Cytochrome P450 and arachidonic acid metabolites: Role in myocardial ischemia/reperfusion injury revisited

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Abstract

Ischemia–reperfusion of the heart and other organs results in the accumulation of unesterified arachidonic acid (AA) via the action of membrane-bound phospholipases, primarily phospholipase A2. AA can be metabolized by the classical cyclooxygenase (COX) and lipoxygenase (LOX) pathways to well-characterized metabolites and their respective cardioprotective end products such as prostacyclin (PGI2) and 12-hydroxyeicosatetraenoic acid (12-HETE). However, it has only been recently recognized that another less well-characterized pathway of AA metabolism, the cytochrome P450 (CYP) pathway, may have important cardiovascular effects. Several lines of data support the possibility that certain CYP metabolites resulting from the hydroxylation of AA such as 20-hydroxyeicosatetraenoic acid (20-HETE) are potent vasoconstrictors and may produce detrimental effects in the heart during ischemia and pro-inflammatory effects during reperfusion. On the other hand, a group of regioisomers resulting from the epoxidation of AA, including 5,6-, 8,9-, 11,12- and 14,15-epoxyeicosatrienoic acid (EETs), have been shown to reduce ischemic and/or reperfusion injury in the heart and vasculature. This review will discuss the detrimental and beneficial actions, including the potential cellular mechanisms responsible as a result of stimulating or inhibiting the two arms of this novel CYP pathway. The therapeutic potential of increasing EET concentrations and/or reducing 20-HETE concentrations will also be addressed.

Keywords: Ischemia; Reperfusion; Infarction; Arachidonic acid; Epoxygenase; N-Hydroxylase; CYP

1. Introduction

Cytochrome P450 (CYP) epoxygenases are known to metabolize AA to four regioisomeric epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EET) and by CYP ω-hydroxylases to 20-hydroxyeicosatetraenoic acid (20-HETE). The EETs are potent dilators of blood vessels as a result of hyperpolarizing vascular smooth muscle cells by activation of large conductance calcium activated potassium channels. In contrast, 20-HETE is a potent vasoconstrictor that activates L-type Ca2+ channels leading to vasoconstriction of vascular smooth muscle. More recently, administration of EETs and the overexpression of mouse CYP2J3 have been shown to result in a cardioprotective effect [1]. Similarly, several nonselective CYP inhibitors and more selective CYP ω-hydroxylase inhibitors have been shown to reduce ischemic injury in rat and rabbit models [2] and to reduce infarct size in a dog model [3]. In a more recent study, performed in CYP2J2 transgenic mice, it appeared that the protective effect observed in the isolated Langendorff mouse heart was at least partly the result of mitochondrial KATP channel opening and the stimulation of the p42/p44 mitogen activated protein kinase (MAPK) pathway [4]. Since many of the pharmacological inhibitors...
used to block these pathways are often nonselective for a given CYP isoform and the observation that there may be important species differences as a result of different tissue expression patterns and location, the mechanisms or exact pathways by which the various CYP isoforms produce their cardiac effects are not well understood. Thus, the main focus of this review article is to discuss the metabolites and the signaling mechanisms involved in determining the results observed by activating or inhibiting various isoforms of the CYP pathway and the potential therapeutic applications that might result from a better understanding of this novel endogenous pathway in the heart.

1.1. Arachidonic acid metabolites in myocardial ischemia–reperfusion injury

In the heart and coronary blood vessels, AA is metabolized by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) to numerous compounds (Fig. 1) with a variety of actions including vasodilation, vasoconstriction, enhanced myocardial injury and cardioprotection [5]. AA is converted by COX to a variety of prostaglandins (PGs) and thromboxanes (TXs) and by LOX to leukotrienes and hydroxyeicosatetraenoic acids (HETEs). Finally, AA is catalyzed by CYP epoxygenases to 4 regioisomers including 5,6-, 8,9-, 11,12- and 14,15-EETs which are subsequently hydrolyzed by epoxide hydrolases to their corresponding dihydroxyeicosatrienoic acids (DHETEs). In addition, CYP hydroxylases convert AA to several HETEs including 19- and 20-HETE [6]. A number of other metabolites of the CYP pathway have also been identified and include 19- and 20-OH PGs [6]. The role of these eicosanoids, particularly the metabolites produced by COX and LOX, has been extensively investigated for their effects in the ischemic/reperfused myocardium and these will be summarized briefly in the next 2 sections.

1.2. Cyclooxygenase pathway

COX-1 expression is constitutively present in endothelial cells of most blood vessels; however, the constitutive expression of COX-2 is not normally found in abundance in blood vessels or heart tissue and is only observed in significant amounts following pathophysiological stimuli such as ischemia [7]. COX-1 and its AA metabolites have been long recognized to possess important roles in the myocardium during acute episodes of myocardial ischemia and reperfusion [8,9]. The enhanced production of prostacyclin (PGI2) produced by defibrotide in pigs was shown to produce a marked reduction of infarct size and a decrease in neutrophil accumulation in the reperfused zone [10]. These effects were abolished by the nonselective COX inhibitor indomethacin [10]. OP-41483, a stable analogue of PGI2 produced myocardial salvage in intact dog hearts, whereas, iloprost, another stable analogue of PGI2, attenuated metabolic derangements [11] and improved mitochondrial respiration in stunned myocardium of canine hearts [12]. Our laboratory also demonstrated that prostaglandin E1 (PGE1) significantly attenuated postischemic contractile dysfunction in stunned canine myocardium [13] and that dazmegrel, a thromboxane synthesis inhibitor, significantly improved regional wall function in stunned myocardium of dog hearts [14]. Based on these results, we hypothesized that a redirection in the synthesis of endogenous cardioprotective prostanooids such as PGI2, was at least partly responsible for the beneficial effect of the thromboxane synthesis inhibitor, dazmegrel. The role of CYP metabolites was not determined at that time as selective agonists and antagonists of this pathway were not available.

More recently, the cardioprotective effects of late or delayed ischemic preconditioning (IPC) in rabbit and mouse hearts [15,16] and by opioids in rabbit [17] and rat hearts [18] was shown to be the result of the upregulation of myocyte COX-2 mRNA and protein expression and the resulting increased synthesis of cardioprotective prostanooids such as PGI2 and PGE2 [15,17]. In support of these findings, it was also shown that selective COX-2 inhibitors such as NS-398 and celecoxib, blocked the effect of delayed IPC to increase tissue concentrations of PGI2 and PGE2 and abolished the effect of delayed IPC to reduce myocardial stunning and infarct size in mice and rabbits [15,17]. These results suggest that COX-2 is a cardioprotective protein and that PGI2 and PGE2 are the most likely AA metabolites responsible for the protective effects of delayed IPC observed in the heart in this model [16,17]. Later studies in COX-1 and COX-2 knockout mice confirmed the observations made in wild-type mice that acute IPC may

Fig. 1. Major metabolic pathways of arachidonic acid metabolism with the CYP450 pathway indicated in bold letters.
also be importantly dependent on COX-2 upregulation [19]. The role of P450 metabolites in acute and delayed IPC have not been tested and will be an interesting future avenue of investigation.

1.3. Lipoxygenase pathway

The role of LOX metabolites of AA has also been investigated in the ischemic/reperfused myocardium. Recently, 12-LOX and 12-HETE have been shown by several investigators, including our laboratory [20,21], to serve a protective role in acute and delayed IPC in rat and mouse hearts. In rat hearts, the LOX inhibitors, nordihydroguaiaretic acid (NDGA), eicosatetraynoic acid (ETYA), baicalein and phenidone, added just before and/or during IPC blocked the protective effects of IPC on the recovery of function during reflow following acute global ischemia [22] or following a delayed preconditioning stimulus produced by the selective delta opioid agonist, SNC-121 [21]. Increases in 12-HETE were observed in the effluent obtained from the isolated rat heart and in the plasma of the intact rat heart, effects that were blocked by NDGA [20,23]. Exogenous 12-HETE was also shown to be protective in the intact and isolated rat heart [22] and the protective effect of IPC was eliminated by inhibition of PKC [24]. In hearts from wild-type (WT) and 12-LOX-knockout (KO) mice, postischemic function was significantly improved by IPC only in the WT mice. In WT mice, acute IPC significantly reduced infarct size and increased the concentration of 12-HETE, however, these effects were absent in 12-LOX-KO hearts [21]. These data clearly indicate that 12-LOX and its major metabolite, 12-HETE, is important in both acute and delayed ischemic and opioid-induced preconditioning.

2. CYP chemistry and pharmacology

CYP metabolizes AA to several biologically active eicosanoids by two distinct enzymatic reactions. CYP epoxygenases convert AA to 4 already described regioisomeric epoxycosatrienoic acids (EETs) and CYP hydrolases convert AA to hydroxyeicosatetraenoic acids (HETEs) including 19- and 20-HETE. The major findings concerning these 2 major CYP pathways in determining the extent of myocardial injury during ischemia and/or reperfusion will be the focus of the remainder of this review.

2.1. CYP epoxygenases and cardioprotection

CYP epoxygenases were first isolated from human heart and liver [25]. Subsequently, many CYP families have been identified in organs of different species [26]. CYP families of CYP1A, CYP2B, CYP2C and CYP2J catalyze the formation of 5,6-, 8,9-, 11,12-, and 14,15-EET from AA [27]. EETs are subsequently further hydrolyzed by epoxide hydrolases to their corresponding dihydroxyeicosatrienoic acids (DHETs) which also have important biological effects. For instance, EETs and DHETs both are potent vasodilators especially in small coronary arteries [6,28] and the EETs have been identified by Campbell et al. [29] as endothelium-derived hyperpolarizing factors (EDHFs). This interesting area has been the subject of several recent reviews and the reader is directed to these references for further information concerning EETs and their role as EDHFs and vascular protectants [30,31]. EET production has been shown to increase in stenosed canine coronary arteries and their production has been shown to be decreased by the nonselective CYP inhibitor, metyrapone. Administration of 11,12-EET and 5,6-EET to the isolated guinea pig heart perfused at normal flow had no effect on global contractility or perfusion pressure, however, these same compounds given during low flow ischemia resulted in a decreased recovery of contractile function after reperfusion [32]. In contrast, the addition of 5 μM of 11,12-EET to the perfusate prior to global ischemia in mouse hearts expressing CYP2J3 resulted in an improvement in the recovery of global contractile function [27]. However, in the same study 14,15-EET and 19-HETE did not improve functional recovery following global ischemia which suggests that all regioisomers of CYP epoxygenases do not have similar effects.

More recently, Granville et al. [2] demonstrated a reduction in ischemic and reperfusion-induced damage following administration of several CYP inhibitors to isolated Langendorff perfused rat hearts and to in vivo rabbit hearts subjected to regional ischemia and reperfusion. These investigators initially used chloramphenicol, an inhibitor of protein synthesis and several CYP isoforms in liver, and found that in both preparations chloramphenicol reduced infarct size by approximately 60% as assessed by CK release and triphenyltetrazolium staining. Interestingly, chloramphenicol reduced infarct size whether administered prior to or just after the start of ischemia. It was initially hypothesized that the reduction in infarct size was a result of the ability of chloramphenicol to reduce mitochondrially released reactive oxygen species (ROS), however, their data did not support this hypothesis. Since it is known that chloramphenicol is also an inhibitor of several CYP isoforms in liver, these authors administered cimetidine, another nonselective CYP inhibitor to the isolated rat heart preparation and found that this histamine-2 receptor blocker was also cardioprotective and produced a concentration-dependent (200–600 μM) reduction in infarct size and CK release. Finally, since it has been shown that both chloramphenicol and cimetidine inhibit liver CYP2C9 at high concentrations [33,34], they tested the effect of a more selective inhibitor of liver and endothelial cell CYP2C9, sulphaphenazole (IC$_{50}$=0.6 μM) and found that 10–300 μM also produced a reduction in infarct size and CK release whether administered either prior to or just after ischemia in the isolated rat heart preparation. Although these authors...
could not demonstrate an effect of chloramphenicol on ROS production by mitochondria, they were able to show that chloramphenicol reduced superoxide generation in rat hearts by using dihydroethidium to estimate ROS production. Based on these results, these authors concluded that a reduction in nonmitochondrial-generated ROS production was responsible for the cardioprotective effects of this structurally unrelated group of compounds [2]. Since CYP2C9 is primarily localized to liver and the vascular endothelium [33] and not the cardiomyocyte and given the nonselectivity of these compounds for multiple CYP isoforms and the high doses and concentrations needed to protect these hearts, definitive conclusions concerning the mechanisms or pathways involved in the protective effects of these agents remain uncertain. However, since CYPs have been shown to be an important source of ROS in many species and organs, it is likely that the protection against ischemia and especially reperfusion injury in the heart as a result of blocking specific CYP isoforms in heart or endothelium may be a result of a reduction in ROS production [34]. The reader is directed to a recent review concerning the role of CYP isoforms in mediating reperfusion injury and the importance of ROS [34].

Interestingly, a recent study performed by Fichtlscherer et al. [35] in humans supports the ROS hypothesis of Granville et al. [2]. In this study, 5 healthy subjects and 16 patients with angiographically documented stable coronary artery disease were treated with sulphaphenozide and it was found that where there was no effect in normal subjects, there was an improved endothelial dependent response to acetylcholine to increase forearm blood flow without any effect on the response to the endothelial-independent dilator sodium nitroprusside. Similar results were obtained with vitamin C and the response to vitamin C was enhanced by sodium nitroprusside. Similar results were obtained with

effect on the response to the endothelial-independent dilator acetylcholine to increase forearm blood flow without any effect on the response to the endothelial-dependent dilator

2.2. CYP hydroxylases

AA can be converted by CYP hydroxylases to hydroxyeicosatetraenoic acids (HETEs) such as 16-, 17-, 18-, 19-, and 20-HETE. CYP ω-hydroxylase converts AA by ω-hydroxylation to 20-HETE [36]. Families of CYP4A, CYP3A and CYP4F are known to catalyze the ω-hydroxylation of AA to 20-HETE [37,38] and canine leukocytes convert AA to 20-HETE [39].

The vascular actions and function of 20-HETE have been thoroughly characterized and its function depends on the species and vascular bed studied. In general, 20-HETE has been shown to be a potent vasoconstrictor in most species including rat, dog and cat [40–42]. The vasoconstrictor effects of 20-HETE have been attributed to its inhibition of smooth muscle potassium channels which result in membrane depolarization and subsequent activation of L-type Ca²⁺ channels [39,40]. Interestingly, 20-HETE has been shown to relax bovine coronary arteries via the production of PGI₂ [43]; however, such an effect has not been investigated in the heart and might be expected to produce cardioprotection if it were to occur.

2.3. CYP ω-hydroxylases and cardioprotection

In an earlier study, we found high coronary venous plasma concentrations of 20-HETE during the late stages of ischemia and particularly during the early stages of reperfusion in the canine heart [44]. In this study, we used a gas chromatographic-mass spectrometric technique to analyze total EETs, DHETs, and 20-HETE released into coronary venous plasma at various times throughout 60 min of ischemia and 3 h of reperfusion. We found that total EETs were increased at 60 min of occlusion and during the first 60 min of reperfusion from approximately 200 to 400 pg/ml, whereas, 14,15 DHET was the major DHET detected in plasma and reached a peak concentration of approximately 12 ng/ml at 30 min of reperfusion. Interestingly, 20-HETE was the most abundant CYP metabolite obtained and peaked at approximately 30 ng/ml at 15 min of reperfusion and remained elevated until 120 min of reperfusion. In 3 experiments, we administered miconazole, a nonselective blocker of both arms of the CYP pathway, 15 min prior to occlusion and observed an overall reduction of coronary function in WT mice. Since CYP2J2 is abundantly found in the heart and is active in producing EETs, these authors concluded that the cardioprotective effects seen were the result of an enhanced production of EETs or their corresponding DHETs. Taken together, the results of Granville et al. [2] and Seubert et al. [4] are promising and suggest that the EETs or DHETs may be a new cardioprotective target. In this regard, more studies are needed to define mechanism(s) responsible for the cardioprotection observed in these 2 studies and in other models of infarction in large animal hearts.
venous plasma concentrations of total EETs, DHETs and 20-HETE. Miconazole also prevented the increases in these same metabolites of AA observed in control dogs during occlusion and reperfusion. A marked reduction in infarct size was also observed in the miconazole-treated dogs. These intriguing preliminary results led us to further evaluate more selective inhibitors of 20-HETE formation in our canine model and in a rat model of myocardial infarction [2,3].

In a recent study performed in our anesthetized dog model of infarction, we characterized the effects of several selective CYP \( \omega \)-hydroxylation inhibitors and the major CYP \( \omega \)-hydroxylase metabolite of AA, 20-HETE, on the extent of infarct size resulting from 60 min of coronary artery occlusion and 3 h of reperfusion [3]. During 60 min of ischemia and particularly during reperfusion, 20-HETE was produced in high concentrations as compared to baseline values prior to occlusion. Following pretreatment with a nonselective CYP inhibitor, miconazole, and two specific CYP \( \omega \)-hydroxylase inhibitors, 17-octadecanoic acid (ODYA) and \( N \)-methylsulfonyl-12,12-dibromomodoc-11-eneamide (DDMS), the coronary venous plasma concentrations of 20-HETE were markedly decreased. These compounds also produced a marked reduction in myocardial infarct size expressed as a percent of the area at risk (IS/AAR,%). Conversely, exogenous 20-HETE administration prior to coronary artery occlusion produced a significant increase in infarct size. We concurrently showed that several CYP \( \omega \)-hydroxylase isoforms including CYP4A1, CYP4A2 and CYP4F were all present in dog heart tissue microsomes and that their activity was markedly reduced by incubation with 17-ODYA. These results confirmed those presented in our earlier study [44] and clearly demonstrated that several endogenous CYP \( \omega \)-hydroxylases produce increased concentrations of the metabolite, 20-HETE, which appears to be involved in exacerbating myocardial injury, at least in the canine heart.

Subsequently, we performed another study in the rat heart [45], to determine if similar results would be obtained to those previously reported in the dog heart [3,44]. Since in all of our previous studies done in the dog, the antagonists of CYP \( \omega \)-hydroxylase were administered as a pretreatment, we also wanted to determine if administration of these inhibitors would be cardioprotective when administered just prior to reperfusion, a situation with more clinical relevance similar to treating patients which are usually seen after the start of a coronary artery occlusion. We also wanted to determine if the sarclemmal \( K_{ATP} \) channel or the mito \( K_{ATP} \) channel was activated in the transgenic CYP2J2 mouse heart and that 5-HD, a selective mito \( K_{ATP} \) channel antagonist, blocked the cardioprotection against myocardial stunning normally observed in the transgene. In our study performed in Sprague–Dawley rats subjected to 30 min of coronary artery occlusion and 2 h of reperfusion, we administered either miconazole (MIC, nonselective CYP inhibitor), 17-ODYA or DDMS (selective CYP \( \omega \)-hydroxylase inhibitors) or \( N \)-methanesulfonyl-6-(2-propargyloxyphenyl) hexamide (MS-PPOH, selective CYP epoxygenase inhibitor) either 10 min prior to occlusion or 5 min prior to reperfusion. A subset of rats was also given either HMR-1098 (selective sarc \( K_{ATP} \) inhibitor) or 5-HD (selective mito \( K_{ATP} \) inhibitor) 10 min prior to reperfusion and 5 min prior to either MIC or 17-ODYA. Rats treated with MIC, 17-ODYA or DDMS but not MS-PPOH produced comparable reductions in infarct size when administered prior to ischemia or just prior to reperfusion. HMR-1098, but not 5-HD blocked the reductions in IS/AAR afforded by MIC and 17-ODYA. These data suggest that in the rat, similar to the dog, inhibition of the CYP \( \omega \)-hydroxylase produced cardioprotection. However, as opposed to the mouse studies with the overexpressing CYP2J2, the sarc \( K_{ATP} \) channel seems to be the major channel in transducing the cardioprotective effects of inhibiting the CYP \( \omega \)-hydroxylase in rats as opposed to the mito \( K_{ATP} \) channel which appears to mediate the effect of the EETs in the CYP2J2 transgenic mouse [4]. This may just be a species difference or it could be due to different mediators of cardioprotection being produced in normal rats and dogs as opposed to the CYP2J2 transgenic mouse. It is also possible that one \( K_{ATP} \) channel is responsible for cardioprotection during the ischemic period as opposed to the other channel which may be protective at reperfusion. Clearly, more studies are needed to sort out these signaling pathways responsible for benefit in these various species.

3. Mechanisms responsible for cardioprotection due to activation or blockade of the CYP pathway

3.1. Role of myocardial \( K_{ATP} \) channels

There are several papers which previously have suggested that the EETs are potent activators of the sarc \( K_{ATP} \) channel [46,47] as well as one which suggests they may also act via the mito \( K_{ATP} \) channel [4]. Initially, Lu et al. [46] determined the effects of the EETs on \( K_{ATP} \) channels of rat cardiac myocytes using the inside-out patch clamp technique. In the presence of 100 \( \mu \)M ATP, the open channel probability (\( P_o \)) of the sarc \( K_{ATP} \) channel was increased by 240% by addition of 0.1 \( \mu \)M 11,12-EET and by 400% by 5 \( \mu \)M 11,12-EET. Neither AA nor 11,12 DHET had any effect on the \( P_o \) whereas, 8,9-EET showed an identical response as that of 11,12-EET. Application of 11,12-EET markedly decreased the channel sensitivity to cytoplasmic ATP from 21.2 \( \pm \)2 \( \mu \)M at baseline to 240 \( \pm \)60 \( \mu \)M in the presence of 0.1 \( \mu \)M and to 780 \( \pm \)30 in the presence of 5 \( \mu \)M of 11,12-EET. The resting membrane potential in rat cardiac myocytes was also hyperpolarized by addition of 11,12-EET. All of these effects of the EETs were completely blocked by gliburide, the \( K_{ATP} \) channel antagonist. Mito \( K_{ATP} \) channel activity was not addressed in this study.
In a more recent study from this laboratory, Lu et al. [47] demonstrated that the 11(S),12(R)-EET regioisomer activated the rat ventricular myocyte sarco K<sub>ATP</sub> channel with an EC<sub>50</sub> of 39.5 nM, whereas, the 11(R),12(S)-EET isomer was inactive. Only the 11(S),12(R)-EET hyperpolarized the resting membrane potential and shortened the cardiac action potential. All the similar analogs and homologs of 11,12-EET were equipotent activators of the sarco K<sub>ATP</sub> channel as a result of their ability to reduce the channel sensitivity to ATP. Using various structural modifications of 11,12-EET these authors indicated that the presence of an epoxide group in a particular three-dimensional configuration is a critical determining factor for K<sub>ATP</sub> channel opening. In particular, a double bond at the ω-3 position adds to its potency for activating K<sub>ATP</sub> channels. These results suggest that the EETs bind to specific sites on the sarco K<sub>ATP</sub> channel and that it may be possible to design more potent and efficacious openers of this channel for potential therapeutic use.

Our recent studies in rats with several CYP ω-hydroxylase inhibitors [45], clearly showed that it seems to be the sarco K<sub>ATP</sub> channel that mediates the cardioprotective effect of reducing 20-HETE concentrations and not the mito K<sub>ATP</sub> channel since HMR-1098, the sarco K<sub>ATP</sub> channel inhibitor, blocks the protective effects of the CYP ω-hydroxylase inhibitors, whereas, 5-HD, the selective mito K<sub>ATP</sub> blocker had no effect. These data seem to support the previous studies of Lu et al. [46,47] performed in rat cardiomyocytes in which they showed that the EETs were potent activators of sarco K<sub>ATP</sub> channels. However, based on our rat studies it is not clear that the EETs are responsible for the cardioprotective effects seen in rats since MS-PPOH, a CYP epoxygenase inhibitor had no effect in this model. In addition, Seubert et al. showed that flavoprotein fluorescence, a marker of mito K<sub>ATP</sub> channel activity [4], was significantly higher in cardiomyocytes obtained from CYP2J2 transgenic animals as opposed to wild-type mice. Furthermore, Seubert et al. [4] showed that 11,12-EET and 14,15-EET increased flavoprotein fluorescence in cardiomyocytes obtained from wild-type mice. These results suggest that the EETs may be able to activate both mito and sarco K<sub>ATP</sub> channels to produce cardioprotection. In agreement, we have recently found that 11,12-EET and 14,15-EET both produce equal degrees of cardioprotection when administered to dog or rat hearts and that glyburide blocked these effects in dogs and 5-HD blocked their effect in rats (unpublished data). Taken together, these results suggest that the beneficial effects of activating the CYP epoxygenase pathway in the heart are mediated, at least partly, via opening the sarco and/or mito K<sub>ATP</sub> channel.

3.2. Other metabolic pathways involved in CYP-induced cardioprotection

The EETs have been shown to activate a number of signaling pathways that appear to be separated from their direct effect on ion channels including activation of the guanine-nucleotide-binding protein, G<sub>α</sub>S [48] by ADP-ribosylation. This activity of the EETs appears to result in activation of BK<sub>Ca</sub> channels in vascular smooth muscle and a resultant vasodilator response. It has been proposed by Spector et al. [49] that activation of protein kinase A (PKA) may be a branch point in EET signaling with one arm translocating to the nucleus and producing changes in gene transcription while the other branch results in activation of the BK<sub>Ca</sub> channel by phosphorylation of a serine residue in vascular smooth muscle.

There is also evidence that EETs may signal via a tyrosine kinase in endothelial cells isolated from pig coronary arteries [50] or in LLCPKc14 cells. The EETs have also been shown to result in phosphorylation of the epidermal growth factor receptor (EGFR), PI3-kinase, cSrc and extracellular signal-regulated kinase (ERK 1 and 2). In this regard, the observation that ERK 1 and 2 are activated by the EETs is particularly important since Seubert et al. [4] showed that ERK was upregulated in the CYP2J2 transgenic mice and that administration of a MEK 1,2 inhibitor, PD98059, during reperfusion blocked the cardioprotective effect of CYP2J2 overexpression. That the EGFR receptor is phosphorylated by the EETs is also of potential importance since the EGFR receptor has recently been shown to mediate the cardioprotective effects of opioids in the heart [51]. Spector et al. [49] proposed that the EETs may regulate EGFR via the upstream activation of a metallocproteinase.

Finally, EETs have been shown to produce antiinflammatory effects via autocrine effects on endothelial cells [52,53]. In particular, 11,12-EET has been demonstrated to reduce leukocyte adhesion to the vascular smooth muscle wall and to inhibit the activation of NF-κB. Whether these effects occur in ischemic/reperfused hearts is open to speculation and should be a target for future studies. Such antiinflammatory effects may explain our observations that inhibition of 20-HETE formation at reperfusion and a possible upregulation of EETs produces a cardioprotective effect in our rat studies [45].

4. Conclusions

Several recent studies performed in transgenic mice overexpressing a CYP2J2 isoform [4] and those performed in intact dogs [3], rats [45], and rabbits [2] have all suggested that the CYP pathway mediates a cardioprotective effect when the CYP epoxygenase pathway is activated during ischemia/reperfusion or when the CYP ω-hydroxylase pathway is blocked during ischemia/reperfusion. At this time it is unclear as to whether the protective effects observed in these studies are due to an increase in EET formation during ischemia/reperfusion or the result of a decrease in the formation of 20-HETE at the same time or the combination of the two effects. Alternatively, another unknown product of the CYP pathway such as nitric oxide (NO) may be involved. Another area of interest which is not
well defined, concerns the cellular mechanisms responsible for the cardioprotection observed via this pathway. Evidence pointing to a role for activation of the sarc K_{ATP} or mito K_{ATP} channel has been observed in several laboratories [4,45] and there is evidence for a role of the ERK pathway in the protective effects observed [4]. Obviously, many additional experiments will be required in the future to ascertain the protective substance(s) involved in the cardioprotection observed and the cellular signaling pathways involved in this novel pathway of AA metabolism.

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References


