell hyperpolarization independent of an increase in cyclic nucleotides together with studies using pharmacological inhibitors of phospholipase $A_2$ ($PLA_2$) and CYP enzymes certainly suggested that CYP-derived metabolites of arachidonic acid may act as an EDHF [18–20]. Moreover, many CYP enzymes are sensitive to inhibition by high concentrations of NO, which fit with observations that EDHF-mediated hyperpolarization and vasodilatation was most prominent when NO production was inhibited [21]. Also, in bioassay systems it was possible to demonstrate the release of a physically transferable endothelium-derived CYP metabolite that could elicit smooth muscle cell hyperpolarization [3]. However, definitive demonstration of the importance of these enzymes came with the report that two epoxides of arachidonic acid i.e. 11,12- and 14,15-epoxyeicosatrienoic acid (EET) were able to elicit the activation of $K_{Ca}$ channels in bovine coronary arteries to induce hyperpolarization and relaxation [5]. A short time later, it was possible to identify a CYP2C enzyme in porcine coronary artery endothelial cells and demonstrate that the downregulation of this enzyme attenuated agonist-induced hyperpolarization and relaxation [4,22].

There is at least circumstantial evidence for a similar CYP-dependent vasodilator pathway in humans as inhibitors such as sulfaphenazole have been found to be both ineffective [23] as well as effective [24–27] in modulating vasodilatation in healthy subjects. The reason for the discrepancy seems to be the vascular bed studied. For example, while CYP inhibitor-sensitive responses in the forearm vasculature in its entirety are difficult to demonstrate, more clear-cut data were obtained when skeletal muscle arterioles [24] and the radial artery [25–27] were studied; tissues in which it was possible to confirm the expression of CYP2C protein [24,27].

How are EETs generated? $Ca^{2+}$ elevating receptor dependent agonists have frequently been used to increase CYP activity and EET production with bradykinin has been particularly effective in the endothelium [4,5]. Adenosine 2A receptors ($A_{2A}R$) seemingly play an important role in the kidney, in particular during the development of salt sensitive hypertension [28]. Following an increase in cell $Ca^{2+}$, $PLA_2$ is activated to liberate arachidonic acid from membrane phospholipids. The latter serves as a substrate for CYP enzymes and results in the generation of epoxides that can affect vascular tone. As metabolism of arachidonic acid seems to take place more or less immediately, it seems that the regulation of CYP activity is largely determined by substrate availability combined with the expression of the enzymes involved. Some of the CYP enzymes are known to be phosphorylated [29,30] but to date there has been no indication that this plays a major role in the acute generation of vasodilator EETs.

As far as endothelial cells are concerned the vast majority of reports indicate that CYP-derived lipid mediators elicit vasodilation, however CYP enzymes in vascular smooth muscle cells also catalyze the generation of hydroxy metabolites such as 20-hydroxyeicosatetraenoic acid (20-HETE) that elicit vasoconstriction — at least in the systemic circulation. The situation is reversed in the pulmonary circulation in which 20-HETE can act as a vasodilator [31] and 11,12-EET as a vasoconstrictor [32,33]. This review focuses on CYP-derived epoxides and the systemic endothelium, but for more detailed information on the role of CYP metabolites in vascular smooth muscle cells see two excellent recent reviews [34,35].