Abstract—Epoxyeicosatrienoic acids (EETs) are epoxides of arachidonic acid generated by cytochrome P450 (CYP) epoxygenases. The activation of CYP epoxygenases in endothelial cells is an important step in the NO and prostacyclin-independent vasodilatation of several vascular beds, and EETs have been identified as an endothelium-derived hyperpolarizing factor. However, EETs also exert membrane potential-independent effects and modulate several signaling cascades that affect endothelial cell proliferation and angiogenesis. This review summarizes the role of CYP-derived EETs in endothelium-derived hyperpolarizing factor–mediated responses and highlights the evidence indicating that EETs are important second messengers involved in endothelial cell signaling pathways related to angiogenesis. (Hypertension. 2006;47:629-633.)

Key Words: calcium channels ■ endothelium-derived factors ■ hypertension ■ vasodilatation

There is now considerable evidence linking the metabolism of arachidonic acid by cytochrome P450 (CYP) enzymes with the regulation of vascular homeostasis and tone, as well as renal function. The enzymes in question fall into 2 classes: (1) the epoxygenases, which generate epoxyeicosatrienoic acids (EETs); and (2) the ω-1 hydroxylases, which generate 20-hydroxyeicosatetraenoic acid (20-HETE).

EETs and Endothelium-Derived Hyperpolarizing Factor

Interest in the vascular actions of EETs was initially related to their identification as an endothelium-derived hyperpolarizing factor (EDHF), that is, compounds responsible for the NO and prostacyclin-independent but endothelium-dependent vasodilatation of some vascular beds. There is now convincing evidence indicating that the hyperpolarizing factor produced by coronary and renal arteries from several species (humans, pigs, cows, dogs, rats, and rabbits) displays characteristics similar to those of a cytochrome CYP-derived metabolite of arachidonic acid.1 A CYP-dependent, EDHF response has also been described in other human arteries/vascular beds including the mammary artery,2 the forearm vasculature,3 and thigh skeletal muscle circulation.4 However, the EDHF-mediated relaxations in human mesenteric arteries5 and renal arteries6 appear to be independent of CYP activity.

For those following the “EDHF story,” the literature has been highly confusing, and for several years the field seemed caught in a pantomime of “it is” versus “it isn’t” reports. One reason for the confusion was that although some EDHF-mediated responses could be attributed to a CYP-derived metabolite of arachidonic acid, there were clearly other responses that occurred entirely independently of the activation of a CYP enzyme. For comprehensive reviews on the nature of the different EDHFs, the reader should consult references.7–9

Part of the reason for some of the controversy was that EETs were initially reported to initiate smooth muscle hyperpolarization by activating iberiotoxin-sensitive large conductance Ca2+-activated K+ (BKCa) channels,10 whereas EDHF-dependent relaxation was more universally sensitive to the combination of charybdotoxin and apamin (inhibitors of small and intermediate conductance KCa channels) than to iberiotoxin.11 Another point that argued against the involvement of EETs in the regulation of vascular tone was that, whereas several of the initial studies aimed at characterizing EDHF used BKCa-expressing cultured smooth muscle cells as a detector, smooth muscle cells from many freshly isolated arteries do not express BKCa channels.12 It is now clear, however, that the decisive charybdotoxin and apamin-sensitive hyperpolarizing event in EDHF-mediated responses is the hyperpolarization of the vascular endothelium and not of smooth muscle cells.7 This realization has forced a reevaluation of the role of CYP products in EDHF-mediated responses.

In line with the definition of an EDHF, EETs were initially assumed to be generated by the vascular endothelium then to leave these cells and travel across the basal lamina to activate BKCa channels on the underlying vascular smooth muscle. This can undoubtedly occur when large amounts of EETs are generated, and EETs can be detected in the perfusate of isolated arteries after exposure to fluid shear stress13 or to agonist stimulation.14 Moreover, a recently developed EET-
selective antagonist, 14,15-epoxyeicosa-5(Z)-monoenoic acid, is able to prevent the endothelium-dependent but NO- and prostacyclin-independent relaxation of bovine coronary arteries in an elegant bioassay model.\textsuperscript{15}

EETs are not all metabolized via the same pathway, and although 5,6-EET is a preferred substrate for cyclooxygenase,\textsuperscript{16} 8,9-, 11,12- and 14,15-EET are metabolized to the corresponding dihydroxy derivatives (the dihydroxyeicosatrienoic acids) by the soluble epoxide hydrolase (sEH),\textsuperscript{17} as well as by \textbeta-oxidation.\textsuperscript{18} This difference accounts for the finding that relaxation of the bovine coronary artery induced by a novel stable agonist of 5,6-EET, 5-(pentadeca-3(Z),6(Z),9(Z)-trienyloxy) pentanoic acid was inhibited by the cyclooxygenase inhibitor indomethacin.\textsuperscript{19} The recent development of sEH inhibitors has helped to determine the physiological consequences of enhanced CYP activity and EET formation. Inhibitors of this enzyme enhance iberiotoxin-sensitive, EET-induced vasodilator responses in isolated human coronary arterioles,\textsuperscript{20} highlighting the potential relevance of this pathway in humans. The latter observation is, however, controversial, and the bradykinin-induced relaxation of human small coronary arteries (diameter \(=300 \mu m\)) has also been recently described as depending on the activation of guanylyl cyclase, inwardly rectifying K\textsuperscript{+} channels, and the Na\textsuperscript{+}-K\textsuperscript{+} ATPase by NO, as well as on the activation of small and intermediate conductance K\textsubscript{Ca} channels by a factor that is not a CYP epoxygenase product or H\textsubscript{2}O\textsubscript{2}.\textsuperscript{21} Given the latter observations together with the consideration that EETs are rapidly metabolized by the sEH and the fact that the CYP-dependent EDHF-mediated relaxation of porcine coronary arteries is also sensitive to the combination of charybdotoxin and apamin, it seemed that EETs may exert additional functions within endothelial cells that ultimately result in the activation of small and intermediate conductance K\textsubscript{Ca} channels.

The effects of EETs on small and intermediate conductance K\textsubscript{Ca} channels need not be direct, and there is evidence indicating that endogenously generated EETs can activate K\textsubscript{Ca} channels by activating membrane-associated second messengers.\textsuperscript{22} For example, EETs activate K\textsubscript{Ca} channels in coronary smooth muscle cells via a G\textalpha\textsubscript{s}-mediated mechanism\textsuperscript{23} and may alter the sensitivity of these channels to Ca\textsuperscript{2+}.\textsuperscript{24} Moreover, the activation of protein kinase A (PKA) by 11,12-EET is implicated in afferent arteriole vasodilatation,\textsuperscript{25} and there is evidence suggesting that K\textsubscript{Ca} channels are modulated by cAMP and PKA.\textsuperscript{26} EETs also play a key role in modulating intracellular Ca\textsuperscript{2+} levels in endothelial cells, as well as the endothelial cell hyperpolarization that occurs as a consequence of the activation of low and intermediate conductance K\textsubscript{Ca} channels.\textsuperscript{27} This implies that CYP-derived EETs are basically intracellular amplifiers of the endothelial hyperpolarization response and goes a long way to explaining why EDHF appears to have different properties in different vessels. One potential mechanism underlying the EET-dependent potentiation of endothelial cell hyperpolarization involves the activation of nonselective cation channels of the transient receptor potential (Trp) family. Some of these channels possess an arachidonic acid binding site and can be activated by EETs.\textsuperscript{28} Indeed, it has been shown recently that the induction of CYP expression with nifedipine increases TrpV4 activity as do sEH inhibitors, which increase the intracellular EET concentration, and that the arachidonic acid–induced activation of TrpV4 can be attributed to a CYP-dependent process.\textsuperscript{29} Although we speculated that EETs may indirectly modulate the open probability of Trp channels via cAMP/PKA,\textsuperscript{27} a recent report suggests that TRPV4 forms a novel Ca\textsuperscript{2+} signaling complex with ryanodine receptors and BK\textsubscript{Ca} channels that elicits smooth muscle hyperpolarization and arterial dilation via Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release.\textsuperscript{30}

More Than Regulators of Vascular Tone?

Because the EETs are known to exert cellular effects that cannot necessarily be linked to the activation of K\textsubscript{Ca} channels, it follows that the CYP-derived metabolites of arachidonic acid may be more than vasodilators/vasoconstrictors. Paracrine and autocrine effects of CYP metabolites have been described in endothelial and smooth muscle cells, but the intracellular second messenger role of EETs (and 20-HETE) may eventually turn out to be the most important function of these arachidonic acid derivatives. EETs, in particular 11,12- and 14,15-EET, activate several intracellular signaling molecules including PKA,\textsuperscript{25} tyrosine kinases and phosphatases,\textsuperscript{31,32} the p38 mitogen-activated protein (MAP) kinase,\textsuperscript{33} extracellular regulated protein kinases 1 and 2, and MAP kinase phosphatases at the same time as inhibiting the c-Jun N-terminal kinase.\textsuperscript{33}

Of particular interest are reports that EETs affect endothelial cell proliferation and angiogenesis.\textsuperscript{34–37} Detailed analysis of the mechanisms involved indicate that EETs can transactivate the epidermal growth factor receptor\textsuperscript{38,39} and interact with fibroblast growth factor-2.\textsuperscript{40} The EET-mediated activation of the epidermal growth factor receptor leads, in turn, to the activation of the kinase Akt and an enhanced expression of cyclin D1. All 4 of the EET regioisomers have been reported to elicit an increase in Akt phosphorylation and cell proliferation in murine endothelial cells, but only the proliferative effects of 5,6- and 14,15-EET are reportedly sensitive to a phosphatidylinositol 3-kinase inhibitor, whereas the 8,9- and 11,12-EET–induced increase in [{\textsuperscript{3}H}]thymidine incorporation seems to be dependent on the activation of the p38 MAP kinase.\textsuperscript{41} In contrast, in bovine aortic endothelial cells, 8,9-, 11,12-, and 14,15-EET–induced cell proliferation can be attenuated by MAP kinase kinase (MEK) and phosphatidylinositol 3-kinase inhibition.\textsuperscript{42} Other signaling pathways also contribute to the increase in cyclin D1 expression, including the MAP kinase phosphatase-1, which decreases c-Jun N-terminal kinase activity.\textsuperscript{33} Activation of Akt by EETs also induces the phosphorylation and, therefore, inhibition of the forkhead factors FOXO1 and FOXO3a and subsequently a decrease in the expression of the cyclin-dependent kinase inhibitor p27\textsuperscript{kip1}.\textsuperscript{43} The involvement of this mechanism in the CYP 2C9–induced endothelial cell proliferation could be demonstrated by the transfection of CYP 2C9–overexpressing cells with either a dominant-negative Akt or a constitutively active FOXO3a, which both inhibit CYP 2C9–induced endothelial cell proliferation.\textsuperscript{43}
EETs and Hypertension

Because EETs play a role in the regulation of vascular tone, it follows that their altered generation and/or degradation may contribute to the development of some forms of hypertension. The relationship between CYP epoxygenase expression/activity and hypertension is, however, not always straightforward, and although it would seem logical to look for a link between decreased CYP epoxygenase expression and hypertension, a number of experimental findings have suggested that quite the opposite is the case. There is evidence suggesting that CYP expression and EET generation are increased in hypertension, during salt loading, as well as in hypercholesterolemia. Certainly, the cyclooxygenase-independent renal vasodilatation produced by arachidonic acid is enhanced rather than blunted in spontaneously hypertensive rats (SHRs) at high-perfusion pressures. The latter response could be characterized pharmacologically as being related to the production of an EET, and 5,6-EET, 8,9-EET, and 11,12-EET all induced renal vasodilatation (5,6-EET was the most potent renal vasodilator), whereas 14,15-EET produced vasoconstriction. However, there are also reports linking salt-induced and angiotensin II–induced hypertension to the failure to increase epoxide hydrolase expression, particularly in the kidney. Genetic hypertension seems to be differentially linked to CYP expression and activity, because there seems to be no difference in the arachidonic acid–induced generation of EETs by kidneys from SHRs and Wistar-Kyoto rats. Such data imply that alternative mechanisms may underlie the increased renal vasodilator effect of arachidonic acid in the SHR kidney. One possibility is that hypertension is associated with alterations in the stability of EETs. This may well be the case, as a marked increase in the renal metabolism of EETs to dihydroxyeicosatetraenoic acids has been reported during the development of hypertension in SHRs, suggesting that either the activity or the expression of the epoxide hydrolase is increased. Moreover, in SHRs and in rats and mice with angiotensin II–induced hypertension, sEH inhibitors, which increase EET levels, markedly attenuate mean arterial blood pressure. Elevated EET levels also help to protect the kidney against hypertension-induced damage in different animal models.

When searching for links between CYP expression and cardiovascular disease, it is perhaps more logical to look for changes in the production of a CYP-derived vasoconstrictor. 20-HETE is currently characterized as a prohypertensive eicosanoid, and has the potential to play a dual role in the regulation of blood pressure by virtue of its ability to induce contraction, as well as to inhibit sodium resorption (reviewed in Reference 59). The global consequences of enhanced renal 20-HETE production seem more closely related to prevention than aggravation of hypertension, as increased renal 20-HETE production after fribate treatment has been linked to CYP 2C epoxygenase induction and attenuation of angiotensin II–induced, as well as desoxycorticosterone acetate salt–induced hypertension in mice. Also, inhibitors of 20-HETE formation promote salt-sensitive hypertension in rats.

That 20-HETE functionally antagonizes the actions of the EETs accounts for the finding that in the absence of cyclooxygenase inhibitors, bradykinin causes the release of a glomerular vasoconstrictor that antagonizes the vasodilator effect of EETs released from the efferent arteriole and perhaps from the glomerulus. Certainly, 20-HETE and EETs elicit opposite effects on the open probability of K⁺ channels, as well as on the Na-K-ATPase in vascular cells.

Several polymorphisms of the CYP enzymes expressed within endothelial cells exist. A polymorphism of the CYP 2J2 epoxygenase, which results in the attenuated binding of the transcription factor Sp1 and reduced promoter activity, has been associated with an enhanced risk of developing coronary artery disease in humans. However, there is also evidence indicating that a CYP 2C epoxygenase expressed in endothelial cells is able to generate superoxide anions in addition to EETs and that in patients with coronary artery disease the inhibition of CYP 2C–derived superoxide anion formation can markedly improve acetycholine-induced vasodilatation in the forearm vasculature. Given this information, it is tempting to speculate that a decrease in CYP 2C activity within the vasculature can protect against the development of hypertension and its consequences. There is circumstantial evidence indicating that this may be the case, as a loss-of-function polymorphism of CYP 2C9 (CYP2C9*3), has been linked with a lower incidence of hypertension in a Chinese population. Other members of the CYP 2 family of enzymes may also participate in the regulation of blood pressure, and expression levels of CYP2E1 are reportedly lower in hypertensive SHRs than in normotensive Wistar rats.

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