Epoxyeicosatrienoic Acids and Dihydroxyeicosatrienoic Acids Are Potent Vasodilators in the Canine Coronary Microcirculation

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Abstract—Cytochrome P<sub>450</sub> epoxygenases convert arachidonic acid into 4 epoxyeicosatrienoic acid (EET) regioisomers, which were recently identified as endothelium-derived hyperpolarizing factors in coronary blood vessels. Both EETs and their dihydroxyeicosatrienoic acid (DHET) metabolites have been shown to relax conduit coronary arteries at micromolar concentrations, whereas the plasma concentrations of EETs are in the nanomolar range. However, the effects of EETs and DHETs on coronary resistance arterioles have not been examined. We administered EETs and DHETs to isolated canine coronary arterioles (diameter, 90.0±3.4 µm; distending pressure, 20 mm Hg) preconstricted by 30% to 60% of the resting diameter with endothelin. All 4 EET regioisomers produced potent, concentration-dependent vasodilation (EC<sub>50</sub> values ranging from −12.7 to −10.1 log [M]) and were approximately 1000 times more potent than reported in conduit coronary arteries. The vasodilation produced by 14,15-EET was not attenuated by removal of the endothelium and indicated a direct action of 14,15-EET on microvascular smooth muscle. Likewise, 14,15-DHET, 11,12-DHET, 8,9-DHET, and the δ-lactone of 5,6-EET produced extremely potent vasodilation (EC<sub>50</sub> values ranging from −15.8 to −13.1 log [M]). The vasodilation produced by these eicosanoids was highly potent in comparison to that produced by other vasodilators, including arachidonic acid (EC<sub>50</sub>=−7.5 log [M]). The epoxide hydrolase inhibitor, 4-phenylchalone oxide, which blocked the conversion of [3H]14,15-EET to [3H]14,15-DHET by canine coronary arteries, did not alter arteriolar dilation to 11,12-EET; thus, the potent vasodilation induced by EETs does not require formation of DHETs. In contrast, charybdotoxin (a KCa channel inhibitor) and KCl (a depolarizing agent) blocked vasodilation by 11,12-EET and 11,12-DHET. We conclude that EETs and DHETs potently dilate canine coronary arterioles via activation of KCa channels. The preferential ability of these compounds to dilate resistance blood vessels suggests that they may be important regulators of coronary circulation. (Circ Res. 1998;83:932-939.)

Key Words: epoxyeicosatrienoic acids ■ dihydroxyeicosatrienoic acids ■ arachidonic acid ■ endothelium-derived hyperpolarizing factor ■ coronary microcirculation

Arachidonic acid (AA) can be converted to vasoactive metabolites through several enzymatic pathways, including the cyclooxygenase, lipoygenase, and cytochrome P<sub>450</sub> pathways. Cytochrome P<sub>450</sub> epoxygenases convert AA to 4 epoxyeicosatrienoic acid (EET) regioisomers, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET, corresponding to the carbons where the epoxide group is formed. EETs have been shown to relax coronary, cerebral, renal, and pial arteries, and they also hyperpolarize vascular smooth muscle cells. In conduit coronary blood vessels, both vasorelaxation and hyperpolarization may be mediated by activation of calcium activated K<sup>+</sup> (Kc<sub>a</sub>) channels. By virtue of these properties, EETs are considered to be candidates for endothelium-derived hyperpolarizing factors (EDHF). EETs are converted rapidly by vascular endothelial and smooth muscle cell epoxide hydrolases to dihydroxyeicosatrienoic acids (DHETs), which are devoid of vasoactive properties in noncoronary blood vessels. However, in porcine conduit coronary arteries contracted with U46619, DHETs produce comparable amounts of relaxation as their parent EETs. The relaxation responses were observed predominately at micromolar concentrations of DHETs, as reported previously for the 4 EET regioisomers in canine conduit coronary arteries.

In all reports to date, the concentrations of EETs and DHETs required to produce significant coronary vasorelaxation were greater than the nanomolar concentrations of EETs in plasma. These findings question the relevance of these eicosanoids as physiological mediators of coronary vascular conductance. In prior studies, the effects of these compounds were examined in conduit coronary arteries rather than in coronary arterioles, which are responsible for the majority of coronary vascular resistance. It was suggested recently that EET sensitivity might increase with decreasing vascular size.
Other investigators have demonstrated that the contribution of EDHF to endothelium-dependent responses is greater in small arteries than in large arteries. Thus, taken together, these studies suggest that the vasodilatory role of EETs and DHETs may be more important in coronary microcirculation than in conduit coronary blood vessels.

In the present study, we examined responses to EETs and DHETs in isolated canine coronary arterioles (diameter, <125 μm) preconstricted with endothelin. In contradistinction to coronary conduit arteries, resistance coronary arterioles were found to dilate to extremely low (picomolar) concentrations of EETs and DHETs, suggesting that these cytochrome P₄₅₀ metabolites may contribute importantly to vasoregulation of the coronary circulation. We also examined potential mechanisms involved in the potent EET-induced coronary microvascular dilatation.

Materials and Methods

All animal protocols were approved by the University of Iowa Animal Care and Use Committee and conform with the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH publication No. 85-23, revised 1985).

Synthesis of EETs and DHETs

Vasoactivity was tested using radiolabeled EETs and DHETs to permit confirmation of the final concentrations by liquid scintillation techniques. The radiolabeled eicosanoids were synthesized, using methods originally developed for unlabeled compounds.

The radiolabeled EET regioisomers were synthesized from methylated arachidonate (American Radiolabeled Chemicals, St Louis, Mo). In brief, [1-¹⁴C]AA was mixed with AA to a specific activity of 12.3 Ci/mol and methylated using ethereal diazomethane. The methylated arachidonate was mixed with 0.2 equivalents of m-chloroperoxybenzoic acid, and the resulting EET methyl esters were extracted, resolved by silicic acid high-performance liquid chromatography (HPLC), and collected. The isolates were saponified, again resolved by silicic acid HPLC, collected, and stored in methanol at −80°C until use.

The radiolabeled DHET regioisomers were synthesized from the corresponding [1-¹⁴C]EET methyl esters. The epoxide ring first was hydroxylated at 40°C for 16 hours in the presence of acetic acid, and the remaining methyl ester was removed by saponification. Each DHET regioisomer was subjected to silicic acid HPLC, collected, and stored in methanol at −80°C until use.

Based on absorption at 192 nm during reversed-phase HPLC and radioassays of collected fractions, the combined normal-phase HPLC procedures produced regioisomers with >98% chemical and radiopurity. The identity of each regioisomer was established by coelution with unlabeled standards during HPLC and gas chromatography and by mass spectrometry.

Procurement of Tissue

Seventy-two adult mongrel dogs of either sex (8 to 25 kg) were euthanized with sodium pentothal (50 mg/kg). The hearts were harvested quickly and placed immediately in cold (4°C) oxygenated (20% O₂; 5% CO₂; 75% N₂) Krebs bicarbonate buffer solution (see details below). Ventricular arterioles (60 to 150 μm; mean diameter, 90±3.4 μm) were dissected, trimmed of fat and connective tissue, and transferred to organ chambers.

Isolated Microvessel Preparation

A pressurized arteriolar preparation was used to study the isolated coronary microvessels. Each end of the arteriole was cannulated with a glass micropipette. The cannulated pipettes were attached to a hydrostatic pressure reservoir under conditions of no flow. The organ chamber was placed on the stage of an inverted microscope, to which a video camera, monitor, and calibrated caliper were attached.

The organ chamber was connected to a rotary pump, which continuously circulated oxygenated Krebs buffer warmed to 37°C. Internal diameters were measured by manually adjusting the video micrometer.

Each arteriole was allowed to equilibrate for 30 minutes at a distending pressure of 20 mm Hg. KCI (50 mmol/L) was added to the bath to test constrictor capacity. When stable levels of constriction were reached, the chambers were rinsed and the arterioles allowed to return to baseline diameter. Endothelin-1 (0.40 to 8.0 nmol/L) was used to constrict the arterioles to 30% to 60% of their resting diameters. Cumulative concentration-response relationships were evaluated for AA (10⁻¹⁰ to 10⁻³ mol/L), acetylcholine ([Ach] 10⁻¹⁰ to 10⁻⁴ mol/L), Ca²⁺ ionophore (A23187; 10⁻¹⁰ to 10⁻⁷ mol/L), and sodium nitroprusside ([SNP] 10⁻¹⁰ to 10⁻⁴ mol/L) by adding the drugs directly to the organ chambers. In separate experiments, concentration-response curves were performed using the 4 EET regioisomers and 8,9-DHET, 11,12-DHET, and 14,15-DHET regioisomers, as well as the 5,6-EET-methyl ester, and the β-lactone of 5,6-EET (10⁻¹⁸ to 10⁻⁴ mol/L). After completing the concentration-response studies, a single dose of SNP (100 μmol/L) was applied; in the KCI depolarization and charybdotoxin experiments, papaverine (100 μmol/L) was also administered. Arterioles were considered to be unacceptable for experimentation if they (1) demonstrated obvious leaks, (2) failed to constrict >50% to 50 mmol/L KCI and >30% to endothelin, or (3) failed to dilate >80% to 100 μmol/L SNP or papaverine.

In some studies, we examined whether the vasodilatory responses produced by 14,15-EET required an intact endothelium. Before microvessel cannulation, the endothelium was denuded by passing a thin wire several times through the vessel lumen. The endothelium was considered to be functionally denuded when bradykinin (1 μmol/L) produced <10% dilation. Damage to underlying smooth muscle was considered minimal if SNP (100 μmol/L) produced >80% dilation.

In other experiments, we examined whether epoxide hydrolase inhibitors altered the dilation response induced by EET. Responses to 11,12-EET were determined in the presence of vehicle (dimethyl sulfoxide [DMSO]) or 20 μmol/L 4-phenylchalone oxide (4-PCO), an inhibitor of epoxide hydrolases.

To examine the role of K⁺ channels in EET- and DHET-induced vasodilation, arteriolar responses to 11,12-EET and 11,12-DHET were performed in vessels depolarized with isotonic KCl solutions (prepared by substituting equimolar amounts of KCl for NaCl) or in endothelin-preconstricted vessels treated with 50 nmol/L charybdotoxin.

Isolated Vascular Rings

Coronary arteries were studied using a common isometric ring technique. Vascular rings (OD, 1.0 to 1.5 mm) were mounted on 2 stirrups made by passing stainless steel wires through the vessel lumen. One stirrup was attached to a force transducer and the other to a micrometer microdrive for stretching the vessel by known increments. Each vessel apparatus was placed in a 10-mL water-jacketed organ bath containing Krebs buffer equilibrated at 37°C and aerated with 20% O₂, 5 %CO₂, and 75% N₂. Isometric contractions and relaxations were measured using a computer. Rings were individually stretched to the maximum of the length-developed tension relationship by repeated test exposures to 75 mmol/L KCl at increasing vessel diameters. The vessels were allowed to stabilize for 30 minutes before performing concentration response curves. PGF₂α was used to constrict the vessels to 30% to 50% of their resting tension. After steady-state tension was achieved, concentration-response relationships were evaluated for several of the vasodilators used in the microvessel studies, including AA (10⁻¹⁰ to 10⁻³ mol/L), Ach (10⁻¹⁰ to 10⁻⁴ mol/L), A23187 (10⁻₁² to 10⁻⁶ mol/L), SNP (10⁻¹⁰ to 10⁻⁴ mol/L), and 11,12-DHET (10⁻⁹ to 10⁻⁷ mol/L), by adding the drugs directly to the organ baths. A single dose of SNP (100 μmol/L) was added at the end of the concentration-response curve to determine maximal relaxation. Acceptable ring experiments developed >1 g of tension to PGF₂α, and diluted 75% to 150% in response to SNP.

Canine epicardial coronary arteries were cleaned and cut into rings 2 to 4 mm in length. The rings were placed into 24-well plates containing 1.0 mL of Krebs buffer solution with 0.1 μmol/L BSA and constantly rotated for 2 hours in a 5% CO$_2$ incubator (37°C). The rings were rinsed with fresh Krebs solution containing 4-PCO (20 μmol/L), or vehicle was added. After 1 hour, the Krebs solution was replaced with fresh solution containing 14,15-EET (5.0 μmol/L) and [3H]14,15-EET (0.3 μCi) along with 4-PCO or vehicle. (Note—[3H]14,15-EET: the ‘H is present at carbons 5, 6, 8, 9, 11, 12, 14, and 15. The “14,15-” refers to the location of the epoxide group.) After 1 hour, 50-μL aliquots of the incubated solution were collected to determine total radioactivity by scintillation counting. Lipids were extracted from the incubation solution using 4-vol water-saturated ethyl acetate. Phases were separated by centrifugation at 600g for 10 minutes, and the ethyl acetate phase was collected and evaporated under N$_2$. The lipid extracts were resuspended in acetonitrile and stored at −20°C until analysis by HPLC.

Lipids were separated by reverse-phase HPLC using a Gilson 302 pump plus 2050 UV detector, and a 2.1×150-mm C$_{18}$ column containing 3-μm spherical particles of EQC (Alltech). The elution profile, developed with a Gilson 715 gradient controller, consisted of water adjusted to pH 3.4 with phosphoric acid and an acetonitrile gradient increasing from 25% to 100% over 60 minutes at a flow rate of 0.4 mL/min. Radioactivity was measured by combining the column effluent with Budget-Solve solution and passing the mixture through a Radiomatic Flo-One Beta isotope detector, as described previously.

The amount of 14,15-DHET formed (normalized to ring wet weight) was calculated from the specific activity of the radiolabeled 14,15-EET used in the incubation. In some experiments, the radiolabeled 14,15-EET was incubated under the same conditions but in the absence of coronary artery rings. No [3H]14,15-DHET was detected under these conditions (data not shown).

Solutions and Test Compounds

All solutions were prepared on the day of the experiment. Krebs solution contained (in mmol/L): NaCl 131.5; KCl 5; CaCl$_2$ 2.5; MgCl$_2$, 1.2; NaHCO$_3$ 23.5; KH$_2$PO$_4$, 1.2; and glucose 11, pH 7.4. Aerated with 20% O$_2$, 5% CO$_2$, and 75% N$_2$. MgCl$_2$, NaHCO$_3$, KH$_2$PO$_4$, and glucose were purchased from Sigma Chemical Co. 4-PCO was purified to 97% purity. All EETs and DHETs produced concentration-dependent vasodilation of pressurized, endothelin-preconstricted canine coronary arterioles. Significant vasodilation to 11,12-EET was observed after administration of subpicomolar concentrations, and the calculated EC$_{10}$ and EC$_{50}$ values were

<table>
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<tr>
<th>Regiosomer</th>
<th>EC$_{10}$ (μmol/L)</th>
<th>EC$_{50}$ (μmol/L)</th>
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<tr>
<td>5, 6</td>
<td>−13.3 ± 1.0</td>
<td>−10.8 ± 0.8$^{(a)}$</td>
<td>−15.3 ± 0.5</td>
<td>−13.3 ± 0.8$^{(a)}$</td>
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<td>8, 9</td>
<td>−13.8 ± 1.0</td>
<td>−11.0 ± 0.4$^{(a)}$</td>
<td>−16.7 ± 0.5$^{*}$</td>
<td>−13.1 ± 0.7$^{(a)}$</td>
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<td>11, 12</td>
<td>−12.8 ± 0.6</td>
<td>−10.8 ± 0.8$^{(a)}$</td>
<td>−17.8 ± 0.6$^{*}$</td>
<td>−15.8 ± 0.7$^{(a)}$</td>
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<tr>
<td>14, 15</td>
<td>−16.0 ± 0.6</td>
<td>−12.7 ± 0.6$^{(a)}$</td>
<td>−16.7 ± 1.0</td>
<td>−14.3 ± 1.2$^{(a)}$</td>
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Endothelium-intact canine coronary arterioles preconstricted with endothelin were exposed to increasing concentrations of one of the EET or DHET regiosomers, and changes in diameter were measured. The δ-lactone of 5,6-DHET was tested (rather than 5,6-DHET) because it is the major 5,6-EET product due to an enhanced chemical stability of the lactone arising from formation of a 6-membered ring. EC$_{10}$ and EC$_{50}$ responses for each of the 4 EET and DHET regiosomers were calculated and compared using the unpaired Student t test. Values are expressed as geometrical mean±SEM (log [M]). Superscript Nos. in parentheses refer to the No. of observations. *P<0.05 vs the corresponding EET regiosomer.

Figure 1. Concentration-dependent vasodilation produced by 11,12-EET and 11,12 DHET. Canine coronary arteries were preconstricted with endothelin to 53.4±4.1% (11,12-EET group) and 51.1±10.7% (11,12-DHET group) of their resting diameters, and 11,12-EET or 11,12-DHET was administered in concentration-dependent fashion. Values represent mean±SEM; n=5 for both groups. *P<0.05.
-12.8±0.6 and -10.8±0.8 log [M], respectively (Figure 1, Table 1). The maximum amount of vasodilation with 1 μmol/L 11,12-EET was 73±6%. In contrast to these findings, the EET vehicle (ethanol) at the highest concentration of vehicle administered produced only a modest amount of vasodilation (25±2%).

Responses to 11,12-DHET, the diol metabolite of 11,12-EET, were determined and compared with those produced by the parent EET. Surprisingly, 11,12-DHET was more potent than its parent compound at every concentration tested (Figure 1). Moreover, with substantial vasodilation occurring at a concentration of ≈10⁻¹⁸ mol/L, 11,12-DHET was the most potent agent tested in this study. The calculated EC₁₀ and EC₅₀ values were -17.8±0.6 and -15.8±0.7 (log [M]), respectively (Table 1). Thus, comparisons of the EC₁₀ and EC₅₀ values indicated that the 11,12-diol was 13 000- to 1 000 000-fold more potent than the parent 11,12-epoxide (Table 1). Maximal vasodilation was observed at 100 pmol/L 11,12-DHET; at this concentration, the diameter of the arteriole was essentially that of the preconstricted state.

Next, we examined responses to 5,6-EET and the δ-lactone of 5,6-EET (5,6-δ-lactone), and the methyl ester of 5,6-EET (5,6-EET-ME) on canine coronary microvessels. Endothelin-induced constrictions were 51.5±7.4%, 45.2±5.6%, and 53.6±7.1% of resting diameter in the 5,6-EET, δ-lactone, and methyl ester groups, respectively. Values represent mean±SEM; n=4 for 5,6-EET; n=3 for the other 2 groups. P=NS vs 5,6-EET. For 5,6-EET vs δ-lactone, P=0.70 and P=0.22 at 0.1 and 10.0 nmol/L, respectively. #P<0.05 for 5,6-δ-lactone vs 5,6-EET-ME.

Responses to the remaining 2 EET regioisomers, 8,9-EET and 14,15-EET, and their diol metabolites, were also determined. All 4 compounds were found to be potent vasodilators of canine coronary arterioles (Figure 3, Table 1). Similar to 11,12-DHET, 8,9-DHET possessed an EC₁₀ that was lower than its EET precursor (Table 1); that is, the 8,9-diol was slightly more potent than its 8,9-epoxide precursor in initiating vasodilation. However, at the higher concentrations the DHET and EET curves tended to overlap (Figure 3B), and the 8,9-DHET EC₁₀ (-13.1±0.7 (log [M])) was essentially the same as that of 8,9-EET (-11.0±0.4 (log [M])). Although the concentration-response curve of 14,15-DHET tended to be to the left of the parent 14,15-EET (Figure 3B), the 2 compounds were equipotent (P=0.09).

To address whether EET-induced vasorelaxation is dependent on the presence of endothelium, we examined responses to 14,15-EET in endothelium-denuded coronary arterioles. Denuded microvessels did not respond to bradykinin (1 μmol/L); however, SNP (100 μmol/L) produced 94±3% relaxation. Despite the absence of functional endothelium, 14,15-EET produced potent vasodilation that did not differ significantly from the responses observed in endothelium-
EETs and DHETs Are Potent Vasodilators

Mechanisms of EET-induced vasodilation. The effects of endothelium denudation (A) and treatment with 4-PCO (B) on the microvascular responses to 14,15-EET are shown. A, Canine coronary arterioles were constricted with endothelin to 39.1±3.4% (intact group) and 43.8±3.1% (denuded group) of their resting diameters, and 14,15-EET was administered in a concentration-dependent fashion. Values represent mean±SEM; n=5 (intact) and n=6 (denuded), *P<0.05. B, Concentration-dependent vasodilation produced by 11,12-EET in the presence of vehicle (DMSO) or 20 μmol/L 4-PCO in canine coronary microvessels. Canine coronary arterioles were pretreated with vehicle or 4-PCO and constricted with endothelin to 48.13±3.91% (vehicle group) and 50.49±4.61% (4-PCO group) of their resting diameters, and 11,12-EET was administered in a concentration-dependent fashion. Values represent mean±SEM; n=5 for both groups. *P<0.05. Inset shows the inhibitory effects of 4-PCO on [3H]14,15-DHET formation by canine coronary arterioles.

Figure 4. Mechanisms of EET-induced vasodilation. The effects of endothelium denudation (A) and treatment with 4-PCO (B) on the microvascular responses to 14,15-EET are shown. A, Canine coronary arterioles were constricted with endothelin to 39.1±3.4% (intact group) and 43.8±3.1% (denuded group) of their resting diameters, and 14,15-EET was administered in a concentration-dependent fashion. Values represent mean±SEM; n=5 (intact) and n=6 (denuded), *P<0.05. B, Concentration-dependent vasodilation produced by 11,12-EET in the presence of vehicle (DMSO) or 20 μmol/L 4-PCO in canine coronary microvessels. Canine coronary arterioles were pretreated with vehicle or 4-PCO and constricted with endothelin to 48.13±3.91% (vehicle group) and 50.49±4.61% (4-PCO group) of their resting diameters, and 11,12-EET was administered in a concentration-dependent fashion. Values represent mean±SEM; n=5 for both groups. *P<0.05. Inset shows the inhibitory effects of 4-PCO on [3H]14,15-DHET formation by canine coronary arterioles.

intact arterioles (Figure 4A) \(EC_{50} (\text{log [M]}) = -10.7 \pm 0.8\); maximal vasodilation=90±3% at 1 μmol/L; n=6). Thus, the dilation did not require a functional endothelial layer.

We also examined the effects of inhibition of epoxide hydrolases with 4-PCO on EET-induced canine coronary microvascular dilation. In preliminary studies, we examined the capacity of 4-PCO to inhibit epoxide hydrolases by pretreating canine coronary arterial rings with 4-PCO (20 μmol/L) or vehicle followed by incubation with 5 μmol/L [3H]14,15-EET. After 1 hour, the incubation buffer was removed, and lipids were extracted and separated by reverse-phase HPLC. 4-PCO inhibited the amount of [3H]14,15-DHET formed during the incubation (Figure 4B, insert), indicating that this dose of 4-PCO compound effectively blocks the epoxide hydrolase–mediated conversion of EETs to DHETs. Coronary microvascular vasodilation produced by 11,12-EET was also studied in the presence of vehicle (DMSO) or 20 μmol/L 4-PCO. In brief, canine coronary arterioles were constricted with endothelin, and 11,12-EET was administered in a concentration-dependent fashion. Responses to 11,12-EET did not differ among arterioles pretreated with 4-PCO as compared with vehicle (Figure 4B): \(EC_{50} (\text{log [M]}) = -12.5 \pm 0.9\); maximal vasodilation=88±8% at 1 μmol/L; and \(EC_{50} (\text{log [M]}) = -11.68 \pm 0.9\); maximal vasodilation=77±3% at 1 μmol/L, respectively (n=5 for both groups). These data suggest that the 11,12-EET-induced vasodilation is not dependent on conversion of the regioisomer to 11,12-DHET.

Further studies were performed to investigate the role of KCa channels in EET- and DHET-induced coronary microvascular dilation. Canine coronary arterioles were constricted with endothelin or isotonic KCl solution (35±2 mmol/L) to 30% to 60% of their resting diameter. 11,12-EET or 11,12-DHET was administered in a concentration-dependent manner. KCl depolarization abolished the dilation to 11,12-EET (Figure 5A) and 11,12-DHET (Figure 5B).

We also examined the effects of charybdotoxin, an inhibitor of KCa channels, on EET- and DHET-induced vasodilation. Responses to 11,12-EET (Figure 5C) and 11,12-DHET (Figure 5D) were attenuated in the presence of 50 mmol/L charybdotoxin by 59% and 56%, respectively. Together, these data suggest that EET- and DHET-induced microvascular relaxation involves activation of vascular smooth muscle KCa channels.

Responses to AA (the precursor of EETs and DHETs), calcium ionophore (A23187), Ach, and SNP were also determined. AA produced concentration-dependent vasodilation, with ≈20% and 90% dilation at concentrations of 1 μmol/L and 10 μmol/L, respectively. The calculated EC50 value for AA was −7.5±0.4 (log [M]) (Table 2), which was 1000- to 10 000-fold less than found for the EETs and their DHET products. All of the other vasodilators also produced concentration-dependent vasodilation of endothelin-preconstricted microvessels. However, 11,12-DHET was far more potent than all of these compounds tested. It is interesting that the potency of A23187 approached those of other EETs and their metabolites (Table 2). A23187 was more potent than Ach, AA, and SNP.

Responses to AA, A23187, Ach, SNP, and 11,12-DHET also were determined in PGF2α-constricted conduit coronary arterial rings. AA and SNP produced complete relaxation of the conduit arterial rings, whereas Ach and A23187 produced 89±4% and 78±7% relaxation, respectively, at the highest concentrations studied (100 and 1 μmol/L respectively). EC50 values of these agents are shown in Table 2. Up to 0.1 μmol/L 11,12-DHET produced minimal relaxation (24±13%) of PGF2α-constricted conduit arterial rings; at higher concentrations, 11,12-DHET actually constricted the vessels. As was observed in microvessels, A23187 was more potent than SNP, Ach, and AA in conduit vessels.

Discussion

There are several major findings in the present study. First, all 4 EET regioisomers produced extremely potent, concentration-dependent dilation of isolated canine coronary arterioles. The vasodilation produced by 14,15-EET was not attenuated by removal of the endothelium, suggesting a direct action of the compound on microvascular smooth muscle cells. Second, the major EET metabolites, DHETs, each produced vasodilation that was at least as potent as that
produced by the parent EETs. However, suppression of DHET formation by inhibition of epoxide hydrolases did not attenuate 11,12-EET–induced vasodilation, suggesting that the potent 11,12-EET–induced vasodilation is not dependent on conversion to 11,12-DHET. Third, vasodilation produced by 11,12-EET and 11,12-DHET was blocked by depolarization with KCl or charybdotoxin, suggesting that the responses were mediated by activation of KCa channels. Finally, the potency of EETs and DHETs to dilate coronary arterioles was found to be far greater than the potency reported for conduit coronary arteries. Together, these findings suggest that EETs and their diol metabolites may importantly regulate the coronary microcirculation.

Rosolowsky et al2 reported that in canine conduit coronary arteries contracted with U46619, the 4 EETs produced endothelium-independent relaxation, with EC50 values ~1 μmol/L for all 4 regioisomers, whereas Campbell et al3 and Weintraub et al18 showed similar results using bovine and porcine conduit coronary arteries. In contrast, we found that the markedly enhanced EET potency in arterioles as compared with conduit coronary arteries, suggesting that responses to SNP did not differ in coronary microvessels and/or through the production of EDHF.30–37 Moreover, pre- suppression of 11,12-EET–induced vasodilation is equipotent vasodilation of endothelium-denuded arterioles, suggesting that the compound acted directly on the vascular smooth muscle cells, an observation that is consistent with reports in canine, bovine, and porcine conduit coronary arteries.1,5,18 It was possible that the markedly enhanced EET potency could have been due to a nonspecific augmentation in the vasodilatory capacity of coronary arterioles as compared with conduit coronary artery responses. Such augmented vasodilatory capacity could be an inherent attribute of the arterioles, or it could stem from differences in the methodology used to examine responses in microvessels as compared with conduit arterial rings. In the present experiments, the coronary microvessels were pressurized, and vascular reactivity was assessed by measuring changes in vessel diameter; in the studies by Rosolowsky et al,1 Campbell et al,5 and Weintraub et al18 arterial rings were studied between conduit coronary arteries and coronary arterioles may differ. It is possible that the markedly enhanced EET potency observed in the present study could have resulted from an indirect effect of EETs to stimulate the production of EDRF, as was suggested by Graier et al.29 However, we found that 14,15-EET produced equipotent vasodilation of endothelium-denuded arterioles, suggesting that the compound acted directly on the vascular smooth muscle cells, an observation that is consistent with reports in canine, bovine, and porcine conduit coronary arteries.5,18

Responses to coronary arteriolar vasodilators were determined as described in Table 1. Conduit arteries were constricted with PGF2α, before exposure to vasodilators. 11,12-DHET produced only minimal (24±13%) relaxation of PGF2α–constricted conduit arterial rings. The EC50 values are expressed as geometrical mean±SEM (log μM). Supernscript numbers refer to the No. of observations. *P<0.05 vs 11,12-DHET.

### Table 2. Sensitivity of Canine Coronary Arterioles and Conduit Vessels to Vasodilators

<table>
<thead>
<tr>
<th>Vasodilator</th>
<th>Arteriolar EC50</th>
<th>Conduit EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>11,12-DHET</td>
<td>−15.8±0.7*</td>
<td>(See legend)</td>
</tr>
<tr>
<td>AA</td>
<td>−7.5±0.4*</td>
<td>−6.0±0.7*</td>
</tr>
<tr>
<td>Ach</td>
<td>−7.9±0.3*</td>
<td>−6.8±0.2*</td>
</tr>
<tr>
<td>SNP</td>
<td>−6.8±0.3*</td>
<td>−6.9±0.3*</td>
</tr>
<tr>
<td>A23187</td>
<td>−9.6±0.3*</td>
<td>−8.9±0.7*</td>
</tr>
</tbody>
</table>

Responses to coronary arteriolar vasodilators were determined as described in Table 1. Conduit arteries were constricted with PGF2α, before exposure to vasodilators. 11,12-DHET produced only minimal (24±13%) relaxation of PGF2α–constricted conduit arterial rings. The EC50 values are expressed as geometrical mean±SEM (log μM). Supernscript numbers refer to the No. of observations. *P<0.05 vs 11,12-DHET.
to EDHF are more prominent in smaller blood vessels in rabbit and rat.\textsuperscript{21,22,38–40} Recently, Nagao et al\textsuperscript{21} reported that smaller blood vessels exhibited enhanced potency to aprilikam, an agonist that produces vasorelaxation through activation of ATP-sensitive K\(^+\) channels, suggesting that small arteries are more sensitive to the actions of hyperpolarizing vasodilators than are large arteries. Therefore, a plausible explanation for the moderately enhanced potencies of AA, Ach, and A23187 in coronary arterioles is that these agonists stimulate the formation of EDHF, which, in turn, produces more potent dilation of arterioles than conduit arteries.

We also report for the first time that 14,15-DHET, 11,12-DHET, 8,9-DHET, and the \(\delta\)-lactone of 5,6-EET are potent dilators of resistance blood vessels. In previous studies, it was reported that DHETs did not produce vasorelaxation of rabbit aorta, rat mesenteric microvessels, or the isolated perfused rat tail artery.\textsuperscript{14–16} Based on these observations, it was proposed that the conversion of EETs to DHETs may serve as a mechanism whereby EETs are rendered inactive within the vasculature.\textsuperscript{12,27} However, we recently reported that DHETs are incorporated into vascular endothelial phospholipids, where they may be released by bradykinin and potentiate vasodilatory responses.\textsuperscript{18,41} However, 11,12-DHET and 14,15-DHET produced comparable amounts of vasorelaxation as their parent EETs in porcine coronary arteries.\textsuperscript{17,18} In the present study, we found that the DHET regioisomers were as potent as (or, in the case of 11,12-DHET, more potent than) their parent EETs in canine coronary arterioles. We are unaware of any endogenous compound that produces vasodilation as potently as that produced by 11,12-DHET. These results suggest that both EETs and DHETs are capable of regulating coronary arteriolar tone by hyperpolarizing vascular smooth muscle. Furthermore, our results with KCl and charybdotoxin suggest that the dilatory responses to 11,12-EET and 11,12-DHET were mediated by activation of K\(_{Ca}\) channels.

Cultured vascular smooth muscle and endothelial cells rapidly convert EETs into DHETs.\textsuperscript{12,13,17,18} We recently reported that after a 3-minute incubation of porcine coronary artery smooth muscle cells with 1 \(\mu\)mol/L \(^{3}H\)14,15-EET, 8% of the radioactivity recovered in the medium already had been converted to \(^{3}H\)14,15-DHET.\textsuperscript{52} These findings, together with the current observation that 11,12-DHET is far more potent than its parent compound, 11,12-EET, in coronary microvessels, raised the possibility that the 11,12-EET–induced vasodilation might be mediated by its diol metabolite. However, the epoxide hydrolase inhibitor 4-PCO, which effectively blocked DHET formation in canine coronary arterioles, did not inhibit vasodilation to 11,12-EET in coronary microvessels. Thus, although conversion of 11,12-EET to 11,12-DHET yields a more potent vasodilator compound in canine coronary microvessels, the mechanism of the 11,12-EET–induced vasodilation is not dependent on the formation of 11,12-DHET.

In summary, the 4 EET regioisomers, and their diol metabolites, produced extremely potent vasodilation of canine coronary arterioles. The vasodilation, which was \(\approx 10,000\)-fold more potent than has been reported in conduit coronary arteries, was mediated by activation of vascular smooth muscle K\(_{Ca}\) channels. The preferential ability of these compounds to dilate resistance arterioles suggests that they may contribute to the physiological and/or pathophysiologic regulation of coronary microcirculation.

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**References**


Epoxyeicosatrienoic Acids and Dihydroxyeicosatrienoic Acids Are Potent Vasodilators in the Canine Coronary Microcirculation

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