Abstract—Since the initial reports that renal cytochrome P450 (CYP) enzymes can metabolize arachidonic acid to substances which affect arterial tone, it has become increasingly clear that CYP enzymes expressed within the cardiovascular system play a crucial role in the modulation of vascular homeostasis. There is strong evidence suggesting that the activation of a CYP epoxygenase in endothelial cells is an essential step in nitric oxide and prostacyclin-independent vasodilatation of several vascular beds, particularly in the heart and kidney. A smooth muscle CYP $\omega$-hydroxylase, on the other hand, generates a vasoconstrictor eicosanoid that is central to the myogenic response. Moreover, CYP epoxygenase and $\omega$-hydroxylase products, as well as CYP-derived reactive oxygen species, are intracellular signal transduction molecules involved in several signaling cascades affecting numerous cellular processes, including vascular cell proliferation and angiogenesis. This review summarizes the vascular effects of epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid, both of which are CYP-derived metabolites of arachidonic acid, endogenously generated within endothelial and vascular smooth muscle cells. Although the link between CYP expression/activity and cardiovascular disease is currently tentative, the evidence being accumulated to suggest that CYP pathways are altered in animal models of hypertension and atherosclerosis can no longer be ignored. The development of selective pharmacological tools is, however, a prerequisite for the analysis of the involvement of specific CYP isoforms in the regulation of vascular homeostasis in human subjects. (Circ Res. 2001;89:753-762.)

Key Words: endothelial dysfunction ■ endothelium-derived hyperpolarizing factor ■ epoxyeicosatrienoic acids ■ free radicals ■ 20-hydroxyeicosatetraenoic acid
unsaturated fatty acids may be potential modulators of vascular function, this review concentrates on the physiological/pathophysiological significance of the CYP-dependent metabolites of arachidonic acid.

Most CYPs are primarily expressed in the liver, with significantly lower levels of expression in extrahepatic tissues. However, some CYPs are predominantly detected in the heart, vasculature, gastrointestinal tract, kidney, and lung, and recent data have suggested that specific CYPs localized in the vascular smooth muscle and endothelium contribute to the regulation of vascular tone and homeostasis. The arachidonic acid metabolizing CYP enzymes with prominent roles in vascular regulation are the epoxygenases of the 2 gene family (eg, CYP 2B, 2C8, 2C9, 2C10, 2J2 in humans; 2C34 in pigs; 2C11, 2C23 and 2J4 in rats) which generate a series of regiospecific and stereospecific epoxides (5,6-, 8,9-, 11,12-, and 14,15-EETs), and the arachidonic acid ω-hydroxylases belonging to the CYP 4A family which form subterminal and ω-terminal hydroxyeicosatetraenoic acids (HETEs, Figure 1). There are, however, some enzymes, eg, the rat CYP 4A2 and 4A3, that are able to generate both 20-HETE and 11,12-EET.

Investigators new to the field are frequently put off by the seemingly incomprehensible nomenclature of the various enzymes. Currently, CYP proteins from all sources with approximately 40% sequence identity or greater are included in the same family which is designated by an Arabic number. Proteins with greater than 55% identity are then grouped together in the same subclass as designated by a capital letter; the last number identifies specific gene products.

EETs and 20-HETE can be processed within vascular cells, and the intermediates thus generated are also active signaling molecules, which, in some cases, elicit opposite effects to the parent compound. Thus, although it has been possible to demonstrate the importance of CYP enzymes in the acute control of vascular tone, exactly which metabolites are involved in the long-term regulation of vascular homeostasis is unclear. In this respect, it is most difficult to determine the direct cellular effects of 5,6-EET and 20-HETE as both these compounds are rapidly metabolized by cyclooxygenase (for review see McGiff and Quilley).

Vascular Tone

EETs

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 (PGI2), 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. The existence of a NO/PGL2-independent component of endothelium-dependent relaxation is particularly prominent in coronary, mesenteric, and renal arteries. Because the NO/PGL2-independent vasodilation 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 (EDHF). An EDHF would be expected, by definition, to be a substance released from endothelial cells that elicits the hyperpolarization of smooth muscle cells and thus induces relaxation.

The link between CYP activity and the generation of an EDHF has been intensively investigated over the last decade. Several EDHFs, demonstrating distinct pharmacological properties, have been reported in different vascular beds and in different species, but the hyperpolarizing factor produced by coronary and renal arteries from humans, pigs, cows, dogs, rats, and rabbits displays characteristics similar to those of a CYP-derived metabolite of arachidonic acid. The arachidonic acid metabolites in question are the EETs generated by endothelial CYP epoxygenases. These substances elicit the hyperpolarization of endothelial and vascular smooth muscle cells by activating calcium-dependent K+ channels as well as the Na-K-ATPase.

Originally, a role for CYP-dependent metabolites of arachidonic acid in EDHF-mediated responses was implied on...
the basis that CYP inhibitors, such as clotrimazole, miconazole, and 17-octadecynoic acid, markedly attenuated NO/PGI\(_2\)-independent hyperpolarization and relaxation in various preparations. However, these conclusions were limited by the fact that the CYP inhibitors used do not discriminate between different CYP isozymes, cannot inhibit the release of preformed pools of EETs incorporated into phospholipids, and some directly interfere with the activation of the K\(_\text{+}\) channels thought to be the main cellular targets of EDHF.\(^{35,36}\) However, more selective epoxygenase inhibitors [6-(2-proparglyloxyphenyl)hexanoic acid and N-methylsulphonyl-6-(2-proparglyoxyphenyl)hexanamide] have since been developed.\(^{37}\) and these compounds have been reported to abolish NO/PGI\(_2\)-independent vasodilatation of renal arterioles.\(^{38}\)

Recently, data relying on techniques other than the pharmacological inhibition of CYP have considerably strengthened the hypothesis that CYP activation is an integral component of the EDHF response. RT-PCR, Western blotting, and immunofluorescence techniques have been used to demonstrate that native coronary endothelial cells express CYP epoxygenases, including CYP 2C8, CYP 2C9, and CYP 2J2.\(^{12,39,41}\) The functional relevance of CYP activity has been addressed by enhancing the expression of CYP enzymes, as well as by attenuating CYP expression with the aid of antisense oligonucleotides. Utilizing the former approach, CYP inducers, such as \(\beta\)-naphthoflavone or nifedipine, were found to increase the synthesis of EETs by cultured and native endothelial cells, and to enhance the agonist-induced, EDHF-mediated hyperpolarization and relaxation of intact coronary arteries.\(^{37,39,41}\) Although changes in NO/PGI\(_2\)-independent relaxation were associated with changes in CYP 2C rather than CY2 J expression, CYP inducers enhance the expression of a spectrum of different enzymes, all of which could contribute to EET/EDHF-mediated responses. The most convincing evidence obtained to date in support of the hypothesis that a CYP 2C epoxygenase is intimately involved in the EDHF response in porcine coronary arteries was provided by the use of antisense oligonucleotides directed against the coding region of CYP 2C8/9. Incubation of porcine coronary arteries with antisense, but not sense or scrambled, oligonucleotides markedly reduced CYP 2C mRNA and protein and attenuated bradykinin-induced, EDHF-mediated hyperpolarization and relaxation without compromising responsiveness to endogenously produced NO or an NO donor.\(^{39}\) Homology among the different CYP 2C isozymes is exceedingly high,\(^{42}\) and using the antisense approach described it was not possible to differentiate between the expression of CYP 2C8 and 2C9. However, the finding that sulfaphenazole, a selective inhibitor of CYP 2C9,\(^{43,44}\) inhibits EDHF-mediated responses\(^{39}\) and potentiates NO-mediated relaxation in the porcine coronary artery\(^{39}\) suggests that the CYP isoform required for the generation of EDHF is a porcine equivalent of CYP 2C9. This inhibitory effect of antisense oligonucleotides provided the first non-pharmacological evidence that a CYP 2C metabolite is an essential permissive factor for EDHF-mediated vascular responses. A similar approach was used to show that EDHF-mediated responses in isolated resistance arteries from hamster gracilis muscle can also be attributed to the activity of a CYP 2C epoxygenase.\(^{46}\)

Like all CYP enzymes, members of the CYP 2C family are inhibited by NO, a phenomenon that may explain why EDHF-mediated responses are barely detectable in the absence of the combined inhibition of NO synthases and cyclooxygenase. The fact that NO intrinsically inhibits CYP activity led to the suggestion that the EET/EDHF pathway may be of minor importance in healthy vessels but acts as a reserve or backup vasodilator mechanism in circumstances associated with a manifest endothelial dysfunction in which the bioavailability of NO is impaired.\(^{47}\)

The renal vasculature seems to be especially tightly controlled by CYP activity, and although classical CYP inhibitors induce little or no alteration in vascular responsiveness in the absence of NO synthase and cyclooxygenase inhibitors, in most arteries studied to date, afferent arteriolar responsiveness to increases in transmural pressure is markedly affected by epoxygenase inhibition.\(^{48}\) In the kidney, EETs also increase sodium excretion\(^{49}\) and decrease cortical renin release\(^{50}\) in addition to modulating vascular diameter.

The tone of cerebral arteries is also markedly affected by EET production, although in this case the EETs seem to be generated by astrocytes in response to glutamate released from adjacent neurons.\(^{51,52}\) The functionally antagonistic effects of EET and 20-HETE on the regulation of cerebral blood flow have been particularly well elucidated (see the following section).

Several CYP isozymes are also abundantly expressed in the lung, and CYP-derived eicosanoid metabolites have recently been shown to be present in lung tissue as well as bronchoalveolar lavage fluid and to possess potent actions in the airway and pulmonary vasculature (for review see Jacobs and Zeldin\(^{53}\)). However, much less is known about the role of CYP epoxygenase products in lung physiology and pathophysiology than in other systems. Indeed, the effects of EETs in the lung are difficult to predict as these eicosanoids can relax or constrict, depending on the experimental model and species under investigation. On the whole, however, the vascular actions of EETs are thought to be contrary to those of the systemic circulation; for example, EETs constrict rather than dilate isolated pressurized pulmonary arteries.\(^{54}\)

20-HETE

20-HETE, an \(\omega\)-hydroxylation product of arachidonic acid catalyzed by cytochrome CYP 4A, is an essential component of the signal transduction cascade activated by several hormonal systems, eg, endothelin-1\(^{55,56}\) and angiotensin II,\(^{57}\) that have central roles in blood pressure regulation (for recent reviews on this topic, see McGiff and Quilley,\(^{11}\) Maier and Roman,\(^{69}\) Harder et al,\(^{52}\) and Imig\(^{58}\)).

Data gathered over the last 5 to 8 years have convincingly shown a link between vascular 20-HETE generation and the myogenic response in renal and cerebral arteries, which is a main determinant of vascular tone in situ. 20-HETE is endogenously produced by smooth muscle cells after an increase in \([\text{Ca}^{2+}]\), and once formed, increases smooth muscle tone by inhibiting large-conductance \(K_{\text{Ca}}\) channels, inducing depolarization, and further increasing \([\text{Ca}^{2+}]\). This
effect is most likely related to the activation of L-type Ca\(^{2+}\) channels,\(^{39}\) although additional effects which may contribute to contraction involve the activation of PKC and inhibition of the Na-K-ATPase.\(^{2,60,61}\) Thus, in CYP 4A-expressing vessels, an increase in transmural pressure would be expected to stimulate stretch-activated Ca\(^{2+}\) channels, thereby enhancing smooth muscle 20-HETE formation and eliciting constriction. Because such a sequence of events would transform the circumferential stretch of an arterial segment resulting from an increase in pressure into the active development of force in the vascular smooth muscle, it could account for the phenomenon of autoregulation (Bayliss effect\(^{62}\)), by which blood flow to organs is maintained constant over a wide range of perfusion pressures. In line with the concept that vascular smooth muscle-derived 20-HETE controls arterial tone is the fact that the myogenic response is endothelium-independent.\(^{63–66}\) Although the hypothesis that an increase in transmural pressure is the physiologically most important stimulus for vascular 20-HETE production is certainly attractive, in spontaneously hypertensive rats (SHR), increases in pulse pressure have been linked with enhanced EET rather than 20-HETE levels.\(^{67}\)

Endothelium-derived factors are able to modulate myogenic contraction\(^{68}\) and at least part of their action can be attributed to interference with the formation and actions of 20-HETE.\(^{69}\) NO, for example, may modulate the formation of 20-HETE by binding to and inactivating the cytochrome P450 heme moiety of CYP 4A. Indeed, the NO-mediated inhibition of 20-HETE formation has been proposed to account for the natriuretic and diuretic actions of NO,\(^{70}\) as well as the cyclic GMP-independent relaxant effects of NO in renal and cerebral arteries.\(^{70–73}\) EETs, on the other hand, enhance the open probability of K\(^{+}\) channels (K\(^{+}\) channels) and inhibit L-type Ca\(^{2+}\) channels, thereby enhancing the open probability of calcium-dependent K\(^{+}\) channels (K\(^{+}\) channels), which leads to depolarization of the smooth muscle cell membrane, the activation of L-type Ca\(^{2+}\) channels, and contraction. In endothelial cells, the activation of PLA\(_{2}\) is also a prerequisite for the generation of EET by CYP 2A. Once formed, EETs are either metabolized to DHETs, incorporated into membrane phospholipids by acyl coenzyme A synthase (CoA-SH), or leave the endothelial cell without modification. EETs, and to a certain extent DHETs, activate K\(^{+}\) channels inducing smooth muscle cell hyperpolarization and inhibiting L-type Ca\(^{2+}\) channels, thus inducing relaxation.

CYP and Free Radical Production

To date, when considering the consequences of vascular CYP epoxygenase and \(\omega\)-hydroxylase activity, most attention has been focused on the generation of vasoactive arachidonic acid metabolites. Little thought has been given to the fact that superoxide anions, hydrogen peroxide, and hydroxyl radicals can also be generated during the CYP reaction cycle when the electrons for the reduction of the central heme iron are transferred on the activated bound oxygen molecule (Figure 3).\(^{10,78,79}\) The continuous production of reactive oxygen species appears to be one of the most important outcomes of NADPH consumption by microsomal monooxygenases implying that these enzymes contribute significantly to the cellular production of oxygen-derived free radicals.\(^{49}\) Given that oxidative stress is now appreciated to play a significant role in the early stages of vascular disease and inflammation, it is more than likely that the CYP epoxygenases expressed in endothelial cells may contribute to the generation of oxygen-derived free radicals within the vascular wall. Indeed, the CYP 2C involved in the generation of the EDHF response in porcine coronary arterioles was recently reported to generate reactive oxygen species in cultured and native endothelial cells.\(^{45}\)
Figure 3. Scheme of the catalytic cycle of cytochrome P450 monoxygenase. The pathways marked as dotted lines produce oxygen-derived free radicals. In one branch, a superoxide anion is released as a result of the decay of the one-electron-reduced ternary complex (*). The second reactive oxygen species-producing pathway is the protonation of the peroxycytochrome P450 (§). For further details see Davydov.

Different CYP isoforms seem to generate varying amounts of oxygen-derived free radicals. For example, the proapoptotic effects of CYP 2E1 in glutathione-depleted cells has been linked to enhanced CYP-associated oxidative stress, whereas bovine aortic endothelial cells transfected with CYP 2J2 are protected against the oxidative stress induced by hypoxia and reoxygenation. Apparent differences in reactive oxygen species generation by different CYP enzymes may also reflect the extent of electron leakage from the protein complex, which has until now been attributed to the activation of the NADPH oxidase.

Vascular Homeostasis

Because the EETs and 20-HETE are known to exert cellular effects that cannot necessarily be linked to the activation of K^+ 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; however, 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. It is even possible that the EET-induced activation of K^+ channels, initially assumed to be a direct effect, is in fact mediated by a cascade of intracellular events involving the ADP ribosylation of cellular proteins including Gα.

EETs (in particular 11,12- and 14,15-EET) and 20-HETE activate several intracellular protein kinases including tyrosine kinases, the p38 MAP kinase, and extracellular regulated protein kinases 1 and 2 (Erk1/2) and increase the proliferation of various cell types, including vascular smooth muscle cells and endothelial cells. There is also evidence to suggest that, in the brain, astrocyte-derived EETs induce the formation of capillary tubes, a process likely to affect capillary density and the long-term regulation of cerebral blood flow. The precise steps between the activation of CYP and the initiation of vascular cell proliferation and angiogenesis remain to be elucidated, but the activation of Erk1/2 is more than likely to be a key step in this process as the phosphorylation and activation of Erk1/2 is generally accepted to be essential for both migration and proliferation of vascular cells. The mitogenic effects of 14,15-EET in epithelial cells also appears to be mediated by the Src family of tyrosine kinases, and 14,15-EET has been proposed to act as an intracellular second messenger after activation of the epidermal growth factor receptor. EETs, on the other hand, has been implicated in the norepinephrine-induced mitogenesis of vascular smooth muscle cells.

Whereas some of the cellular responses to EETs are nonregioisomer specific, other effects are clearly selective. For example, 11,12-EET has also been reported to exert an antiinflammatory effect in endothelial cells by inhibiting the activation of NF-κB and decreasing the cytokine-induced expression of VCAM-1, effects which were not observed in response to 14,15-EET. EETs are unique signaling molecules in that not only their regiochemical, but also their stereochromic, selectivity is under regulatory control and can be manipulated in vivo using the CYP inducer phenobarbital. However, a recent investigation failed to uncover any stereospecificity in the dilator responses elicited by stereoisomers of 9,10-, 11,12-, or 14,15-EET.

At this point, it is necessary to state a word of caution because the effects observed after the exogenous application of a CYP product to vascular cells may be different to those elicited as a consequence of endogenous CYP activation. For example, although the direct application of EETs to endothelial cells attenuates NF-κB activity, overexpressing CYP 2C9 protein in these cells to increase endogenous CYP activity has the opposite effect. The reason for this apparently contradictory effect is most probably that, as mentioned above, the endothelial CYP 2C generates reactive oxygen species that modulate NF-κB activity. Thus, despite the potential antiinflammatory properties of the EETs, enhanced activation of vascular CYPs may eventually be detrimental to vascular homeostasis as a consequence of the simultaneous generation of EETs and free radicals.
unclear whether or not a membrane receptor, such as the protein kinase A (PKA)-linked protein with a high affinity for 14,15-EET described in guinea pig monocytes, is involved in this process. Being lipophilic substances, it is also possible that EETs and HETEs can incorporate directly into the cell membrane and bind to effector molecules such as ADP ribosyltransferases, the small GTP-binding protein Ras, and PKA. Additional intracellular receptors for CYP products have not yet been identified, but one possibility is that these oxidized fatty acids bind to fatty acid binding proteins (FABPs) such as H-FABP, which in turn mediate some of the physiologically relevant actions of these intermediates, such as the activation of PPAR. The inclusion of EETs and 20-HETE into the lipid bilayer could also provide cells with a powerful tool for the control of the structural properties of individual membrane domains. Indeed, a functional role for CYP enzymes in the control of the cell membrane microenvironment has been proposed on the basis of reports showing that novel lipid-derived mediators, such as a synthetic 8,9-epoxyeicosatrienoyl-phosphatidylcholine and 1-palmityl-2-(11,12)-epoxyeicosatrienoyl phosphatidylcholine alter the Ca²⁺ permeability of synthetic liposomes and inhibit reconstituted L-type calcium channels, respectively. This idea is certainly worth investigating especially given the importance of membrane microenvironment and lipid rafting for cell signal transduction.

**CYP and Cardiovascular Disease**

As the functional relevance of vascular CYP enzymes has only been really appreciated in the last 10 to 15 years, the pathophysiological role played by CYP-derived metabolites in the regulation of vascular homeostasis has not yet been fully elucidated. Although some data are available from animal models of hypertension and heart failure, most investigations into the importance of EETs in human cardiovascular regulation have been little more than simple pharmacological characterizations of the NO/PGI₂-independent changes in vascular diameter and blood flow. Despite the limitations associated with studying human tissue, convincing evidence has recently been presented to suggest that a CYP-dependent EDHF plays a significant role in the regulation of coronary arteriolar tone by affecting K⁺ channel activation and smooth muscle hyperpolarization. Moreover, in coronary arterioles from healthy subjects, a CYP-dependent mechanism seems to account for flow-induced dilatation, with NO playing only a minor role in this response.

Given that NO interacts with hemoproteins, such as CYP to throttle enzyme activity, and EDHF-mediated responses are only unequivocally detectable when NO synthase is inhibited, it was proposed that a decrease in the bioavailability of NO (e.g., in endothelial dysfunction) would be associated with an increase in CYP activity as well as EDHF-mediated responses. Experimental evidence supports this proposal, as a shift away from NO-mediated endothelium-dependent relaxation toward EDHF-dependent relaxation has been reported in microvessels from cardiomyopathic hamsters, as well as in coronary arteries from rats with congestive heart failure. A similar phenomenon has been described for bradykinin-induced changes in forearm blood flow in essential hypertensive patients and in arterioles removed from patients with coronary artery disease, where vasodilatation is mediated entirely by a mechanism sensitive to both CYP and K⁺ channel inhibitors. Such findings indicate that in the absence of NO, vascular tone can be regulated by an EDHF-like mechanism. These observations are particularly important as they demonstrate a link between coronary artery disease/heart failure and an abnormality in the regulation of the coronary microcirculation.

There is evidence suggesting that CYP expression and EET generation are increased in hypertension, during salt loading, and in hypercholesterolemia. However, although a compensatory increase in EET formation may acutely affect vascular tone, the long-term consequences of enhanced endothelial CYP-activity on vascular homeostasis are unknown. It may be possible, however, to attribute the positive cardiovascular effects of some therapeutic agents to the upregulation of vascular CYPs. For example, nifedipine is reported to increase CYP expression, and to increase endothelial EET generation, as well as to improve EDHF-mediated hyperpolarization and relaxation of the porcine coronary artery.

The hydrolysis of EETs to DHETs is catalyzed by the epoxide hydrolases, and 11,12-DHET has been shown to relax porcine coronary arteries to a similar extent as 11,12-EET. As the DHETs are not as readily incorporated into membrane lipids as the EETs, this is thought to be the form in which the majority of endothelium-derived EETs leave the cell. A marked increase in the renal metabolism of EETs to DHETs has been reported during the development of hypertension in SHR, suggesting that either the activity or the expression of the epoxide hydrolase is increased. Such a switch may prove detrimental to vascular function as overactivity of the epoxide hydrolases could be expected to deplete the cellular pool of lipid-stored EETs. A link between the soluble epoxide hydrolase (sEH) and blood pressure was recently demonstrated in that the deletion of the sEH gene lowered systolic blood pressure and altered arachidonic acid metabolism in male mice. Moreover, the expression of sEH in renal microsomes from SHR was found to be markedly higher than in Wistar rats, and a sEH inhibitor, which decreased DHET formation, markedly reduced blood pressure in adult hypertensive animals. However, the expression of sEH, unlike the generation of EET, was elevated in SHR from birth and was not a phenomenon that could be directly correlated to the age of the animals or, more importantly, to the development of manifest hypertension.

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 reabsorption. Depending on the specific site of its generation, increased 20-HETE formation can be linked to the development of hypertension as well as to blood pressure reduction. In the Sprague-Dawley rat, for example, four independently regu-
lated CYP 4A isoforms are differentially expressed along the nephron, each of which display critical differences in their ability to generate 20-HETE (eg, two of the rat renal CYP 4A isozymes [CYP 4A2 and CYP 4A3] are reported to generate the functionally antagonistic products 20-HETE and 11,12-EET). Thus, although a decrease in the generation of 20-HETE by CYP 4A enzymes, predominantly localized in the thick ascending limb, may result in an increase in blood pressure (as reported in the salt-sensitive Dahl rat), an increase in the generation of 20-HETE in the vicinity of preglomerular microvessels would also be expected to increase afferent arteriolar tone and result in hypertension.

Several reports have demonstrated that the expression of CYP 4A and the production of 20-HETE are altered in genetic and experimental models of hypertension. For example, in SHR, deoxycorticosterone acetate (DOCA-salt, angiotensin II-infused, and Lyon hypertensive rats the renal production of 20-HETE is elevated and inhibitors of the formation of 20-HETE decrease arterial pressure (for a recent review see Moreno). The best characterized link between ω-hydroxylase activity and blood pressure has been established in the SHR. In these animals, ω-hydroxylase activity and intrarenal levels of 20-HETE are at their highest during the hypertension development phase. Selective inhibition of ω-hydroxylase activity in SHR reduces 20-HETE production and blood pressure, and in vivo administration of CYP 4A1 antisense oligonucleotides has also been reported to reduce blood pressure as well as vascular reactivity in the mesenteric bed.

A convincing demonstration of the prohypertensive effects of 20-HETE was recently provided in a study of mice lacking a CYP 4A gene (CYP 4A14). Although the finding that CYP 4A14 mice demonstrated a manifest hypertension at first appeared to contradict the hypothesis that 20-HETE is prohypertensive, these animals exhibited a paradoxical increase in renal 20-HETE production. The increase in 20-HETE production, however, was a direct consequence of CYP 4A14 deletion, as the lack of this enzyme was associated with an increase in circulating levels of androgens, as well as the renal expression of a second CYP 4A gene (CYP 4A12) in close proximity to afferent arterioles. Enhanced CYP 4A12 expression and 20-HETE formation coincided with increased afferent arteriolar resistance and an altered autoregulatory capacity. Hypertension was more pronounced in males, and castration normalized blood pressure and attenuated both CYP 4A12 expression and 20-HETE production whereas androgen replacement restored hypertension. These results demonstrate a novel chain of events that make a pronounced impact on the cardiovascular system consisting of a CYP 4A-dependent regulation of androgen production, an androgen-dependent regulation of renal 20-HETE production, and 20-HETE-induced alterations in vascular resistance leading to hypertension.

**CYP Polymorphisms**

Of the CYP enzymes currently thought to have a cardiovascular relevance, the 2J and 2C families are reported to be polymorphic. At least 5 single nucleotide polymorphisms of CYP 2C9 have been identified and appear to be population specific as CYP2C9*2 has been detected in approximately 22% of Caucasians but not in the Japanese population, whereas CYP2C9*3 is reported to occur with a frequency of about 8% in both races. The CYP2C9*5 variant, on the other hand, was found to be expressed only in African Americans. Single nucleotide polymorphisms are known to alter CYP activity and reduce substrate metabolism, and in vitro data suggest that carriers of the mutant CYP 2C9 would eliminate CYP 2C9 substrates at slower rates than individuals expressing the wild-type protein. Clinical importance of CYP polymorphisms for cardiovascular or endothelial function has not yet been studied and work has concentrated on the association of polymorphism with altered drug metabolism and carcinogenesis. There is, for example, evidence indicating that CYP polymorphisms alter the metabolism of the angiotensin II receptor antagonist, losartan, and of the HMG-CoA reductase inhibitor, fluvastatin. Both agents are reported to affect (albeit not exclusively), CYP 2C expression and/or activity. Therefore, in addition to affecting the function of CYP enzymes expressed in the vasculature, CYP polymorphism may determine the response of a given individual to a cardiovascular drug.

Polymorphisms within the coding or promoter regions of CYP genes could also affect the metabolism of arachidonic acid resulting in an altered EET- 20-HETE–generating capacity. It therefore seems logical to propose a link between alterations in CYP expression and/or activity and cardiovascular disease. Identification of polymorphisms and the associated phenotypes will almost certainly help to elucidate the role of CYPs and their products in the modulation of cardiovascular function.

**Acknowledgments**

Experiments performed in the author’s laboratory were supported by the Deutsche Forschungsgemeinschaft (SFB 553, B5) and Institut de Recherches Internationales Servier.

**References**

5. Scarbrough PE, Ma J, Qu W, Zeldin DC. P450 subfamily CYP2J2 and their role in the bioactivation of arachidonic acid in extrapluetic tissues. Drug Metab Rev. 1999;31:205–234.


Fleming


101. Imig JD, Inscho EW, Deichmann PC, Reddy KM, Falck JR. Afferent arteriolar vasodilation to the sulfinamide analog of 11,12-
dehydoxyicosatrienonic acid involves protein kinase A. Hypertension. 1999;
33:408–413.
102. Widstrom RL, Norris AW, Spector AA. Binding of cytochrome P450
monooxygenase and lipoxygenase pathway products by heart fatty acid-
103. Wolfrum C, Bormann CM, Borchers T, Spener F. Fatty acids and
hypolipidemic drugs regulate peroxisome proliferator-activated receptors α-
and γ-mediated gene expression via liver fatty acid binding protein: a signaling
104. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic
cacid bioactivation: molecular and functional properties of the arachidonic
105. Chen J, Capdevila JH, Zeldin DC, Rosenberg RL. Inhibition of cardiac
106. Clark SG, Fuchs LC. BK Ca channels compensate for loss of NOS-
ulator of the CYP4A genes in the spontaneously hypertensive

deleted expression of the CYP4A genes in the spontaneously hyper-
108. Frisbee JC, Falck JR, Lombard JH. Contribution of cytochrome P-450
109. Frisbee JC, Falck JR, Lombard JH. Contribution of cytochrome P-450
spontaneously hypertensive rat kidney. Mol Pharmacol. 2000;57:
1011–1020.
110. Imig JD, Inscho EW, Deichmann PC, Reddy KM, Falck JR. Afferent arteriolar vasodilation to the sulfinamide analog of 11,12-
dehydoxyicosatrienonic acid involves protein kinase A. Hypertension. 1999;
33:408–413.
102. Widstrom RL, Norris AW, Spector AA. Binding of cytochrome P450
monooxygenase and lipoxygenase pathway products by heart fatty acid-
103. Wolfrum C, Bormann CM, Borchers T, Spener F. Fatty acids and
hypolipidemic drugs regulate peroxisome proliferator-activated receptors α-
and γ-mediated gene expression via liver fatty acid binding protein: a signaling
104. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic
cacid bioactivation: molecular and functional properties of the arachidonic
105. Chen J, Capdevila JH, Zeldin DC, Rosenberg RL. Inhibition of cardiac
106. Clark SG, Fuchs LC. BK Ca channels compensate for loss of NOS-
ulator of the CYP4A genes in the spontaneously hypertensive

deleted expression of the CYP4A genes in the spontaneously hyper-
108. Frisbee JC, Falck JR, Lombard JH. Contribution of cytochrome P-450
109. Frisbee JC, Falck JR, Lombard JH. Contribution of cytochrome P-450
spontaneously hypertensive rat kidney. Mol Pharmacol. 2000;57:
1011–1020.
Cytochrome P450 and Vascular Homeostasis
Ingrid Fleming

Circ Res. 2001;89:753-762
doi: 10.1161/hh2101.099268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/89/9/753

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/