Epoxideicosatrienoic acids in cardioprotection: ischemic versus reperfusion injury

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Submitted 17 January 2006; accepted in final form 8 February 2006

RECENTLY, THE INFLUENCE of the cytochrome P-450 (CYP) epoxygenases and their arachidonic acid (AA) metabolites, the epoxideicosatrienoic acids (EETs), have been shown to produce increases in postischemic function via ATP-sensitive potassium channels ($K_{\text{ATP}}$); however, the direct effects of EETs on infarct size (IS) have not been investigated. We demonstrate that two major regioisomers of CYP epoxygenases, 11,12-EET and 14,15-EET, significantly reduced IS in dogs compared to control (22.1 ± 1.8%), whether administered 15 min before 60 min of coronary occlusion (6.4 ± 1.9%, 11,12-EET; and 8.4 ± 2.4%, 14,15-EET) or 5 min before 3 h of reperfusion (8.8 ± 2.1%, 11,12-EET; and 9.7 ± 1.4%, 14,15-EET). Pretreatment with the epoxide hydrolase metabolite of 14,15-EET, 14,15-dihydroxyeicosatrienoic acid, had no effect. The protective effect of 11,12-EET was abolished (24.3 ± 4.6%) by the $K_{\text{ATP}}$ channel antagonist glibenclamide. Furthermore, one 5-min period of ischemic preconditioning (IPC) reduced IS to a similar extent (8.7 ± 2.8%) to that observed with the EETs. The selective CYP epoxygenase inhibitor, N-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH), did not block the effect of IPC. However, administration of MS-PPOH concomitantly with N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), a selective inhibitor of endogenous CYP ω-hydroxylases, abolished the reduction in myocardial IS expressed as a percentage of area at risk (IS/AAR) produced by DDMS (4.6 ± 1.2%, DDMS; and 22.2 ± 3.4%, MS-PPOH + DDMS). These data suggest that 11,12-EET and 14,15-EET produce reductions in IS/AAR primarily at reperfusion. Conversely, inhibition of CYP epoxygenases and endogenous EET formation by MS-PPOH, in the presence of the CYP ω-hydroxylase inhibitor DDMS blocked cardioprotection, which suggests that endogenous EETs are important for the beneficial effects observed when CYP ω-hydroxylases are inhibited. Finally, the protective effects of EETs are mediated by cardiac $K_{\text{ATP}}$ channels.

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MS-PPOH, a selective CYP epoxide hydrolase inhibitor, and DDMS, a selective CYP \( \alpha \)-hydroxylase inhibitor.

Finally, several studies have suggested that the EETs are direct activators of sarcolemmal ATP-sensitive potassium (sarcK\(_{\text{ATP}}\)) channels in membrane patches isolated from rat myocardium (6,7) and have been shown to activate mitochondrial K\(_{\text{ATP}}\) (mitoK\(_{\text{ATP}}\)) channels in mouse hearts (13). Because both of these channels have been shown to be important in mediating cardioprotection produced by numerous mediators in many laboratories, including our own (5), we decided to test the importance of this channel in mediating the cardioprotective effects of the EETs in our canine model of infarction.

**MATERIALS AND METHODS**

**Materials.** Arachidonic acid (AA), EETs, dihydroxyicosatetraenoic acids (DHETs), and 20-HETE (for eicosanoid determination by mass spectrometry) were obtained from Cayman Chemical (Ann Arbor, MI). DDMS, EETs, 20-HEDE, 20-HETE, MS-PPOH, and \([^{2}H]_{8}\)20-HETE were synthesized in the laboratory of J. R. Falck. \([^{7}H]_{8}\)14,15-DHET and \([^{9}H]_{8}\)EETs were synthesized in our laboratory (11). Glibenclamide was obtained from Sigma Chemical (St. Louis, MO). C18 Bond Elut solid phase extraction (SPE) columns were obtained from Varian (Harbor City, CA). 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 performed in accordance with the “Position of the American Heart Association on Research and Animal Use,” adopted by the American Heart Association, and approved by the Biomedical Resource Center and the Institutional Animal Care Committee of the Medical College of Wisconsin. The Medical College of Wisconsin is accredited by the American Association of Laboratory Animal Care.

The protocol for dog preparation and experiments has been previously described in detail (12). Briefly, adult mongrel dogs of either sex, weighing 18 to 25 kg, were fasted overnight, anesthetized with the combination of barbitral sodium (200 mg/kg) and pentobarbital sodium (15 mg/kg), and ventilated with room air that was 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 5–7 cmH\(_{2}\)O with a trap. Arterial blood pH, PCO\(_{2}\), and PO\(_{2}\) were monitored at selected intervals by an AVL automatic blood-gas system and maintained within normal physiological limits (pH, 7.35 to 7.45; PCO\(_{2}\), 30 to 40 mmHg; and PO\(_{2}\), 85 to 120 mmHg) 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 coronary artery (LAD) 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. Hemodynamics, heart rate, and coronary blood flow were monitored throughout the experiment. The left atrium was cannulated via the appendage for radioactive microsphere injections.

**Experimental design.** Dogs were sequentially assigned to 12 groups for different treatments. Typically, eight dogs were used in each group of experiments. At 15 min before the 60-min LAD occlusion period, 11,12-EET (0.128 mg/kg), 14,15-EET (0.128 mg/kg), 14,15-DHET (0.155 mg/kg), DDMS (0.81 mg/kg), or vehicle were administered by intracoronary infusion for 2–3 min as shown in protocol 1 (Fig. 1). For IPC experimental groups, a brief occlusion of 5 min was performed 15 min after the administration of drugs (or vehicle) and the subsequent 60-min LAD occlusion (10 min after IPC) as shown in protocol 2 (Fig. 1). In two other groups, these same doses of EETs were administered by intratracheal infusions over 2–3 min at 5 min before reperfusion to determine whether EETs block reperfusion injury. Glibenclamide (1.0 mg/kg iv) was administered either alone 20 min before occlusion or concomitantly with IPC or DDMS. In all groups, hemodynamic measurements, blood-gas analyses, and myocardial blood flow measurements were performed at baseline and at 30 min into the 60-min occlusion period. After reperfusion, hemodynamics were measured every hour and myocardial blood flow was determined at the end of the 3-h reperfusion period. At the end of the experiment, the hearts were electrically fibrillated, removed, and prepared for IS determination and regional myocardial blood flow measurement.

**IS determination.** IS was determined as previously described (8). Briefly, at the end of the 3-h 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 of saline were injected at equal pressure into the left atrium and LAD, respectively. The heart was then immediately fibrillated and removed. The left ventricle (LV) was dissected and sliced into serial transverse sections 6–7 mm in width. The nonstained ischemic area and the blue-stained normal area were separated and incubated with 1% 2,3,5-triphenyl-2H-tetrazolium chloride (Sigma) in 0.1 mol/l phosphate buffer, pH 7.4 at 37°C for 15 min. After incubation overnight in 10% formaldehyde, the nonischemic and ischemic tissues within the AAR were separated and determined gravimetrically. IS was expressed as a percentage of the AAR (IS/AAR).

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**Fig. 1.** Protocols for drug administration and ischemia-reperfusion of dogs. **Protocol 1:** drug treatment, occlusion (Occ), and after reperfusion (Rep). **Protocol 2:** drug treatment combined with ischemic preconditioning (IPC), occlusion, and after reperfusion. Lower arrows in both panels represent time points for collecting blood samples for determination of eicosanoids. BL, baseline; DS, drug sample.
**Regional myocardial blood flow.** Regional myocardial blood flow (in ml·min⁻¹·g⁻¹) was measured by the radioactive microsphere technique as previously reported (1). Microspheres were administered at 30 min into the 60-min occlusion period and at the end of 3 h of reperfusion. Transmural blood flow was calculated as the weighted average of the subepicardium, midmyocardium, and subendocardium in each region.

Sample collection and preparation for CYP metabolites of AA determination. In some experiments, coronary venous plasma (great cardiac vein) samples were collected as previously described (9). Briefly, blood was drawn through an 8-Fr single lumen (100 cm length) catheter. Once the catheter was cleared of residual, stagnant blood, 5 ml of blood was aspirated over a 3- to 5-s period into a chilled sample tube containing heparin (1,000 U/ml) and miconazole (5 × 10⁻⁵ mol/l), mixed thoroughly and placed in an ice bucket. Blood samples were collected at different times as indicated in protocols 1 and 2 (Fig. 1). The samples were centrifuged at 3,000 g at 0°C for 10 min to separate plasma. Plasma was removed and transferred to a tube, purged with nitrogen gas, and capped and stored at −80°C or extracted.

The internal standards, 1.0 ng each for [¹³C₆]EETs, [¹³C₆]DHETs, and 20-[¹³C₆]HETE, were added to each sample and mixed. Ethanol was then added to a final concentration of 15% and mixed and centrifuged at 1,500 rpm for 3 min. Plasma samples were extracted by SPE as previously described (11).

**Liquid chromatography-electrospray ionization-mass spectrometry determination of CYP metabolites of AA.** CYP metabolites of AA in plasma samples were analyzed by liquid chromatography-electrospray ionization-mass spectrometry (Agilent 1100 LC/MSD, SL Model) as previously described (11). Selected ion monitoring in the negative detection mode was used for determination of the CYP metabolites of AA.

**Statistical analysis.** All values are expressed as means ± SD. Differences between groups in hemodynamics were compared by using a two-way ANOVA. Differences between groups in tissue blood flows, AAR, and IS were compared by one-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 two-way repeated-measures ANOVA, followed by a Tukey’s post hoc test. Differences between groups were considered significant if P < 0.05.

## RESULTS

**Regional myocardial blood flow.** Transmural blood flows in the nonischemic (left circumflex coronary) and the ischemic (LAD) regions were measured during 60 min of occlusion and after 3 h of reperfusion. There was no significant difference in nonischemic or transmural collateral blood flow (Table 1) between groups, indicating that all groups were subjected to similar degrees of ischemia. Similarly, there were no significant differences in the AAR between groups (data not shown).

Thus the two major determinants of IS in the dog, collateral blood flow and the AAR, were not different, so that any changes in IS observed in the drug- or IPC-treated groups were the result of their direct or indirect effects on other determinants of IS, such as cell signaling pathways. Six animals were excluded from data analysis due to a high collateral blood flow (endocardial, >0.15 ml·min⁻¹·g⁻¹).

**Hemodynamics.** Heart rate and mean arterial blood pressure at baseline between groups or at 30 min after occlusion or 3 h after reperfusion were not significantly different (Table 2). These data further suggest that changes in IS were not the result of changes in peripheral hemodynamics.

### Effects of 11,12- and 14,15-EET on myocardial IS. IS/AAR in the four treatment groups was significantly reduced compared with that in the control group (Fig. 2A). 11,12-EET at 0.128 mg/kg markedly reduced IS/AAR (6.4 ± 1.9 and 8.8 ± 2.1%, respectively) compared with that in the control group (22.1 ± 1.8%), whether administered 15 min before occlusion or 5 min before reperfusion. Similarly, 14,15-EET at 0.128 mg/kg also produced a marked reduction in IS/AAR (8.4 ± 2.4% and 9.7 ± 1.4%, respectively). Exogenous administration of the major metabolite of 14,15-HETE, 14,15-DHET, had no effect on IS/AAR (Fig. 2A). The beneficial effect of 11,12-EET pretreatment on IS/AAR (6.4 ± 1.9%) was completely abolished by pretreatment with the KATP channel blocker glibenclamide (24.3 ± 4.6%, Fig. 2B).

### Effects of MS-PPOH on IPC- and DDMS-induced cardioprotection. A brief 5-min period of IPC of the heart 10 min before the 60-min occlusion decreased IS/AAR to 8.7 ± 1.8%, similar to previous results obtained in our laboratory (10). Pretreatment with MS-PPOH did not block the effects of IPC to reduce IS/AAR (10.5 ± 2.0%, Fig. 3A). In contrast, DDMS produced a marked reduction in IS/AAR (4.9 ± 2.9%, Fig. 3B), similar to that previously observed in our laboratory (10); however, the protective effects of DDMS were completely

### Table 1. Transmural blood flow values

<table>
<thead>
<tr>
<th>Group</th>
<th>Nonischemic Region (30-min Occ)</th>
<th>Ischemic Region (30-min Occ)</th>
<th>Nonischemic Region (3-h Rep)</th>
<th>Ischemic Region (3-h Rep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.80 ± 0.17</td>
<td>0.94 ± 0.16</td>
<td>0.08 ± 0.01</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>11,12-EET (Occ)</td>
<td>0.50 ± 0.05</td>
<td>0.81 ± 0.10</td>
<td>0.12 ± 0.02</td>
<td>0.88 ± 0.10</td>
</tr>
<tr>
<td>11,12-EET (Rep)</td>
<td>1.03 ± 0.13</td>
<td>0.91 ± 0.09</td>
<td>0.13 ± 0.02</td>
<td>1.05 ± 0.18</td>
</tr>
<tr>
<td>14,15-EET (Occ)</td>
<td>0.69 ± 0.03</td>
<td>1.07 ± 0.08</td>
<td>0.12 ± 0.02</td>
<td>1.30 ± 0.16</td>
</tr>
<tr>
<td>14,15-EET (Rep)</td>
<td>0.80 ± 0.09</td>
<td>0.68 ± 0.08</td>
<td>0.11 ± 0.01</td>
<td>0.91 ± 0.17</td>
</tr>
<tr>
<td>14,15-DHET (Occ)</td>
<td>0.98 ± 0.17</td>
<td>1.17 ± 0.16</td>
<td>0.11 ± 0.01</td>
<td>1.21 ± 0.09</td>
</tr>
<tr>
<td>11,12-EET (Occ) + GLIB</td>
<td>0.72 ± 0.15</td>
<td>0.69 ± 0.07</td>
<td>0.13 ± 0.02</td>
<td>0.55 ± 0.12</td>
</tr>
<tr>
<td>MS-PPOH (Occ)</td>
<td>0.66 ± 0.12</td>
<td>0.84 ± 0.12</td>
<td>0.08 ± 0.02</td>
<td>0.65 ± 0.14</td>
</tr>
<tr>
<td>IPC</td>
<td>0.70 ± 0.09</td>
<td>0.90 ± 0.05</td>
<td>0.11 ± 0.02</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>MS-PPOH + IPC</td>
<td>0.85 ± 0.11</td>
<td>0.92 ± 0.12</td>
<td>0.11 ± 0.01</td>
<td>0.95 ± 0.23</td>
</tr>
<tr>
<td>DDMS (Occ)</td>
<td>0.79 ± 0.14</td>
<td>1.06 ± 0.12</td>
<td>0.11 ± 0.02</td>
<td>1.20 ± 0.22</td>
</tr>
<tr>
<td>MS-PPOH + DDMS (Occ)</td>
<td>0.98 ± 0.09</td>
<td>1.18 ± 0.13</td>
<td>0.12 ± 0.02</td>
<td>1.68 ± 0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–9 dogs. Shown are transmural blood flow values (in ml·min⁻¹·g⁻¹). Occ, occlusion; Rep, reperfusion; EET, epoxycosatrienoic acid; DHET, dihydroxyicosatrienoic acid; Glib, glibenclamide; MS-PPOH, N-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide; IPC, ischemic preconditioning; DDMS, N-methylsulfonyl-12,12-dibromododec-11-enamide.
abolished by pretreatment with MS-PPOH (22.2 ± 3.4%, Fig. 3B).

Plasma CYP metabolites of AA, 14,15-EET, and 14,15-DHET. When dogs were pretreated with 14,15-EET, there was an increase from nondetectable concentrations in coronary venous blood to a peak of 0.5 ng/ml immediately after drug injection, which remained at a concentration between 0.25 and 0.5 ng/ml throughout occlusion and reperfusion (Fig. 4B). 14,15-DHET, the metabolite of 14,15-EET, also increased to a peak of ~5 ng/ml, which remained at that level throughout occlusion and fell during the 3 h of reperfusion to ~1 to 2 ng/ml at 3 h. Infusion of 14,15-DHET at a dose calculated to approximate the concentration of 14,15-DHET, obtained with the injection of 14,15-EET, peaked at ~4.5 ng/ml and remained at this concentration throughout reperfusion (Fig. 4A). These data suggest that the protective effect of 14,15-EET is not due to increases in its metabolite 14,15-DHET.

**DISCUSSION**

We have previously demonstrated that CYP ω-hydroxylases and their AA metabolite 20-HETE have a detrimental role in enhancing myocardial ischemia-reperfusion injury in the canine myocardium (10, 12). In the present study, we demonstrate that exogenous administration of two CYP epoxygenase products, 11,12-EET and 14,15-EET, markedly reduce IS in canine hearts subjected to a 60-min occlusion period followed by 3 h of reperfusion. The reduction of IS was observed whether the EET was administered 15 min before occlusion or at 5 min before reperfusion. This latter observation, which suggests that the EETs may reduce reperfusion injury, has important clinical implications because most patients suffering an acute ischemic event would be administered cardioprotective therapies after a certain period of occlusion and not before the coronary occlusion. Of additional importance, the magnitude of the reduction in IS/AAR after EET administration was equivalent to that of IPC, thought to be the most powerful cardioprotective intervention thus far demonstrated. Similar to IPC (5), the protective effects of the EETs are at least partially the result of opening of K_ATP channels, although the results with glibenclamide do not discern between the importance of the sarc- or mitoK_ATP channel. Taken together, these results suggest that activation of CYP ω-hydroxylases and administration of exogenous 20-HETE have significant detrimental effects on the canine myocardium during ischemia and/or after reperfusion and that exogenous administration of two regiosomers of the CYP epoxygenase pathway, 11,12- and 14,15-EET, significantly reduced IS, and this effect was completely blocked by Glib. Values are means ± SE, n = 6 – 8 dogs. There were no significant differences among groups (data not shown). 11,12-EET significantly reduced IS/AAR (Fig. 2A). B: IS/AAR for the following treatments: control, 11,12-EET (Occ) (0.128 mg/kg); glibenclamide (Glib) (1.0 mg/kg) + 11,12-EET (Occ) (0.128 mg/kg). AAR/LV was not significantly different among groups (data not shown). 11,12-EET significantly reduced IS, and this effect was completely blocked by Glib. Values are means ± SE, n = 6 – 8 dogs/group. *p < 0.01, significantly lower than control; #p < 0.05, significantly higher than control. Pre, pretreatment.

Table 2. Hemodynamic values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Occ 30 min</th>
<th>Rep. 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>152 ± 2</td>
<td>158 ± 4</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>11,12-EET (Occ)</td>
<td>148 ± 2</td>
<td>152 ± 5</td>
<td>152 ± 5</td>
</tr>
<tr>
<td>14,15-DHET (Occ)</td>
<td>160 ± 6</td>
<td>153 ± 4</td>
<td>148 ± 3</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>102 ± 7</td>
<td>101 ± 6</td>
<td>108 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 – 9 dogs. There were no significant differences among groups by ANOVA, followed by the Newman-Keuls post hoc test.

Fig. 2. A: myocardial infarct size (IS) expressed as percentage of area at risk (IS/AAR, in %) for the following treatments: 11,12-epoxyeicosatrienoic acid (EET) (Occ), 0.128 mg/kg; 14,15-EET (Occ), 0.128 mg/kg; 11,12-EET (Rep), 0.128 mg/kg; and 14,15-dihydroxyeicosatetraenoic acid (DHET), 0.155 mg/kg. AAR/LV was not significantly different among groups (data not shown). 11,12-EET and 14,15-EET produced marked reductions in IS/AAR whether given before occlusion or just before reperfusion. 14,15-DHET had no significant effect on IS/AAR(%). B: IS/AAR for the following treatments: control, 11,12-EET (Occ) (0.128 mg/kg); glibenclamide (Glib) (1.0 mg/kg) + 11,12-EET (Occ) (0.128 mg/kg). AAR/LV was not significantly different among groups (data not shown). 11,12-EET significantly reduced IS, and this effect was completely blocked by Glib. Values are means ± SE, n = 6 – 8 dogs/group. *p < 0.01, significantly lower than control; #p < 0.05, significantly higher than control. Pre, pretreatment.
EET, demonstrated potent cardioprotective effects in canine hearts. Because the protective effects of the EETs and IPC were nearly equally effective, we initially hypothesized that the effects of IPC may be the result of an increase in EET synthesis; however, our results with the CYP epoxygenase inhibitor MS-PPOH suggests that this is not likely. MS-PPOH did not block the protective effect of IPC, whereas the effect of a CYP ω-hydroxylase inhibitor DDMS, which we (10) had previously shown to also be markedly cardioprotective in dogs, presumably by reducing the production of 20-HETE and shifting the endogenous balance in favor of EET production, was completely blocked by MS-PPOH. The additive effects of DDMS and IPC in our previous work also support the hypothesis that IPC and inhibition of the CYP ω-hydroxylase pathway each have distinct signaling pathways, although they may converge on the KATP channel downstream.

Several recent studies (13, 15) indicate that CYP epoxygenases and their AA metabolites, the EETs, are cardioprotective in mice. In plasma samples, obtained and published in several recent references from our laboratory (10, 12), only 11,12- and 14,15-DHET but not the parent compounds were detected, suggesting the possibility that the EETs were rapidly hydrolyzed by soluble epoxide hydrolase (sEH). Despite their rapid hydrolysis and avid protein binding, the cardioprotective effect of the exogenous EETs appears to be very long lasting, which suggests that like IPC, their effect turns on a signaling pathway that is not immediately inactivated. Nevertheless, the synthesis of new EET analogs that are resistant to breakdown by sEH may be desirable for future clinical applications. In this regard, the effect of several sEH inhibitors has recently been suggested to have protective effects as hypotensive and lipid-lowering agents and may also be expected to have cardioprotective effects.
effects by enhancing or preserving EET concentrations in the ischemic and/or reperfused myocardium (14).

Interestingly, the protective effect of the EETs was completely blocked by the \( K_{ATP} \) channel blocker glibenclamide. This finding in dogs was not that surprising because the EETs have been previously shown to activate sarco\( K_{ATP} \) channels in membrane patches (6, 7) isolated from rat hearts and mito\( K_{ATP} \) channels as assessed by changes in flavoprotein fluorescence in mitochondria isolated from mouse hearts (13). Further studies are necessary to determine the relative importance and timing of opening of these two channels in dogs or other species.

In conclusion, the present results indicate that exogenous administration of two of the major regioisomers of the CYP epoxygenase pathway, 11,12-EET and 14,15-EET, both produce equivalent and markedly significant reductions in IS/AAR in the canine heart, whether given before occlusion or at reperfusion, and that these effects are mediated by opening of cardiac \( K_{ATP} \) channels. The cardioprotective effects observed were nearly equivalent to that of IPC, although the present results using the CYP epoxygenase inhibitor MS-PPOH suggest that these two interventions may have some fundamental differences in their signaling pathways. Although the cellular mechanism(s) responsible for the protection observed after EET administration are yet to be determined, these data suggest potential therapeutic targets for intervention in myocardial ischemia-reperfusion injury. Blockade of the CYP \( \omega \)-hydroxylase pathway by agents such as DDMS or activation of the CYP epoxygenase pathway and subsequent increase in EET metabolism may be two interrelated mechanisms, which, when optimally combined, may result in a powerful novel approach for combating ischemic heart disease.

**REFERENCES**


