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Review Article

Cytochrome P-450: a new target in the heart and coronary circulation

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CONTENTS

Abstract

Cytochrome P-450 enzymes (CYPs) are present in the heart and coronary circulation. The activation of CYP2J2/3 to increase the production of epoxyeicosatrienoic acids (EETs) may be cardioprotective in cardiac ischemia and reperfusion. On the other hand, inhibition of CYP2C9 with cimetidine, or more selectively with sulfaphenazole, is cardioprotective in the perfused rat heart. In a dog model of cardiac ischemia and reperfusion, inhibitors of CYP ω-hydroxylases (*e.g.,* CYP4A, CYP4F) reduce infarct size. Inhibition of selected CYPs as an approach to the treatment of myocardial infarction should therefore be further developed. The function of CYPs in the coronary artery and other blood vessels is varied and complex, and only just beginning to be elucidated. In the coronary artery, when nitric oxide (NO) bioavailability is inhibited, the activity of CYPs may be increased, with the production of vasodilatory EETs. Conversely, when CYP2C9 is inactivated in humans with coronary artery disease (CAD), acetylcholine-induced NO-mediated vasodilatation is enhanced. In the presence of CAD/oxidized LDL, the EET vasodilatory system may be inhibited. Drugs that target CYPs and/or EETs are thus expected to be useful in the elucidation of the role of this system, and may also have therapeutic utility.

Introduction

Coronary artery disease is the leading cause of death in Americans, with half a million deaths every year, and is associated with an annual incidence of myocardial infarction of about 1.5 million (1). After myocardial infarction, long-term damage can be reduced by rapid reperfusion. However, further damage is also inflicted during reperfusion. Recent experimental evidence suggests that inhibiting selected cytochrome P-450 enzymes (CYPs) may be useful in myocardial ischemia and reperfusion.

CYPs are membrane-bound terminal oxidases that exist in a multienzyme system, many of which are expressed in the liver. There are currently about 1,000 known CYPs, and 50 have been shown to be functionally active in humans. These are categorized into 17 families (*e.g.*, CYP1), which are further divided on the basis of subfamily (*e.g.,* CYP1A) and isoforms (*e.g.*, CYP1A1). In the liver, the CYPs metabolize many endogenous and exogenous compounds, including environmental chemicals and drugs. Arachidonic acid can be metabolized by CYP epoxygenases to epoxyeicosatrienoic acids (EETs; 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) and by CYP ω-hydroxylases to 20-hydroxyeicosatetraenoic acid (20-HETE). CYPs have recently been identified in the heart and blood vessels, but our understanding of the role of these CYPs is only just beginning to be unraveled.

Part of our understanding of the role of CYPs in the cardiovascular system comes from polymorphism studies. A polymorphism leading to reduced gene activity of CYP2J2 is associated with an increased risk of CAD, indicating a cardioprotective role for CYP2J2 (2). The possession of rare genetic variants of CYP2C8 or CYP2C9 is associated with a small increase in the risk of myocardial infarction (3). This may be related to genetic differences in the formation of endogenous CYP2C products in the heart or blood vessels. A genetic variant in CYP1A1 in cigarette smokers increases the risk for triple-vessel disease to a small extent, suggesting that CYP1A1 may participate in the pathogenesis of atherosclerosis (4).

This review focuses on CYPs in the heart and blood vessels, particularly the coronary artery, and their functional roles in the cardiac and vascular systems. Their potential as new targets for drugs for cardiovascular disease is also considered.

Cytochrome P-450 enzymes in the heart and blood vessels

CYP2J2 is highly expressed in the human heart (5), and recently, the presence of other CYPs was detected in

the explanted human heart. mRNA for CYP1A1, CYP2B6/7, CYP2C8-19, CYP2D6 and CYP4B1 was predominantly expressed in the right ventricle. Verapamil can be metabolized by CYP2C and by the human heart, also indicating the presence of this enzyme. However, the gene for CYP3A, the major drug-metabolizing enzyme in the liver and lung, and those for CYP2F1 and CYP2A6/7 are not expressed in the heart (6). In cardiac hypertrophy, there is increased gene expression of CYP2A6/7 and CYP4A11 (7), and in heart failure, the following CYPs are upregulated: CYP2J2, CYP1B1, CYP2EA, CYP4A10 and CYP2F2 (reviewed in 8).

Human and porcine coronary artery endothelial cells express CYP2C8, CYP2C9 and CYP2J2 (9). More recently, the expression of CYP1A1, CYP2A6/7, CYP2B6/7, CYP2C8, CYP2C9, CYP2E1 and CYP2J2 was detected in human coronary artery endothelial cells, whereas transcript levels of CYP2C18, CYP2D6, CYP3A4, CYP3A5, CYP3A7 and CYP4B1 were below the limit of detection (10).

Tobacco smoke and cocaine are known to increase the incidence of myocardial infarction. In addition to inducing liver CYPs, tobacco smoke also induces heart CYPs, which may be involved in myocardial infarction (8). In mice, exposure to cocaine induces cardiac CYP2J2 and CYP1A1, but not CYP1A2 (11). In contrast, certain cardioprotective agents inhibit CYPs. Resveratrol, an antiatherogenic and cardioprotective polyphenolic compound in red wine, inhibits CYP1A1. It is well known that the cardioprotective effects of the statins cannot solely be explained in terms of inhibition of HMG-CoA reductase. As some of the statins also inhibit CYPs, this may contribute to their cardioprotective effects (8). Clinical trials have recently demonstrated that intense lipid lowering with atorvastatin is more effective than modest lipid lowering with pravastatin in reducing clinical events (reviewed in 12). Atorvastatin inhibits CYP2C19 and CYP3A4, and to a lesser extent CYP2D6, whereas pravastatin has a negligible effect on CYPs (8). Thus, it is possible that the added benefits of atorvastatin may be related to its inhibition of CYPs.

Functions of cytochrome P-450 enzymes in the heart

Little is known about the physiological role of heart CYPs. Studies using CYP inhibitors in rat ventricular myocytes suggest that CYPs may modulate the L-type Ca^{2+} current, as inhibition of CYPs is associated with inhibition of the current and cell shortening (13). In mice with cardiac-specific overexpression of CYP2J2, there is an enhancement of the L-type Ca^{2+} current (14). Evidence that the EET metabolites of CYPs (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) directly inhibit cardiac L-type Ca^{2+} channels has been obtained using reconstituted channels (15). EETs are potent activators of K_{ATP} channels in rat ventricular myocytes (16), whereas high concentrations of EETs are required to inhibit cardiac sodium channels (17). CYPs have a role in steroid metabolism, and there may be a relationship between testosterone, CYPs and cardiac hypertrophy (7), but this has yet to be confirmed.

EETs have no effect in isolated guinea pig hearts, but when the hearts are subjected to low-flow ischemia, 5,6-EET and 11,12-EET delay recovery, suggesting that these metabolites have a detrimental effect (18). The levels of EETs and 20-HETE are increased in coronary venous plasma during ischemia and reperfusion in dogs (19).

The pathological role of the heart CYPs has also been investigated using CYP inhibitors. As there are fewer CYPs in the heart and they are present in small amounts, it has been suggested that inhibitors may be able to have a more profound effect on the heart than on the liver (8).

CYP2J2/3 and cardioprotection

Although multiple CYPs are expressed in the heart, CYP2J2 is unique in that it is primarily expressed in the heart, abundant in cardiomyocytes and active in the biosynthesis of EETs (reviewed in 20). The EETs produced by CYP2J2/3 may be cardioprotective. Rat heart CYP2J3 has 70% homology with human heart CYP2J2. In isolated perfused rat hearts, 11,12-EET and 14,15-EET had no effect, whereas in hearts subjected to ischemia and reperfusion with impaired contractility, the administration of 11,12-EET (but not 14,15-EET) prior to ischemia improved the recovery of cardiac contractility (21). The effects of EET administration after ischemia were not reported in this study, and thus it is not known whether EETs are effective postischemia.

Transgenic mice overexpressing CYP2J2 have an increased capacity for EET biosynthesis. The transgenic mice show improved recovery of cardiac function after 20 min of ischemia and 40 min of reperfusion compared to wild-type mice. Results with the selective cytochrome P-450 epoxygenase inhibitor MS-PPOH, which reduces the levels of EETs, confirmed that the improvement was due to the EETs. Thus, MS-PPOH inhibited postischemic recovery in wild-type mice to a small extent, indicating that EETs may be cardioprotective under normal conditions. MS-PPOH abolished the benefit seen in the mice overexpressing CYP2J2 after ischemia. The cardioprotective effects of CYP2J2 overexpression were also abolished by treatment with the K_{ATP} channel inhibitor glibenclamide or the mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate, indicating that the mechanism of cardioprotection with the CYP2J2-derived EETs involves the mitochondrial K_{ATP} channel (20).

CYP2C9 and reperfusion injury

The histamine H_2 receptor blocker cimetidine also inhibits CYP2C9 and protects against reperfusion injury. Thus, in a rabbit model of lung reperfusion injury, cimetidine and other CYP inhibitors decreased lung edema and

prevented the reperfusion-related increase in lung microvascular permeability (22). In the isolated perfused rat heart after ischemia, continuous perfusion with cimetidine reduced the incidence of ischemia-induced ventricular fibrillation from 57% in controls to 8%, and the incidence of reperfusion-induced fibrillation from 78% to 38%. In contrast, another histamine H_2 receptor blocker, ranitidine, had no effect on ischemia/reperfusion-induced ventricular fibrillation. This suggests that inhibition of CYPs, rather than H_2 receptor antagonism, underlies the ability of cimetidine to reduce ventricular arrhythmias (23).

During reperfusion, the heart undergoes further damage, characterized by excessive production of reactive oxygen species (ROS), which damage DNA, proteins and lipids. Mitochondria are generally considered to be the main source of the ROS, but other enzyme systems (*e.g.*, NAD(P)H oxidase, NO synthase [NOS] and xanthine oxidase) may contribute to ROS production in reperfusion injury, although of these, only NOS has been shown to have a role to date. It was recently postulated that cardiac CYPs may be the source of the ROS (24).

Inhibition of heart CYP2C9 may be a useful approach to preventing ischemia/reperfusion injury, as drugs that inhibit heart CPY2C9 are cardioprotective in animal models of ischemia/reperfusion injury. Although it was initially thought that cardioprotection might be due to inhibition of mitochondrial protein synthesis, it was later discovered that inhibition of heart CYP2C9 was responsible for the cardioprotective effect (24).

Mitochondrial dysfunction is a characteristic of ischemic injury and recovery of mitochondrial function is essential for myocardial contractility. Ischemia and reperfusion profoundly affect mitochondria, and one of the many effects observed is ROS production. The antibiotic chloramphenicol is known to inhibit mitochondrial protein synthesis, probably because mitochondrial ribosomes resemble bacterial ribosomes. Consequently, a cardioprotective effect of chloramphenicol would implicate mitochondrial protein synthesis and the subsequent production of ROS in reperfusion damage (24). Perfused rat hearts were treated with chloramphenicol (300 μM) for 20 min before ischemia or upon reperfusion. No-flow ischemia was maintained for 30 min prior to reperfusion for either 15 min (to measure creatine kinase release) or for 120 min prior to determination of infarct size. Chloramphenicol pretreatment before ischemia decreased the release of creatine kinase, a measure of heart damage. When administered prior to ischemia, it also reduced infarct size from 43% (untreated) to 16%, and treatment during reperfusion reduced infarct size from 43% to 22%, illustrating that much of the protection afforded by chloramphenicol was during reperfusion (24).

Chloramphenicol was also cardioprotective in a rabbit model of ischemia and reperfusion. In this model, anesthetized rabbits received i.v. chloramphenicol (20 mg/kg) 30 min prior to 30 min of coronary occlusion and 4 h of reperfusion. The infarct size was smaller in the chloramphenicol-treated (18%) than the untreated rabbits (49%). These results fit with the mitochondrial protein synthesis hypothesis. However, when the levels of mitochondrial protein synthesis or mitochondrial respiratory chain activity were measured directly, chloramphenicol showed no major effects (24).

Chloramphenicol also inhibits CYPs (25), which may account for the cardioprotection. When cardiac microsomes from the perfused rat heart were analyzed for CYP activity, this activity was found to be inhibited by 95% in microsomes from hearts treated with chloramphenicol. If inhibition of CYP activity underlies the cardioprotective effect of chloramphenicol, other agents that inhibit CYPs but not mitochondrial protein synthesis should also be cardioprotective. Cimetidine, an inhibitor of CYP2C9, is also cardioprotective, as demonstrated in the perfused rat heart, where it reduced creatine kinase release and infarct size. In contrast, ketoconazole, a potent inhibitor of CYP3A4 and a weak inhibitor of CYP2C9, was not cardioprotective. Sulfaphenazole is another potent and selective inhibitor of CYP2C9 (26). In the perfused rat heart, sulfaphenazole (10 and 30 μM) reduced creatine kinase release and reduced the infarct size from 45% (control) to ~16% and 6%, respectively. A similar effect was observed when sulfaphenazole was administered after ischemia (24). These findings confirm that inhibition of CYP2C9 underlies the cardioprotective effect observed in these experiments.

20-HETE-producing CYPs and ischemia/reperfusion injury

In addition to being metabolized to EETs, arachidonic acid can be metabolized by CYP ω-hydroxylases to 20-HETE. In anesthetized dogs, during 60 min of coronary artery occlusion and reperfusion, the predominant CYP metabolite of arachidonic acid in the venous plasma was 20-HETE, with only low concentrations of EET metabolites. The production of 20-HETE could be inhibited by intracoronary administration of CYP inhibitors prior to occlusion. Inhibition was observed with both a nonspecific CYP inhibitor (miconazole) and specific CYP ω-hydroxylase inhibitors (17-octadecanoic acid and *N*-methylsulfonyl-12,12-dibromododec-11-enamide [DDMS]) (27). At the end of 3 h of reperfusion, myocardial infarct size was determined and expressed as a percentage of the area at risk, which was 19.6% in control dogs. Inhibition of CYPs with miconazole, 17-octadecanoic acid and DDMS reduced the infarct size to 8.4%, 5.9% and 10.8%, respectively. Conversely, exogenously administered 20-HETE increased the infarct size to 26.9% (27).

Several CYPs, including CYP4A and CYP4F, can ω-hydroxylate arachidonic acid to 20-HETE. The dog heart was shown to express CYP4A1, CYP4A2 and CYP4F protein. The heart tissue microsomes produced 20-HETE, which was inhibited by 17-octadecanoic acid (27). These results suggest that inhibition of CYP ω-hydroxylase is cardioprotective.

Functions of cytochrome P-450 enzymes in the coronary artery and other blood vessels

The function of CYPs in the coronary artery and other blood vessels is varied and complex, and only just beginning to be elucidated. In addition to NO and prostacyclin, other vasodilators are produced by the endothelium lining the blood vessels. These endothelium-derived hyperpolarizing factors (EDHFs) are synthesized and released from the endothelium in response to acetylcholine or bradykinin, and then hyperpolarize vascular smooth muscle cells to induce vasodilatation. The EDHF produced in some vascular beds, including the coronary circulation, may be a CYP-derived metabolite of arachidonic acid, and CYP2C9 may be EDHF synthase. The CYP products proposed to be EDHFs are: 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET (reviewed in 28). In addition to relaxing the main coronary arteries of most species (*e.g.*, 29), these EETs have been shown to be potent dilators of the canine coronary microcirculation (30). 14,15-EET can be metabolized by human coronary artery endothelial cells to 10,11-epoxyhexadecadienoic acid, which is a potent dilator in coronary microvessels (31). Whereas the EET products from the CYP epoxygenase pathways relax some blood vessels, the product of the CYP ω-hydroxylase pathway, 20-HETE, has been shown to constrict certain blood vessels (*e.g.*, small porcine coronary arteries) (32).

EETs activate high-conductance Ca^{2+}-dependent K^+ channels on pig coronary artery endothelial cells, and this effect may be associated with their ability to relax coronary arteries (33). Opening of vascular K_{ATP} channels is associated with vasodilatation, and 11,12-EET has been shown to activate these channels in isolated smooth muscle cells from rat mesenteric arteries (34). In addition to vasodilatation, EETs may have other effects on blood vessels, including activation of mitogen-activated protein kinases (MAPKs) and antiinflammatory effects in endothelial cells (28). Recently, 11,12-EET, the product of CYP2C, has been shown to transiently increase and then uncouple gap junction communication between porcine coronary endothelial cells (35). 11,12-EET also inhibits vascular smooth muscle cell migration in cell culture (36).

To obtain responses mediated by EDHF alone, the synthesis of prostacyclin and NO must be inhibited by cyclooxygenase inhibitors (such as diclofenac) and NOS inhibitors (*e.g.*, L-NAME). Under these conditions, a role for CYPs in relaxation responses to EDHF in the coronary and renal vasculature has been demonstrated. As the role of CYPs can only be demonstrated when the prostacyclin and NO pathways are blocked, it has been suggested that the CYP system of coronary vasodilatation acts as a back-up when the other systems fail.

In other vascular beds, including the canine femoral artery (37), guinea pig carotid artery (38, 39), rat hepatic artery (40) and guinea pig basilar artery (41), it is unlikely that CYPs have a role in EDHF relaxation. Also, there may be some species differences, as although EETs have been shown to relax bovine, porcine and human coronary arteries, they did not relax guinea pig coronary arteries (42). Moreover, the EETs may be vasoconstrictors in some vascular beds, *e.g.*, porcine aortic smooth muscle (43), the rat mesenteric artery response to angiotensin II (44), the rat afferent arteriolar response to endothelin-1 (45), rabbit (46) and rat pulmonary arteries (47).

Coronary artery relaxation

The ability of a CYP inhibitor (SKF-525a) to inhibit the relaxation responses to arachidonic acid in bovine coronary arteries was reported as long ago as 1993 (48). Subsequently, it was suggested that the EETs were the EDHF of the bovine coronary artery, as least partly based on the fact that methacholine-induced hyperpolarization was inhibited by SKF-525a (49). EET antagonists have also been useful in identifying the EDHF of the bovine coronary artery as EET. A 14,15-EET analogue, 14,15-epoxyeicosa-5(*Z*)-enoic acid (14,15-EEZE), acts as an EET antagonist to inhibit both EET-induced relaxation and the EDHF component of methacholine-, bradykinin- or arachidonic acid-induced relaxation (50).

The induction of CYPs in native porcine coronary artery endothelial cells enhances the formation of 11,12-EET, as well as EDHF-mediated hyperpolarization and relaxation. Incubation of porcine coronary arteries with an antisense oligonucleotide against the coding region of CY2C8/9 reduced CYP2C mRNA and protein, and selectively attenuated bradykinin-induced EDHF-mediated hyperpolarization and relaxation (9).

CYP2B, CYP2C and CYP2J have been shown to be present in native porcine and cultured human coronary endothelial cells. The expression of CYP2C mRNA, but not CYP2B or CYP2J mRNA, was increased by the CYP inducer β-naphthoflavone and by nifedipine. Nifedipine also increased the production of 11,12-EET from native porcine endothelial cells. In contractility experiments, nifedipine enhanced bradykinin-induced EDHF-mediated hyperpolarization and relaxation. The effects of nifedipine in this study were not due to Ca^{2+} channel-blocking activity, but possibly to downregulation of NOS and NO regulation of CYP. In support of this, the CYP2C9 inhibitor sulfaphenazole attenuated EDHF-mediated hyperpolarization and relaxation. This suggests that CYP2C is the CYP-dependent EDHF synthase in porcine coronary arteries (51).

In the porcine coronary artery, NO inhibits the activity of CYP/EDHF synthase. Thus, when NO bioavailability is decreased, the activity of the CYP/EDHF synthase system and the production of vasodilatory EETs are increased. Consequently, CYP/EDHF/EET-mediated vasodilatation can compensate in the absence of NO-mediated vasodilatation (52). CYP2C9 is also found in human coronary artery endothelial cells, where it has been shown to generate the ROS superoxide anion, which in turn attenuates the bioavailability of NO. In the presence of diclofenac to inhibit the production of prosta-

cyclin, the coronary artery relaxant response to bradykinin is predominantly mediated by NO, and when CYP2C is downregulated in the arteries by antisense oligonucleotides, these responses to NO are potentiated. Sulfaphenazole and superoxide scavengers (Tiron and nordihydroguaretic acid) also potentiated these responses to NO. In microsomes from cells overexpressing CYP2C9, CYP2C9 and superoxide activity was inhibited by sulfaphenazole. ROS generation in coronary artery rings was attenuated by CYP2C antisense treatment or sulfaphenazole. These results suggest that CYP2C9 regulates ROS production and NO relaxation in porcine coronary arteries (53).

Following exposure to cortisol, the EDHF-mediated relaxation of U-46619-constricted porcine coronary arteries (in the presence of cyclooxygenase and NOS inhibition) was enhanced. Cortisol also upregulated the expression of CYP2C in porcine coronary endothelial cells, with the same time course as for the enhancement of relaxation, providing evidence that CYP2C plays a role in the generation of the EDHF-mediated response in the coronary endothelium (54).

At supratherapeutic concentrations, the statins cerivastatin and fluvastatin induce CYP2C in porcine coronary artery endothelial cells. This induction was associated with an increase in the EDHF-mediated bradykinin-induced relaxation of porcine coronary arteries in the presence of diclofenac and L-NAME (55). This evidence further supports a role for CYP2C metabolites in EDHF-mediated relaxation.

Stretch may be a stimulus for upregulation of CYPs and the generation of EETs. In cultured porcine coronary and human umbilical vein endothelial cells, stretch elicited the generation of 8,9-EET, 11,12-EET and 14,15-EET. Prolonged stretch increased both EET generation and the expression of CYP2C mRNA and protein. There was also an increase in the expression of CYP2C mRNA and protein in pressurized segments of porcine coronary artery perfused under pulsatile conditions (56).

Recently, a link was found between endothelial CYPs and oxidized LDL. Oxidized LDL is associated with vascular injury and atherosclerotic plaque formation, and this association is probably mediated through the lectin-like oxidized LDL receptor (LOX-1). In this study, the expression of CYP1A1, CYP2A6/7, CYP2B6/7, CYP2C8, CYP2C9, CYP2E1 and CYP2J2 was detected in human coronary artery endothelial cells, whereas transcript levels of CYP2C18, CYP2D6, CYP3A4, CYP3A5, CYP3A7 and CYP4B1 were below the limit of detection. Oxidized LDL treatment caused regression of all the detectable CYPs in these endothelial cells. CYP 1A1, CYP2B6/7, CYP2E1, CYP2J2, CYP2C8 and CYP2C9 transcripts were also suppressed in diseased human aortic tissue. Treatment of human coronary artery endothelial cells with the oxidized LDL receptor inhibitor κ-carrageenan or with a specific LOX-1 antibody restored the gene expression levels of CYP1A1, CYP2C8, CYP2C9, CYP2E1 and CYP2J2 to normal. Both oxidized LDL and sulfaphenazole decreased the production of 11,12-EET from the endothelial cells. The production levels of this CYP metabolite in the presence of oxidized LDL were restored by κ-carrageenan. These results suggest a mechanistic role for CYPs in oxidized LDL-induced vascular injury (10).

Coronary artery endothelial CYPs may contribute to effects observed in intact hearts. Bradykinin is a vasodilator in the isolated rat heart, and this response is similar to that of 5,6-EET (57). In rat hearts perfused at constant pressure, bradykinin is a negative inotrope, and this response is absent after CYPs are inhibited with aminobenzotriazole or proadifen, or after the coronary endothelium has been rendered dysfunctional with Triton X-100. The CYP metabolite 14,15-EET mimics the effect of bradykinin. These results suggest that endothelial CYP products mediate the negative inotropic effects of bradykinin (58).

Acetylcholine is another well-known cardiodepressant, and the effect of acetylcholine is considered to be mediated by cardiac muscarinic receptors. However, acetylcholine also acts on endothelial cells to initiate the release of NO, prostacyclin and EDHF. In isolated rat hearts, the cardiodepressant effect of acetylcholine can be partially attenuated by inhibiting CYPs with 1-aminobenzotriazole or by inducing endothelial dysfunction with Triton X-100. In contrast, inhibition of NOS or cyclooxygenase does not influence the cardiodepressant effect of acetylcholine (59). These results suggest that endothelial CYP products contribute to the negative inotropic effects of acetylcholine.

Other blood vessels where EDRF may be an EET

As the main emphasis of this review is on the heart and coronary circulation, blood vessels other than the coronary arteries where EDRF may be an EET are only considered briefly. There is extensive literature showing that the EETs have both a physiological and pathological role in the renal vasculature, and a role in relaxing the human internal mammary arteries, rat small mesenteric arteries and the cerebral microvasculature is emerging.

1. Renal arteries

In the presence of an AT_1 receptor antagonist, angiotensin II causes an AT_2 receptor-mediated vasodilatation of rabbit renal afferent arterioles, which could be abolished by inhibiting CYPs, suggesting that the vasodilatation is EET-mediated (60). In the absence of an AT_1 receptor antagonist, the vasoconstrictor response to angiotensin II is augmented when CYPs are inhibited with miconazole. This suggests that the vasodilating response to endogenous EETs modulates the angiotensin II-induced constriction in the rabbit afferent arteriole (61). The NO-independent bradykinin-induced relaxation in the rat renal afferent arterioles (62) and rabbit efferent arterioles (63) is also mediated by EETs. The

acetylcholine-induced relaxation in rabbit renal afferent arterioles is also partly mediated by EETs (64).

EETs are metabolized to dihydroxyeicosatrienoic acids (DHETs), which do not have effects on the preglomerular vasculature. This conversion can be inhibited with the epoxide hydrolase inhibitor *N*-cyclohexyl-*N*-dodecylurea (NCND), which should lead to an accumulation of vasodilatory EETs. When hypertension is induced by angiotensin infusion in the rat, NCND lowers blood pressure (65).

2. Mammary arteries

CYP2C is present in the endothelium and smooth muscle cells of human left internal mammary arteries. The predominant EET synthesized by human internal mammary arteries is 11,12-EET, which relaxes the artery. EDRF-mediated acetylcholine- or bradykinin-induced relaxation of phenylephrine-contracted internal mammary arteries was determined in the presence of meclofenamate and L-NAME to inhibit cyclooxygenase and scavenge any residual NO. The EDRF-mediated relaxation was inhibited by the CYP inhibitors 17-octadecanoic acid or *N*-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide, and by the selective EET antagonist 4-15-epoxyeicosa-5(*Z*)-enoic acid. 11,12-EET activates the large-conductance Ca^{2+}-dependent K^+ channel (BK_{Ca}) to induce hyperpolarization, and this can be blocked by a specific BK_{Ca} blocker (iberiotoxin), but not by inhibitors of other K^+ channels (66).

3. Mesenteric arteries

EETs induce relaxation of rat small mesenteric arteries, but not mesenteric arteries from rats with insulin resistance (67). Compared to lean rats, mesenteric arterial CYP2C11 and CYP2J protein levels were decreased in obese Zucker rats. Soluble epoxide hydroxylase mRNA and protein expression was increased in obese mesenteric arteries. NO-independent (EDRF) acetylcholine-induced dilatation was attenuated in mesenteric arteries from the obese rats (68).

There is some evidence that the attenuation of vasoconstrictor responses after chronic hypoxia is due to enhanced CYP2C9 expression. Superior mesenteric arteries isolated from rats subjected to chronic hypoxia are hyperpolarized, which can be reversed by CYP inhibition with 17-octadecanoic acid and SKF-525A, or the CYP2C9 inhibitor sulfaphenazole. CYP2C9 protein levels were greater in arteries from hypoxic rats, as were 11,12-EET levels in the endothelial cells (69).

4. Cerebral vessels

EETs may also have an important role in the cerebral microvasculature, where in addition to being produced by the vascular endothelium, they are synthesized by astrocytes (reviewed in 70).

Human studies

In healthy volunteers, inhibition of CYP2C9 with sulfaphenazole alone did not affect forearm blood flow, as measured by venous occlusion plethysmography. Sulfaphenazole also had no effect on NO- or prostacyclin-induced vasodilatation (71). This suggests that CYP2C9 has no effect on forearm blood flow under normal physiological conditions. In contrast, in patients with stable CAD, although sulfaphenazole had no effect alone, it enhanced the forearm blood flow response to acetylcholine, which is mediated by NO. When NOS was inhibited with L-NMMA, sulfaphenazole had no effect on the response to acetylcholine, suggesting that sulfaphenazole enhances the response to acetylcholine by increasing the bioavailability of NO, probably as a consequence of an attenuated generation of ROS by CYP2C9 in endothelial cells (72).

CYPs may have a role in the regulation of hyperemia and oxygen uptake during exercise. In humans performing exercise, neither sulfaphenazole alone nor the NOS inhibitor L-NMMA alone had any effect on skeletal blood flow. However, with the combination of sulfaphenazole and L-NMMA, there was a decrease in blood flow and oxygen uptake during exercise. As inhibition of CYP2C9 or NOS alone had no effect, these results suggest an interaction between CYP2C9 and NOS, such that CYP-mediated vasodilatation takes over when NO production is compromised (73).

Conclusions

Although research has clearly shown that inhibition of CYP2CP or CYP ω-hydoxylases is associated with a reduction in myocardial infarct size in animal models, effects on cardiac function have not been described (24, 27). *In vitro* and *in vivo* studies of the effects of inhibiting CYP2C9 or CYP ω-hydoxylases on cardiac function are clearly needed.

In the development of cardioprotective agents, one of the major problems is that many of the agents are effective when administered prior to ischemia, but not so effective when administered postischemia (*e.g.*, inhibitors of Na^+ transporters) (74). In terms of practical application, where drugs would be administered after a myocardial infarction, this limits their potential. One of the major advantages suggested from the experimental data is that the benefits of CYP2C9 inhibitors occurred during reperfusion, and consequently, these agents have potential for use after myocardial infarction (24). The effects on infarct size of inhibiting CYP ω-hydoxylases after ischemia have not been assessed to date, and would be of interest. As initial experimental studies have shown beneficial effects from inhibiting selected CYPs during ischemia/reperfu-

sion in animal models of myocardial infarction, this promising approach should be further investigated for the treatment of myocardial infarction.

Both CYP2C9 and CYP2J2 generate EETs, but whereas inhibition of CYP2C9 has been shown to be beneficial in an animal model of cardiac ischemia/reperfusion, overexpression of CYP2J2 has also been shown to be beneficial. How can this be explained? The authors of the study showing inhibition of CYP2C9 to be beneficial suggest that reduced ROS, rather than EETs, underlies the beneficial effects. On the other hand, it seems likely that the EETs are responsible for the benefits of overexpressing CYP2J2.

The epoxide hydrolase inhibitor NCND has been used to increase vasodilatory EETs and has been shown to reverse angiotensin-induced hypertension in rats (65). It would be of interest to determine whether enhancing the levels of EETs is associated with cardioprotective effects in animal models of cardiac ischemia and reperfusion.

The function of CYPs in the coronary artery and other blood vessels is varied and complex, and only just beginning to be elucidated. One interesting aspect is that the vasodilatory effect of the EETs is not common to all vascular beds. Thus, drugs that target the CYP/EET system may be selective for certain vascular beds, which could represent an advantage over nonselective drugs.

A pathological role for CYP in the coronary circulation is beginning to emerge. In the bovine coronary artery, when NO bioavailability is inhibited, the activity of the CYPs may be increased, with the production of vasodilatory EETs. Conversely, when CYP2C9 is inactivated in humans with CAD, NO-mediated acetylcholine-induced vasodilatation is enhanced. In the presence of CAD/oxidized LDL, the EET vasodilatory system may be inhibited. Clearly, further elucidation of the influence of the CYP/EET system on the vasculature is needed. Drugs that target the vascular CYP/EET system will not only be of value for elucidating the function of this system, but may also have therapeutic applications, *i.e.*, mimicking the vasodilatation of EETs may be useful in the treatment of CAD.

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