Prostaglandins & other Lipid Mediators xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Prostaglandins and Other Lipid Mediators

Review

Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, and renal microvascular function

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ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 5 December 2012
Accepted 9 January 2013
Available online xxx

Keywords:
Kidney
Hypertension
Eicosatrienoic acid
Hydroxysatetraenoic acid
Afferent arteriole

ABSTRACT

The development of pharmacological, genetic, and biochemical tools have allowed for detailed studies to determine the contribution of cytochrome P450 (CYP) metabolites of arachidonic acid to renal microvascular function. Renal microvessels can generate CYP hydroxylase metabolites including 20-hydroxyeicosatetraenoic acid (20-HETE) and CYP epoxygenase metabolites, epoxyeicosatrienoic acids (EETs). 20-HETE constricts afferent arterioles and contributes to renal blood flow autoregulation. EETs act as endothelium-dependent hyperpolarizing factors (EDHFs) on the renal microcirculation. 20-HETE inhibits whereas EETs activate renal microvascular smooth muscle cell large-conductance calcium-activated K^+ channels (K_{Ca}). Likewise, 20-HETE renal microvascular actions are pro-hypertensive and EET actions are anti-hypertensive. These findings in the renal microvasculature and those of others have provided impetus for the development of enzymatic inhibitors, agonists, and antagonists for 20-HETE and EETs to determine their potential therapeutic value. Initial genetic studies and experimental studies with soluble epoxide hydrolase inhibitors to increase EETs, EET analogs, and 20-HETE inhibitors have demonstrated improved renal microvascular function in hypertension. These findings have demonstrated the important contributions that 20-HETE and EETs play in the regulation of renal microvascular function.

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1. Introduction

The recognition that cytochrome P450 (CYP) enzymes had the capacity to metabolize arachidonic acid and generate epoxyeicosatrienoic acids (EETs) and hydroxysatetraenoic acids (HETEs) ignited curiosity to determine their biological actions [1,2]. As the identification of the CYP enzymes that catalyzed the reactions were being identified and further characterized in the 1980s, there was slower progress with the determination of the physiological actions for EETs and HETEs. Early studies demonstrated that kidneys had significant expression of CYP enzymes and that EETs and HETEs had actions on epithelial cells to alter sodium transport [3,4]. Vascular actions for EETs as dilators were first described toward the end of 1980s [5]. Around this same time period it was becoming evident that nitric oxide was an endothelial-derived relaxing factor [6,7]. It was also apparent that the endothelial cells released a hyperpolarizing factor (EDHF) that was speculated to be a non-cyclooxygenase arachidonic acid metabolite [6,7]. EETs became a candidate for being an EDHF and a number of laboratories pursued this idea during the 1990s [8–10]. On the other hand, 20-HETE was determined to be a vasoconstrictor in the early 1990s [11,12]. A point of contention was that the epithelial actions attributed to 20-HETE were anti-hypertensive whereas the vascular actions were pro-hypertensive [13]. Therefore, the 1990s were an era that took CYP generated EETs and HETEs from a biological curiosity to

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1098-8823/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.prostaglandins.2013.01.002

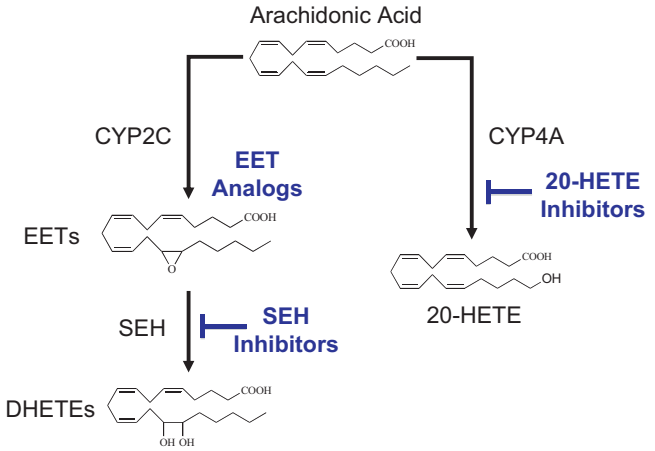

Fig. 1. Therapeutic targeting for the epoxygenase and hydroxylase pathways: epoxyeicosatrienoic acids (EETs) are generated from arachidonic acid by cytochrome P450 (CYP2C) enzymes. EETs are converted to dihydroxyeicosatrienoic acids (DHETEs) by the soluble epoxide hydrolase (sEH) enzyme. 20-Hydroxysatetraenoic acid (20-HETE) is generated by cytochrome P450 (CYP4A) enzymes. EET analogs, sEH inhibitors, and 20-HETE inhibitors are therapeutic targets for hypertension, renal, and cardiovascular diseases.

a metabolic pathway that could significantly impact physiological and pathophysiological states.

There were numerous hurdles to overcome to determine the physiological and pathophysiological importance of CYP arachidonic acid metabolites. Pharmacological, molecular biological, and analytical tools had to be developed to determine the biological actions attributed to CYP enzymes, EETs, and 20-HETE. The laboratories of Jorge Capdevila and John Falck developed many of the tools necessary for investigators to determine the biological importance of this pathway [13,14]. These tools led to a number of experimental studies in my laboratory to determine the impact of CYP enzymes, EETs, and 20-HETE on renal microvascular function (Fig. 1). This review article will focus on findings demonstrating renal microvascular actions for EETs and 20-HETE and their contribution to hypertension.

2. 20-HETE and afferent arteriolar autoregulatory responses

Early experimental studies determined that renal arterioles, glomeruli, and vasa recta capillaries expressed CYP4A hydroxylase enzymes that are primarily responsible for generating 20-HETE [12,13]. Other experimental studies determined that 20-HETE levels were elevated in spontaneously hypertensive rats and 20-HETE constricted canine renal arteries [11,15,16]. 20-HETE afferent arteriolar constriction was determined to be due to inhibition of calcium-activated K^+ (K_{Ca}) channels, membrane depolarization, activation of L-type calcium channels, and an increase in intracellular calcium [11–13] (Fig. 2). Besides the direct action of 20-HETE to constrict afferent arterioles, a central role for 20-HETE is its contribution to renal blood flow autoregulation [17,18].

Renal blood flow autoregulation is the ability to keep blood flow and glomerular filtration rate constant in the face of changes in perfusion pressure. The kidney is able to maintain a constant renal blood flow between 80 and 160 mmHg through two mechanisms, the myogenic response and tubuloglomerular feedback. The contribution of CYP metabolites to renal blood flow autoregulation was demonstrated by infusing the non-selective CYP inhibitor 17-ODYA into the renal artery [18]. Renal blood flow and cortical blood flow increased in response to increases in mean arterial pressure in the presence of CYP inhibition [18]. Experiments in the juxtamedullary nephron preparation determined that the afferent arteriolar constriction was attenuated and that glomerular capillary pressure increased as perfusion pressure increased from 80 to 160 mmHg [17]. Additional studies established a contribution for CYP metabolites to the afferent arteriolar myogenic and tubuloglomerular feedback responses [17–19]. Renal microvascular myogenic responses in the absence of tubuloglomerular feedback were attenuated by CYP inhibition [17]. Micopuncture studies demonstrated that CYP inhibition abolished the tubuloglomerular feedback response and the addition of 20-HETE to the tubular perfusate restored the feedback response [19]. One concern with these studies was that 17-ODYA is unable to determine the contribution of hydroxylase metabolite 20-HETE *versus* epoxygenase EET metabolites because 17-ODYA inhibits the renal generation of EETs as well as 20-HETE [17–19]. Relatively selective inhibition of the epoxygenase pathway with imidazole derivatives including miconazole and clotrimazole had no effect on renal blood flow autoregulation [18]. This finding provides support to the notion that EETs were not contributing to autoregulation and more clearly established a contribution of the hydroxylase metabolite 20-HETE to renal blood flow autoregulation.

The development of selective CYP epoxygenase and hydroxylase inhibitors, as well as, EET and 20-HETE antagonists allowed for more direct evaluation of the specific CYP metabolites that contributed to afferent arteriolar autoregulation. The development of selective CYP epoxygenase (PPOH and MS-PPOH) and hydroxylase (DDMS) inhibitors and EET (14,15-EEZE) and 20-HETE (20-HEDE) antagonists allowed for experimental studies to delineate the contributions of EETs and 20-HETE to renal microvascular function [20,21]. The selective hydroxylase inhibitor DDMS attenuated the decrease in afferent arteriolar diameter to increasing perfusion pressure [21]. Moreover, the selective epoxygenase inhibitors PPOH and MS-PPOH had the opposite effect and in response to elevations in perfusion pressure resulted in enhanced afferent arteriolar constriction [21]. These findings provided significant support that 20-HETE is a critical component of the afferent arteriolar response to increases in perfusion pressure and that dilator EETs attenuates this vasoconstriction.

A parallel concept that purines adenosine and ATP and their receptors contributed to renal blood flow autoregulation and afferent arteriolar autoregulatory responses was gaining substantial experimental support [22,23]. This led to the idea that interactions between purinergic receptors and CYP metabolites may exist at a level that controls afferent arteriolar autoregulation. Similar to CYP hydroxylase inhibition, inactivation of ATP P2X receptors on preglomerular microvessels abolishes autoregulatory behavior [22,23]. Interestingly, hydroxylase inhibition or 20-HETE antagonism attenuated the initial afferent arteriolar constriction and abolished the sustained constriction to the P2 receptor agonist ATP [24]. The afferent arteriolar constriction to the P2X receptor agonist α,β-methylene ATP was also greatly attenuated by DDMS or 20-HEDE [24]. Additionally, the increase in renal microvascular cell calcium evoked by ATP and the P2X receptor agonist, α,β-methylene ATP, but not the P2Y agonist, UTP, were markedly reduced by 20-HETE inhibition [25]. These studies provided compelling evidence that endogenous 20-HETE contributes to the P2X receptor-mediated afferent arteriolar autoregulatory response by influencing vascular smooth muscle cell calcium influx.

Overall, 20-HETE is an important autocrine factor that contributes to the regulation of renal blood flow and afferent arteriolar function. There is significant evidence that there is a connection between ATP P2X receptor activation, 20-HETE, and afferent arteriolar autoregulatory responses. Future studies are required to fully establish the contribution and cell signaling mechanisms by which 20-HETE regulates afferent arteriolar function.

Please cite this article in press as: Imig JD. Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, and renal microvascular function. Prostaglandins Other Lipid Mediat (2013), http://dx.doi.org/10.1016/j.prostaglandins.2013.01.002