Inhibition of Cytochrome P450ω-Hydroxylase
A Novel Endogenous Cardioprotective Pathway


Abstract—Cytochrome P450s (CYP) and their arachidonic acid (AA) metabolites have important roles in regulating vascular tone, but their function and specific pathways involved in modulating myocardial ischemia–reperfusion injury have not been clearly established. Thus, we characterized the effects of several selective CYPω-hydroxylase inhibitors and a CYPω-hydroxylase metabolite of AA, 20-hydroxyeicosatetraenoic acid (20-HETE), on the extent of ischemia–reperfusion injury in canine hearts. During 60 minutes of ischemia and particularly after 3 hours of reperfusion, 20-HETE was produced at high concentrations. A nonspecific CYP inhibitor, miconazole, and 2 specific CYPω-hydroxylase inhibitors, 17-octadecanoic acid (17-ODYA) and N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), markedly inhibited 20-HETE production during ischemia–reperfusion and produced a profound reduction in myocardial infarct size (expressed as a percent of the area at risk) (19.6±1.7% [control], 8.4±2.5% [0.96 mg/kg miconazole], 5.9±2.2% [0.28 mg/kg 17-ODYA], and 10.8±1.8% [0.40 mg/kg DDMS], P<0.05, respectively). Conversely, exogenous 20-HETE administration significantly increased infarct size (26.9±1.9%, P<0.05). Several CYPω-hydroxylase isoforms, which are known to produce 20-HETE such as CYP4A1, CYP4A2, and CYP4F, were demonstrated to be present in canine heart tissue and their activity was markedly inhibited by incubation with 17-ODYA. These results indicate an important endogenous role for CYPω-hydroxylases and in particular their product, 20-HETE, in exacerbating myocardial injury in canine myocardium. The full text of this article is available online at http://circres.ahajournals.org. (Circ Res. 2004;95:e65-e71.)

Key Words: arachidonic acid metabolites • cytochrome p450 • 20-HETE • ischemia • reperfusion

It has long been recognized that ischemia–reperfusion of the canine heart results in an accumulation of unesterified arachidonic acid (AA).1,2 AA can be metabolized by cytochrome P450 (CYP) epoxygenases to 4 regioisomeric epoxyeicosatrienoic acids (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) and by CYPω-hydroxylases to 20-hydroxyeicosatetraenoic acid (20-HETE).3–6 Subsequently, EETs can be further metabolized to their corresponding dihydroxyeicosatrienoic acids (DHETs) by epoxide hydrolases.7,8 Although EETs relax many blood vessels, including coronary, cerebral, renal, and pial arteries, and hyperpolarize vascular smooth muscle cells possibly by activation of calcium activated potassium (Kca) channels.9 DHETs produce relaxation only in some vessels.10,11 However, 20-HETE is a potent vasoconstrictor12 that activates L-type Ca2+ channels leading to vasoconstriction of renal afferent arterioles.13,14

As noted, roles of these eicosanoids in the regulation of peripheral vascular tone have been extensively investigated. However, their functions during ischemia and after reperfusion in the heart and coronary circulation, as well as mechanisms responsible for their vascular and myocardial properties are not well-understood. It is known that CYP metabolites are involved in AA-induced relaxation of canine coronary arteries.13 Both EETs and DHETs have been shown to be potent vasodilators in the canine coronary microcirculation.14 The production of EETs and DHETs was increased in stenosed canine coronary arteries.15 Recently, nonspecific CYP inhibitors such as chloramphenicol, cimetidine, and sulfaphenazole have been reported to reduce ischemia–reperfusion injury, as measured by recovery of contractile function and reduction of infarct size, in rat and rabbit hearts.16 However, because the drugs used to inhibit CYP were not selective inhibitors, it was difficult to determine the precise pathway or metabolite responsible.

Previously, we found that high plasma concentrations of CYP metabolites of AA, particularly the CYPω-hydroxylase metabolite, 20-HETE, were released during ischemia and after reperfusion from the canine heart.17 These results led us to investigate the CYP isoforms and their AA metabolites responsible for mediating ischemia–reperfusion injury in canine hearts.
Materials and Methods

Materials
['H]14,15-DHET and [2H8]EETs were synthesized in our laboratory.20 Primary antibodies against CYP4A1 and CYP4A2 were generous gifts from Dr J. H. Capdevila at Vanderbilt University Medical School. The primary antibody against CYP4F was a gift from Dr D. R. Harder at the Medical College of Wisconsin. Horseradish peroxidase-conjugated secondary antibody was obtained from BioRad (Hercules, Calif). C18 Bond Elut solid phase extraction columns were obtained from Varian (Harbor City, Calif). All other chemicals and solvents were of analytical or highest purity grades. Distilled, deionized water was used in all experiments.

General Preparation of Dogs

All experiments conducted in this study were in accordance with the Position of the American Heart Association on Research and Animal Use adopted by the American Heart Association and the guidelines of the Biomedical Resource Center of the Medical College of Wisconsin. The Medical College of Wisconsin is accredited by the American Association of Laboratory Animal Care (AALAC).

The protocol for dog preparation and experiments have been previously established in our laboratory.21 Briefly, adult mongrel dogs of either sex, weighing 15 to 25 kg, were fasted overnight, anesthetized with the combination of sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg), and ventilated with room air supplemented with 100% oxygen. Body temperature was maintained at 38±1°C with a heating pad. Atelectasis was prevented by maintaining an end-expiratory pressure of 5 to 7 cm–H2O with a trap. Arterial blood pH, pCO2, and pO2 were monitored at selected intervals by an AVL automatic blood gas system and maintained within normal physiological limits (pH, 7.35 to 7.45; pCO2, 30 to 35 mm Hg; pO2, 85 to 100 mm Hg) by adjusting the respiration rate and oxygen flow or by intravenous administration of 1.5% sodium bicarbonate if necessary. A flowmeter (Statham 2202) was used to measure left anterior descending (LAD) coronary blood flow. A mechanical occluder was placed distal to the flow probe such that there were no branches between the flow probe and the occluder. Hemodynamic variables, heart rate, and coronary blood flow were monitored and recorded by a polygraph throughout the experiment. The left atrium was cannulated via the appendage for radioactive microsphere injections.

Experimental Design

Dogs were sequentially assigned to 1 of 6 groups. Normally, 8 dogs were used in each group of experiments. At 15 minutes before the 60-minute LAD occlusion period, miconazole (0.96 mg/kg), 17-HETE (0.01 mmol/L), or vehicle were administered by intracoronary infusion for 2 to 3 minutes. In all groups, hemodynamic measurements, blood gas analyses, and myocardial blood flow measurements were performed at baseline and at 30 minutes into the 60-minute occlusion period. After reperfusion, hemodynamics were measured every hour and myocardial blood flow was determined at the end of the 3-hour reperfusion period. At the end of the experiment, the hearts were electrically fibrillated, removed, and prepared for infarct size determination and regional myocardial blood flow measurement.

Infarct Size Determination

The protocol for infarct size determination has been previously described. Briefly, at the end of the 3-hour reperfusion period, the LAD was cannulated. To determine the anatomic area at risk (AAR) and the nonischemic area, 5 mL of Patent blue dye and 5 mL saline were injected at equal pressure into the left atrium and LAD, respectively. The heart was then immediately fibrillated and removed. The left ventricle was dissected and sliced into serial transverse sections 6 to 7 mm in width. The nonstained ischemic area and the blue-stained normal area were separated and incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) in 0.1 mol/L phosphate buffer, pH 7.4 at 37°C for 15 minutes. After incubation overnight in 10% formaldehyde, the noninfarcted and infarcted tissues within the AAR were separated and determined gravimetrically. Infarct size was expressed as a percentage of the AAR.

Regional Myocardial Blood Flow

Regional myocardial blood flow was measured by the radioactive microsphere technique developed in this laboratory as previously reported.22 Microspheres were administered at 30 minutes into the 60-minute occlusion period and at the end of 3-hour reperfusion. Transmural blood flow was calculated as the weighted average of the subepicardium, midmyocardium, and subendocardium in each region.

Sample Collection and Preparation of CYP Metabolites of AA

A detailed protocol for obtaining arterial (femoral artery) and venous plasma (great cardiac vein) samples was previously described.19 Blood was drawn through a 8-French single lumen (100 cm in length) catheter. Once the catheter was cleared of residual stagnant blood, 2 mL of blood was aspirated over a 3- to 5-second period into a chilled sample tube containing heparin (1000 U/mL) and miconazole (5×10−5 mol/L), mixed thoroughly and placed in an ice bucket. The samples were centrifuged at 3000g at 0°C for 10 minutes to separate plasma. Plasma was removed and transferred to a tube, purged with nitrogen gas, capped, and stored at −80°C or extracted. The internal standards, 1.0 ng each for [14]EETs, [14]DHETs, and [14]HETE, were added to each sample and mixed. Then, ethanol was added to a final concentration of 15% ethanol, mixed, and centrifuged at 1500 rpm for 3 minutes. The solid phase extraction protocol was previously described.20

Liquid Chromatographic-Electrospray Ionization–Mass Spectrometry Determination of CYP Metabolites of AA

CYP metabolites of AA in plasma samples were analyzed by liquid chromatographic-electrospray ionization–mass spectrometry (Agilent 1100 LC/MSD, SL Model) as previously described. Selected ion monitoring was used for quantitation of these CYP metabolites of AA.

Micromere Preparation and High-Performance Liquid Chromatography Analysis of 20-HETE Production

Heart tissue was homogenized in buffer containing 0.1 mol/L phosphate buffer, pH 7.7, sucrose 250 mmol/L, EDTA 1 mmol/L, and complete protease inhibitor, and centrifuged at 9000g at 4°C for 10 minutes. Then, the supernatant was centrifuged at 100 000g at 4°C for 1.5 hours. The pellet was resuspended in 0.5 mol/L phosphate buffer, pH 7.25, containing 1 mmol/L EDTA, 0.01 mmol/L dithiothreitol, 30% glycerol, and complete protease inhibitor. The reaction was incubated in 400 μL assay buffer (0.1 mol/L phosphate buffer, pH 7.4, 1 mmol/L EDTA, 10 mmol/L MgCl2) containing 100 μg protein, 1 mmol/L NADPH, 10 mmol/L isocitrate, and 0.1 U/mL isocitrate dehydrogenase. The solution was added with or without 10−7 mol/L 17-ODYA for 5 minutes before the incubation with [14]CAA for 30 minutes. Samples were extracted with solid phase extraction as described.20 Samples were separated on a C18 reverse phase column (10×250 mm, Nucleosil, Phenomenex) using water:acetonitrile containing 0.1% acetic acid as a mobile phase at the flow rate of 1.0 mL/min. The mobile phase gradient started with 50% acetonitril and linearly increased to 100% acetonitril in 35 minutes. The eluent was collected at 5 fractions per minute and the radioactivity was measured.
counted on the scintillation counter. The retention time of the radioactive peak of 20-HETE in the sample was compared with the retention time of the 20-HETE standard.

**Western Blot Analysis**
Heart tissues were homogenized in lysis buffer and centrifuged at 100,000 g at 4°C for 60 minutes and the pellets were resuspended in lysis buffer. Proteins (35 µg) were separated by 10% SDS-PAGE (Criterion Gels; BioRad, Hercules, Calif) at 150 V for 90 minutes and 50 V for 2 hours, and then transferred to a 0.2-µm polyvinylidene difluoride membrane. Proteins were blocked with 3% bovine serum albumin and 3% dry milk for 90 minutes. Primary antibodies against CYP isoforms (1:2000 dilution for CYP4A1 and 2; and 1:15,000 dilution for CYP4F) were incubated at 4°C overnight. The membrane was washed 3 times with Tris-buffered saline buffer and 2 times with Tris-buffered saline–Tween 20 (containing 0.1% Tween 20). Then, goat anti-rabbit IgG-HRP (BioRad) at 1:10,000 dilution was used to complex with the primary antibodies at room temperature for 70 minutes. The detection was made by using ECL Western blotting detection kit (Amersham, Mass) and captured by Fuji film X-ray (Tokyo, Japan).

**Statistical Analysis**
All values are expressed as mean±SEM. Differences between groups in hemodynamics were compared by using 2-way analysis of variance (ANOVA), differences between groups in tissue blood flows, AAR, and infarct size were compared by 1-way ANOVA, and differences in concentrations of AA metabolites at various times during occlusion and after reperfusion between treatment groups and the control group were compared by using a 2-way repeated measures ANOVA followed by a Tukey post hoc test. Differences between groups were considered significant if $P<0.05$.

**Results**

**Hemodynamic Responses**
There were no significant differences in heart rate, mean arterial blood pressure, and pressure–rate product at baseline between groups or at subsequent times throughout the experiment.

**Regional Myocardial Blood Flow**
Transmural blood flow values in the nonischemic (left circumflex coronary) and the ischemic (LAD) regions were measured during occlusion and after 3 hours of reperfusion. There were no significant differences in transmural collateral blood flow between groups indicating that all groups were subjected to similar degrees of ischemia.

**Plasma CYP Metabolites of AA**
CYP metabolites of AA detected in dog coronary venous plasma collected during ischemia and reperfusion are predominantly 20-HETE and lower concentrations of 11,12-DHET and 14,15-DHET as shown in Figure 1. The coronary venous plasma concentrations of 20-HETE, 14,15-DHET, and 11,12-DHET in all groups taken 10 minutes before drug administration were identical to the baseline concentrations of the control group. Administration of a nonspecific CYP inhibitor (miconazole) and specific CYP4A-hydroxylase inhibitors (17-ODYA and DDMS) significantly reduced the concentration of 20-HETE (except 17-ODYA at 0.07 mg/kg, which did not significantly reduce 20-HETE concentration; # represents significant difference from control group for all groups except 17-ODYA at 0.07 mg/kg). 5,6-DHET and 8,9-DHET were not detectable in most dogs. EETs were not
detectable in these venous plasma samples, indicating that EETs may be rapidly hydrolyzed to DHETs. Surprisingly, the nonspecific CYP inhibitor, miconazole, did not significantly reduce the 11,12-DHET and 14,15-DHET concentrations. Furthermore, the concentration of 14,15-DHET increased in the plasma of dogs treated with DDMS as compared with the control dogs.

**Myocardial Infarct Size**

Area at risk and myocardial infarct size data are shown in Figure 2. AAR expressed as a percentage of LV (A) and infarct size (IS) expressed as a percentage of AAR (B). Miconazole = 0.96 mg/kg; 17-ODYA(1) = 0.07 mg/kg; 17-ODYA(2) = 0.28 mg/kg; DDMS = 0.41 mg/kg; 20-HETE = 0.032 mg/kg. AAR/LV is not significantly different among groups as shown in (A). Miconazole, 17-ODYA at 0.28 mg/kg, and DDMS significantly reduced infarct size (B). However, 20-HETE significantly increased infarct size. Values are mean ± SEM, n = 8 per group. *Significantly lower than the control with P < 0.05; #significantly higher than the control with P < 0.05.

Figure 2. Area at risk (AAR) expressed as a percentage of LV (A) and infarct size (IS) expressed as a percentage of AAR (B). Miconazole = 0.96 mg/kg; 17-ODYA(1) = 0.07 mg/kg; 17-ODYA(2) = 0.28 mg/kg; DDMS = 0.41 mg/kg; 20-HETE = 0.032 mg/kg. AAR/LV is not significantly different among groups as shown in (A). Miconazole, 17-ODYA at 0.28 mg/kg, and DDMS significantly reduced infarct size (B). However, 20-HETE significantly increased infarct size. Values are mean ± SEM, n = 8 per group. *Significantly lower than the control with P < 0.05; #significantly higher than the control with P < 0.05.

**CYPω-Hydroxylases**

Several CYP families such as CYP4A and CYP4F can induce the ω-hydroxylation of AA to form 20-HETE. Because 20-HETE was the major CYP metabolite of AA in plasma and specific CYPω-hydroxylase inhibitors markedly reduced infarct size, we determined the expression of CYPω-hydroxylases in heart tissue. Figure 3 shows the immunoreactive bands of proteins of CYP4A in membrane and cytosolic fractions corresponding to the molecular weight marker at 50 kDa. The intensity of immunoreactive bands in the membrane fraction is greater than the cytosolic fractions. C. Immunoreactive bands of CYP4F in membrane and cytosolic fractions corresponding to the molecular weight marker at 48 kDa. The intensity of immunoreactive bands in the membrane fraction is greater than the cytosolic fractions. I indicates ischemic area; N, nonischemic area.

Figure 3. Western blots of membrane and cytosolic proteins of heart tissue microsomes from different dogs. A, Immunoreactive bands of CYP4A1 in membrane and cytosolic fractions corresponding to the molecular weight marker at 50 kDa. The intensity of immunoreactive bands in the membrane fraction is greater than the cytosolic fractions. B, Immunoreactive bands of CYP4A2 in membrane and cytosolic fractions corresponding to the molecular weight marker at 48 kDa. The intensity of immunoreactive bands in the membrane fraction is greater than the cytosolic fractions. C, Immunoreactive bands of CYP4F in membrane and cytosolic fractions corresponding to the molecular weight marker at 50 kDa. The intensity of immunoreactive bands in the membrane fraction is greater than that in the cytosolic fraction. I indicates ischemic area; N, nonischemic area.

Figure 2. Area at risk (AAR) expressed as a percentage of LV (A) and infarct size (IS) expressed as a percentage of AAR (B). Miconazole = 0.96 mg/kg; 17-ODYA at 0.28 mg/kg, and DDMS at 0.41 mg/kg markedly reduced infarct size (8.4 ± 2.5%, 5.9 ± 2.2%, and 10.8 ± 1.8%, respectively) as compared with the control group (19.6 ± 1.7%). Conversely, exogenously administered 20-HETE at 0.032 mg/kg significantly increased infarct size (26.9 ± 1.9%).
an immunoreactive band for CYP4F at 50 kDa as shown in Figure 3C. These immunoreactive bands were also present in the cytosolic protein fractions but at much lower intensity. These results indicate that the canine heart expresses these CYPω-hydroxylases.

Activity and Production of 20-HETE of Heart Microsomes

Heart tissue microsomes were incubated with [14C]AA in the absence of a specific CYPω-hydroxylase inhibitor, 17-ODYA, and then the samples were analyzed by high-performance liquid chromatography and fractions were counted for radioactivity. A radioactive peak was observed at fraction number 75 concomitantly with the 20-HETE standard. AA indicates unmetabolized [14C]AA.

This is the first study to our knowledge to clearly demonstrate that CYPω-hydroxylases and their major AA metabolite, 20-HETE, mediate ischemia–reperfusion injury of the canine heart. We further demonstrated that several CYPω-hydroxylases that can produce 20-HETE are present in the canine heart. These CYPω-hydroxylases and 20-HETE have been extensively studied in other tissues, particularly in the kidney. In the heart, CYP epoxygenases and their AA metabolites, EETs, have been investigated as potential cardioprotective agents because of their vasodilatory and/or direct cardiac effects. In this study, the plasma concentration of 20-HETE during ischemia and after reperfusion was much greater than the concentrations of EETs and/or DHETs. More importantly, the inhibition of CYP by a nonspecific CYP inhibitor, miconazole resulted in protection against myocardial injury. These results indicate that the CYPω-hydroxylases and 20-HETE may have a more significant detrimental role in myocardial ischemia–reperfusion injury than that proposed for the cardioprotection produced by CYP epoxygenases and their AA metabolites, the EETs. Alternatively, it is most likely that a decrease in 20-HETE and a concomitant increase in EETs would produce the maximal cardiac protective effect obtained from blocking and activating this system, respectively. The mechanism(s) responsible for the adverse effect of 20-HETE in myocardial ischemia–reperfusion injury are unknown but may be possibly attributable to its vasoconstrictor effects33,29 resulting from the blockade of calcium-activated potassium channels41 and/or ATP-sensitive potassium channels and these possibilities are undergoing investigation. Other actions on oxygen-free radical formation and release or enhanced production of proinflammatory cytokines or mediators may be other factors that might also be responsible for the adverse effect of 20-HETE on infarct size, although these possibilities have not been previously addressed.

Only 11,12-DHET and 14,15-DHET but not the EETs were detected in plasma samples, indicating that EETs were readily hydrolyzed. Furthermore, only the 11,12-regioisomers and 14,15-regioisomers were the most abundant isomers produced by the heart. However, 20-HETE concentrations were much greater than those of the DHETs. A nonspecific CYP inhibitor, miconazole, and the 2 selective CYPω-hydroxylase inhibitors, 17-ODYA and DDMS, markedly reduced the production of 20-HETE in plasma during ischemia and reperfusion (Figure 1A). Interestingly, miconazole reduced 20-HETE concentrations, but it did not significantly reduce the production of EETs (or DHETs) as expected. 17-ODYA has been widely recognized as a specific CYPω-hydroxylase inhibitor, but it has also been shown to inhibit CYP epoxygenases in rat renal microsomes.30,31 However, we did not observe a decrease in DHET concentrations with 17-ODYA administration. However, DDMS at 0.41 mg/kg reduced the 20-HETE concentration, but it also increased the production of 14,15-DHET. An increase of 14,15-DHET produced by DDMS may have occurred because DDMS is a more specific CYPω-hydroxylase inhibitor,30 and the blockade of CYPω-hydroxylases resulted in a shift in the AA substrate to the CYP epoxygenase pathway. It is not clear why the 11,12-DHET concentration did not increase with DDMS. It has been shown that CYP4A2 and CYP4A3 can produce 20-HETE as well as 11,12-EET (precursor of 11,12-DHET).27,32 Thus it is possible that DDMS also inhibits CYP4A2 and thus 11,12-EET (or 11,12-DHET) production. The extent of the contribution of 14,15-DHET in cardioprotection, if any, remains unknown. Most recently, CYP epoxygenase (CYP2J2) and its AA metabolites have been shown to be cardioprotective after ischemia,33 thus the increase of 14,15-DHET concentration by DDMS may also contribute to cardioprotection in addition to the reduction of 20-HETE. Furthermore, blockade of the CYP pathway and a shift in AA substrate to other oxygenase pathways such as cyclooxygenases and lipoxygenases cannot be ruled out.

The inhibition of 20-HETE production appears to be closely associated with the reduction of infarct size. Further-

![Image](https://circres.ahajournals.org/content/11005/1/14/fig4)

**Figure 4.** Chromatograms of heart tissue microsomes incubated with [14C]AA in the absence (solid line) and presence (dash line) of 10–6 mol/L 17-ODYA. A radioactive peak at fraction number 75 comigrated with the 20-HETE standard. AA indicates unmetabolized [14C]AA.
more, all 3 CYP inhibitors significantly reduced infarct size when the 20-HETE concentration was significantly lower than control during late ischemia and throughout reperfusion. 17-ODYA at a lower dose of 0.07 mg/kg exhibited a reduction in 20-HETE concentration and a decrease in infarct size; however, these changes were not significantly different from control at this dose. At a higher dose, 17-ODYA markedly reduced the 20-HETE concentration and infarct size. Nonspecific CYP inhibitors have been recently shown to reduce infarct size of rat and rabbit hearts.18 However, the reduction in infarct size produced by these inhibitors was attributed to the stimulation of a CYP2C9-like enzyme and increases in its AA metabolite, 11,12-EET.

20-HETE is primarily catalyzed in kidney by several isoforms of the CYP4A family, particularly CYP4A1, CYP4A2, and CYP4A3,24,34,35 and CYP4F.28 Although CYP4A1 and CYP4A3 can metabolize AA to 20-HETE and 11,12-EET, CYP4A1 specifically metabolizes AA to 20-HETE with greater catalytic activity.32 Western blot analysis indicated immunoreactive bands associated with the primary antibodies against CYP4A1, CYP4A2, and CYP4F proteins from microsomes of heart tissue. As expected, the intensity of the immunoreactive bands was more intense from the membrane protein fractions than from the cytosolic protein fractions. The very weak immunoreactive bands of proteins in the cytosolic fractions may be from contamination by proteins that were not completely separated by centrifugation. To the best of our knowledge, this is the first report of the expression of CYPo-hydroxylases in the canine heart. The relative specific activity and contribution of each of these CYPo-hydroxylases in the synthesis of 20-HETE in the canine heart remains to be elucidated.

20-HETE synthesis in vitro in heart microsomes was investigated by incubating these microsomes with exogenous [4C]AA and analyzing by HPLC. A radioactive peak that comigrated with standard 20-HETE was observed and the peak was decreased when the microsomes were treated with 10-5 mol/L of 17-ODYA before incubation with [4C]AA. These data indicate that heart tissue can produce 20-HETE and that its activity could be inhibited by 17-ODYA. Radioactive peaks corresponding to the EETs and DHETs were not detected under these conditions.

Taken together, the results of these studies indicate a key important endogenous role of CYPo-hydroxylases and their major AA metabolite, 20-HETE, in mediating ischemia–reperfusion injury of the canine heart. These data also suggest a potential new therapeutic target for intervention in reducing the severity of ischemic or reperfusion injury in patients with ischemic heart disease.

Acknowledgments

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References


24. Schwartzman ML, da Silva JL, Lin F, Nishimura M, Abraham NG. Cytochrome P450 4A expression and arachidonic acid omega-


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