Comparison of vasodilatory properties of 14,15-EET analogs: structural requirements for dilation

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Comparison of vasodilatory properties of 14,15-EET analogs: structural requirements for dilation. Am J Physiol Heart Circ Physiol 284: H337–H349, 2003. First published September 19, 2002; 10.1152/ajpheart.00831.2001.—Epoxideicosatrienoic acids (EETs) are endothelium-derived eicosanoids that activate potassium channels, hyperpolarize the membrane, and cause relaxation. We tested 19 analogs of 14,15-EET on vascular tone to determine the structural features required for activity. 14,15-EET relaxed bovine coronary arterial rings in a concentration-related manner (ED50 = 10−6 M). Changing the carboxyl to an alcohol eliminated dilator activity, whereas 14,15-EET-methyl ester and 14,15-EET-methylsulfonamide retained full activity. Shortening the distance between the carboxyl and epoxy groups reduced the agonist potency and activity. Removal of all three double bonds decreased potency. An analog with a Δ8 double bond had full activity and potency. However, the analogs with only a Δ5 or Δ11 double bond had reduced potency. Conversion of the epoxy oxygen to a sulfur or nitrogen resulted in loss of activity. 14(S),15(R)-EET was more potent than 14(R),15(S)-EET, and 14,15-(cis)-EET was more potent than 14,15-(trans)-EET. These studies indicate that the structural features of 14,15-EET required for relaxation of the bovine coronary artery include a carbon-1 acidic group, a Δ8 double bond, and a 14(S),15(R)-(cis)-epoxy group.

endothelium-derived hyperpolarizing factor; cytochrome P-450; arachidonic acid; epoxideicosatrienoic acid

ENDOTHELIAL CELLS MEDIATE relaxation of vascular smooth muscle through the release of a number of soluble mediators such as nitric oxide, prosta
cyclin, and endothelium-derived hyperpolarizing factor (EDHF) (6, 19, 31). The identity of EDHF remains controversial, with studies that implicate metabolites of arachidonic acid, potassium ion, and hydrogen peroxide (3, 8, 15, 23, 30, 34, 37). In the coronary artery, a number of laboratories have shown that EDHF is an epoxideicosatrienoic acid (EET), a cytochrome P-450 metabolite of arachidonic acid (3, 15, 18, 20, 21, 23, 39). The coronary endothelium synthesizes the EETs, and agonists such as acetylcholine and bradykinin stimulate their release (3, 32, 39, 40). The acetylcholine- and bradykinin-stimulated, endothelium-dependent relaxation and hyperpolarization of coronary smooth muscle are blocked by inhibitors of cytochrome P-450, an EET antagonist, and inhibitors of calcium-activated potassium (KCa) channels (3, 15, 20, 23). The EETs open large-conductance KCa channels in smooth muscle cells, which causes hyperpolarization of coronary smooth muscle and relaxation of the coronary artery (3, 35, 39). Thus EETs mimic the effects of EDHF. The activation of the KCa channel by the EETs involves a guanine nucleotide-binding protein, most likely Gs (16, 21, 27).

Although the EETs represent important mediators of coronary vascular tone, it is not known which structural component(s) of the EET molecule is necessary for vasorelaxation. Previous studies indicate that all four regioisomeric EETs, 14,15-, 11,12-, 8,9-, and 5,6-EET, relax bovine and canine coronary arteries equally (3, 39, 41). Thus the location of the epoxy group is not critical for vasodilation in these arteries. In contrast, 5,6-EET was more active than the other regioisomeric EETs in relaxing the rat tail artery, rabbit and pig cerebral arteries, and rat mesenteric artery (4, 9, 26, 36). The EETs are hydrolyzed by epoxide hydrolase to dihydroxyicosatrienoic acids (DHETs) (46, 50). In some studies, the DHETs relax coronary arteries and are equipotent to the EETs (33, 45). The relaxations to DHETs are blocked by inhibitors of KCa channels. In other studies, the DHETs were not active (4) or were less active than the EET (2).

In the present study, we synthesized 19 analogs of 14,15-EET (Fig. 1) and tested the analogs for their ability to relax the bovine coronary artery. The analogs were specifically made with changes in the epoxy group, the carboxyl group, carbon chain length, and the double bonds to determine the contributions of these
structures to vasorelaxation. As in previous studies (33, 45), we found that 14,15-DHET relaxed the bovine coronary artery but was approximately fivefold less potent than 14,15-EET. These studies indicate the importance of the carboxyl group, the epoxy group, the carboxyl-to-epoxy group distance, and double bonds in the relaxant effect of 14,15-EET.

**METHODS**

**Vascular Reactivity of Bovine Coronary Arteries**

Bovine hearts were purchased from a local slaughterhouse, and the left anterior descending coronary artery was dissected and cleaned of connective tissue. Vessels of 1 mm diameter were cut into rings of 3 mm width as previously described (3, 27, 39). Vessels were stored in Krebs buffer consisting of (in mM) 119 NaCl, 4.8 KCl, 24 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, 11 glucose, 0.02 EDTA, and 3.2 CaCl2. The vessels were suspended from a pair of stainless steel hooks in a 6-ml water-jacketed organ chamber. The organ chamber was filled with Krebs buffer and bubbled with 95% O2-5% CO2 at 37°C. One hook was anchored to a stainless steel rod and the other hook to a force transducer (model FT-03C; Grass Instruments, West Warwick, RI). Tension of the vessel was measured by an ETH-400 bridge amplifier, and the data were acquired with a MacLab 8e analog-to-digital converter and MacLab software version 3.5.6 (AD Instruments, Milford, MA) and stored on a Macintosh computer for subsequent data analysis.

Basal tension was set at the length-tension maximum of 3.5 g and equilibrated for 1.5 h. KCl (40 mM) was added to the chamber until reproducible maximal contractions were maintained. U-46619 (10–20 nM), a thromboxane receptor agonist, was used to precontract the vessels from basal tension to between 50% and 90% of the maximal KCl contraction. Cumulative additions of 14,15-EET, 14,15-DHET, or analogs of 14,15-EET were added to the chamber. Between concentration-response curves, the chambers were rinsed with fresh Krebs buffer, 40 mM KCl was administered to determine the maximum contraction, and the vessels were rinsed. Consecutive concentration-response curves were performed with 14,15-EET followed by a concentration-response curve to a 14,15-EET analog. The experiment was always repeated with the order of the agonists reversed. In control experiments with consecutive concentration-response curves
Syntheses of 14,15-EET Analogs

**General.** All reactions were conducted under an argon atmosphere unless otherwise stated. 14,15-EET (7, 17), 14,15-EET-thirane (11, 12), 14,15-EET-aziridine (11, 12), 14,15-epoxyeicosanoic acid [14,15-EEA (51)], 14,15-(trans)-EET (24), 8,9-epoxybutadecenoic acid [8,9-EBDE (10)], and 14,15-EET-methylsulfonylimide [14,15-EET-5-ZE (5)] were prepared according to published procedures.

**Preparation of cis-14,15-oxideicoso-5(Z)-enoic acid (8,9,11,12-tetrahydro-14,15-EET).** cis-14,15-Oxideicoso-5(Z)-enoic acid (8,9,11,12-tetrahydro-14,15-EET) (14,15-EE-5-ZE; acid 70) was prepared as follows. n-BuLi (0.66 ml, 1.6 M hexane solution, 1.05 mmol) was added dropwise to a stirring, -40°C solution of 1-(tert-butylidiphenyllylidyloxyl)hex-5-ynyl (acytlenyl 1; Ref. 29) (320 mg, 1.05 mmol) in anhydrous tetrahydrofuran (THF)-hexane (1:9), Rf 0.33; 1H NMR (400 MHz, CDCl3-TMS): δ 6.4 Hz, 2 H, 3.46 – 3.50 (m, 1 H), 3.67 (t, J = 6.4 Hz, 2 H), 7.41 (m, 6 H), 7.66 (dd, J = 1.6, 7.6 Hz, 2 H), 9.08 (s, 9 H), 1.29–1.41 (m, 6 H), 1.42–1.77 (m, 12 H), 1.78–1.88 (m, 2 H), 2.11–2.16 (m, 4 H), 3.34–3.39 (m, 1 H), 3.46–3.50 (m, 1 H), 1.61 (t, J = 6.0 Hz, 2 H), 3.71–3.75 (m, 1 H), 3.83–3.88 (m, 1 H), 4.58 (apparent t, J = 2.8 Hz, 1 H), 7.35–7.42 (m, 6 H), 7.66 (dd, J = 1.6, 7.6 Hz, 4 H).

**Alkylation of bromide 4 using 1-heptyne as described for the preparation of diol 2 gave rise to bis-acetylenic 5 (70% yield).** The properties of the product are as follows. TLC: EtOAc-hexane (1:9), Rf 0.68; 1H NMR (400 MHz, CDCl3-TMS): δ 6.4 Hz, 2 H, 3.10–3.14 (m, 2 H), 1.42–1.52 (m, 8 H), 1.58–1.61 (m, 8 H), 1.62–1.68 (m, 2 H), 1.84 (apparent quintet, J = 7.4 Hz, 2 H), 2.11–2.17 (m, 4 H), 3.38 (t, J = 6.4 Hz, 2 H), 3.67 (t, J = 6.4 Hz, 2 H), 7.35–7.41 (m, 6 H), 7.66 (dd, J = 1.6, 7.6 Hz, 4 H).

**Partial reduction of bis-acetylene 5 was achieved via NaBH4 (1 mg, 0.02 mmol) addition to a stirring suspension of Ni(OAc)2 (3 mg, 0.01 mmol) in EtOH (10 ml) at room temperature under an argon atmosphere. After 30 min, neat ethylenediamine (1.64 μl, 0.024 mmol) was introduced, followed 10 min later by an ethanolic solution (2 ml) of bis-acetylene 5 (130 mg, 0.24 mmol). The reaction was purged

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with H₂ and maintained under a H₂ atmosphere for the next 1 h with a H₂-filled balloon. The reaction mixture was diluted with ether (20 ml) and filtered through a silica gel pad, and the filtrate was evaporated in vacuo, yielding pure diene 6 (119 mg, 91%). The properties of the product are as follows. TLC: EtOAc-hexane (1:5), Rₜ ~ 0.66; ¹H NMR (400 MHz, CDCl₃-TMS): δ 0.90 (t, J = 7.2 Hz, 3 H), 1.06 (s, 9 H), 1.25–2.05 (m, 6 H), 1.35–2.15 (m, 2 H), 1.56–1.61 (m, 2 H), 1.94–2.05 (m, 2 H), 3.65 (t, J = 6.4 Hz, 2 H), 5.30–6.40 (m, 4 H), 7.35–7.44 (m, 6 H), 7.66 (dd, J = 1.6, 7.6 Hz, 4 H).

Tetra-n-butylammonium fluoride (1.11 ml, 1.11 mmol, 1.0 M solution in THF) was added to a room temperature solution of diene 6 (119 mg, 0.22 mmol) in dry THF (4 ml). After 3 h, all volatiles were evaporated in vacuo and the crude product was dissolved in EtOAc (25 ml), washed with water (2 × 50 ml) and brine (20 ml), and dried over Na₂SO₄. Silicone gel column chromatography (20% EtOAc-hexane) of the residue, obtained after evaporation, furnished alcohol 7 (56.3 mg) in 97% yield. The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rₜ ~ 0.25; ¹H NMR (400 MHz, CDCl₃-TMS): δ 0.88 (t, J = 7.2 Hz, 3 H), 1.25–1.40 (m, 18 H), 1.99 (s, 3 H), 2.89–2.95 (m, 8 H), 3.64 (t, J = 6.4 Hz, 2 H), 5.28–5.42 (m, 4 H).

Alcohol 7 (56 mg, 0.217 mmol) was slowly added to a stirring 0°C solution of Jones reagent [1 ml; prepared by heating 0.677 g of CrO₃ (0.677 g) in 2.5 ml of H₂O cooled to 0°C, and adding in one portion 18 mg of NHS-ester 11 (0.154 mmol) dissolved in 2.5 ml of CH₂Cl₂] (50 ml) (Fig. 3). After being stirred at room temperature for 12 h, all volatiles were moved in vacuo, and the residue was dissolved in Et₂O-MeOH (20 ml) and brine until the aqueous layer tested negative with starch-I₂ test. The mixture was then acidified with H₂ and maintained under a H₂ atmosphere for the next 6 h. The combined organic extracts were dried (Na₂SO₄) and concentrated in vacuo to give acid 10 (45 mg, 95%) as a pale yellow oil. The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rₜ ~ 0.21; ¹H NMR (400 MHz, CDCl₃-TMS): δ 0.91 (t, J = 7.0 Hz, 3 H), 1.23–1.58 (m, 20 H), 1.76 (apparent quintet, J = 7.6 Hz, 2 H), 2.02 (dd, J = 6.8, 6.8 Hz, 2 H), 2.15 (dd, J = 7.2, 7.2 Hz, 2 H), 2.35 (dd, J = 7.2, 7.2 Hz, 2 H), 2.86–2.91 (m, 2 H), 5.27–5.68 (m, 2 H).

Preparation of N-methylsulfonimidamide. Acid 10 (45 mg, 0.14 mmol) and N-hydroxysuccinimide (NHS; 18 mg, 0.154 mmol) were mixed and azetrotopically dried with anhydrous benzene (scheme 1). The mixture was dissolved in dry THF (10 ml) to which 1,3-dicyclohexylcarbodiimide (DCC; 32 mg, 0.156 mmol) was added all at once. After being stirred at room temperature for 12 h, all volatiles were moved in vacuo and the residue was purified by SiO₂ column chromatography to give NHS-ester 11 (47 mg, 75%) as a colorless gum. The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rₜ ~ 0.25; ¹H NMR (400 MHz, CDCl₃-TMS): δ 0.91 (t, J = 7.0 Hz, 3 H), 1.23–1.58 (m, 20 H), 1.76 (apparent quintet, J = 7.6 Hz, 2 H), 2.02 (dd, J = 6.8, 6.8 Hz, 2 H), 2.15 (dd, J = 7.2, 7.2 Hz, 2 H), 2.35 (dd, J = 7.2, 7.2 Hz, 2 H), 2.86–2.91 (m, 2 H), 5.27–5.68 (m, 2 H).

NHS-ester 11 (47 mg, 0.12 mmol), methanesulfonamide (116 mg, 1.2 mmol) and N,N-dimethylaminopyridine (DMAP; 16 mg, 0.13 mmol) were mixed and then kept under vacuum for 2 h. Dry HMPA (0.5 ml) was added, and the resultant homogeneous solution was heated at 90°C for 2 h. The contents were cooled to room temperature and dissolved in EtOAc (50 ml), washed with H₂O (50 ml), dried (Na₂SO₄), and concentrated in vacuo. Purification of the residue by SiO₂ column chromatography provided N-methylsulfonimidamide 12 (32 mg, 73%). The properties of the product are as follows. TLC: EtOAc-hexane (4:6), Rₜ ~ 0.28; ¹H NMR (300 MHz, CDCl₃-TMS): δ 0.91 (t, J = 7.0 Hz, 3 H), 1.23–1.59 (m, 20 H), 1.76 (apparent quintet, J = 7.6 Hz, 2 H), 2.02 (dd, J = 6.8, 6.8 Hz, 2 H), 2.13 (dd, J = 7.2, 7.2 Hz, 2 H), 2.33 (dd, J = 7.2, 7.2 Hz, 2 H), 2.86–2.91 (m, 2 H), 3.29 (s, 3 H), 5.27–5.48 (m, 2 H).

Preparation of cis-14,15-oxidoeicosa-8(Z)-enoic acid (5,6,11,12-tetrahydro-14,15-EET) (14,15-EE-8-ZE; Ref. 22) (650 mg, 119 mg, 0.61 mmol) in anhydrous CH₂Cl₂ (40 ml) were added to a stirred 0°C solution of 5,6,11,12-tetrahydro-14,15-EET (cis-14,15-Oxidoeicosa-8(Z)-enoic acid (5,6,11,12-tetrahydro-14,15-EET) (14,15-EE-8-ZE; acid 20) was prepared as follows. Seventy percent m-chloroperbenzoic acid (m-CPBA; 990 mg, 5.72 mmol) was added in portions to a 0°C solution of unc-5-5-1-8 (41); Ref. 22) (650 mg, 3.80 mmol) in CH₂Cl₂ (20 ml) (scheme 2; Fig. 3). After being stirred for 3 h, the reaction mixture was diluted with CH₂Cl₂ (100 ml), washed with saturated aqueous NaHCO₃ (2 × 50 ml), water (50 ml), and brine (50 ml), dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by column chromatography over silica gel to afford epoxide 15 (640 mg, 90%) as a colorless syrup. The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rₜ ~ 0.24; ¹H NMR (300 MHz, CDCl₃-TMS): δ 0.89 (t, J = 7.0 Hz, 3 H), 1.21–1.68 (m, 14 H), 1.99 (bs, 1 H), 2.89–2.97 (m, 2 H), 3.68 (dd, J = 6.1, 6.1 Hz, 2 H); ¹³C NMR (75 MHz, CDCl₃-TMS): δ 13.98, 22.59, 22.97, 26.26, 27.51, 27.74, 31.70, 32.39, 57.29, 57.41, 62.31.

To a continuously stirred 0°C solution of epoxide 15 (640 mg, 3.44 mmol) in anhydrous CH₂Cl₂ (40 ml) were added 640 mg, 3.44 mmol) and 76.4 mg, 0.13 mmol) of LiOH (0.45 of 1.0 M aqueous sol, 0.45 mmol) at room temperature in THF-H₂O (5:1, 5 ml) for 12 h. The reaction mixture was then acidified with 1.0 M aqueous oxalic acid to pH 4 and extracted with ethyl acetate (2 × 40 ml). The combined organic extracts were dried (Na₂SO₄) and concentrated in vacuo to give acid 10 (45 mg, 95%) as a pale yellow oil. The properties of the product are as follows. TLC: EtOAc-hexane (1:9), Rₜ ~ 0.54; ¹H NMR (300 MHz, CDCl₃-TMS): δ 0.89
Following the protocol used to couple acetylene 1 with bromide 13 (see scheme 1), bromide 16 was alkylated with 1-(tert-butyldiphenylsilyloxy)non-8-yl (21: Ref. 38) to give 17 (77%). The properties of the product are as follows. TLC: EtOAc-hexane (1:9), Rf 0.20; 1H NMR (300 MHz, CDCl3-TMS): δ 0.89 (t, J = 7.0 Hz, 3 H), 1.04 (s, 9 H), 1.24–1.59 (m, 24 H), 2.08–2.19 (m, 4 H), 2.89–2.97 (m, 2 H), 3.65 (dd, J = 6.6, 6.6 Hz, 2 H), 7.26–7.41 (m, 6 H), 7.62–7.71 (m, 4 H); 13C NMR (75 MHz, CDCl3-TMS): δ 4.19, 18.89, 18.93, 19.39, 22.78, 25.86, 25.99, 26.47, 27.05, 27.59, 27.96, 29.04, 29.07, 29.13, 29.28, 31.91, 32.70, 57.20, 57.33, 64.11, 79.86, 80.71, 127.74, 129.66, 134.32, 135.74.

The desilylation of 17 to alcohol 18 (90%) was carried out as described for the conversion of diene 5 to alcohol 7 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rf 0.22; 1H NMR (400 MHz, CDCl3-TMS): δ 0.89 (t, J = 7.0 Hz, 3 H), 1.24–1.62 (m, 24 H), 2.10–2.22 (m, 4 H), 2.88–2.94 (m, 2 H), 3.63 (t, J = 6.4 Hz, 2 H).

The P-2 Ni hydrogenation of alcohol 18 generating cisolefin 19 (91%) was carried out as described for the conversion of bis-acetylene 5 to diene 6 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.26; 1H NMR (400 MHz, CDCl3-TMS): δ 0.90 (t, J = 7.0 Hz, 3 H), 1.24–1.60 (m, 24 H), 1.95–2.10 (m, 4 H), 2.86–2.96 (m, 2 H), 3.62 (t, J = 6.7 Hz, 2 H), 5.29–5.41 (m, 2 H).

Pyridinium dichromate (PDC; 1.39 g, 3.70 mmol) was added to a continuously stirred room temperature solution of alcohol 19 (230 mg, 0.74 mmol) in dry N,N-dimethylformamide (10 ml). After 16 h, the reaction mixture then was diluted with EtOAc (20 ml), washed with water (3 × 20 ml) and brine (20 ml), dried over Na2SO4, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give acid 20 (26 mg, 67%). The properties of the product are as follows. TLC: EtOAc-hexane (1:1), Rf 0.20; 1H NMR (400 MHz, CDCl3-TMS): δ 0.90 (t, J = 7.0 Hz, 3 H), 1.24–1.55 (m, 20 H), 1.56–1.66 (m, 2 H), 1.89–2.01 (m, 4 H), 2.30 (t, J = 7.6 Hz, 2 H), 2.86–2.95 (m, 2 H), 3.67 (s, 3 H), 5.30–5.40 (m, 2 H); 13C NMR (75 MHz, CDCl3-TMS): δ 14.20, 22.81, 25.12, 26.45, 26.50, 27.31, 27.36, 27.95, 27.99, 29.11, 29.26, 29.72, 29.81, 31.94, 34.29, 51.66, 57.38, 57.46, 129.73, 130.25, 174.51.

Preparation of cis-14,15-oxidoexcicos-11(Z)-enoic acid (5,6,8,9-tetraydro-14,15-EE). cis-14,15-Oxidoexcicos-11(Z)-enoic acid (5,6,8,9-tetraydro-14,15-EE) (14,15-EE-11-ZE; 28) was prepared as follows. Epoxy-alcohol 23 was prepared from oct-2-en-1-ol (22; Refs. 43 and 49) (90%) (scheme 3; Fig. 4) as described for the conversion of undec-5-en-1-ol 14 to epoxide 15 (see scheme 2). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.34; 1H NMR (400 MHz, CDCl3-TMS): δ 0.92 (t, J = 7.2 Hz, 3 H), 1.22–1.62 (m, 8 H), 3.01–3.10 (m, 1 H), 3.18–3.20 (m, 1 H), 3.62–3.71 (m, 1 H), 3.82–3.91 (m, 1 H).

Epoxy-bromide 24 was obtained in 80% yield from epoxy-alcohol 23 as described for the conversion of epoxide 15 to bromide 16 (see scheme 2). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.72; 1H NMR (400 MHz, CDCl3-TMS): δ 0.92 (t, J = 7.2 Hz, 3 H), 1.29–1.38 (m, 3 H), 1.46–1.60 (m, 5 H), 3.06–3.10 (m, 1 H), 3.24–3.31 (m, 2 H), 3.43–3.55 (m, 1 H); 13C NMR (300 MHz, CDCl3-TMS): δ 13.93, 22.49, 26.14, 27.26, 29.07, 31.58, 55.60, 58.81.
Preparation of cis-14,15-oxidoicosa-5(Z),11(Z)-dienoic acid (8,9-tetrahydro-14,15-EET). cis-14,15-Oxidoicosa-5(Z),11(Z)-dienoic acid (8,9-tetrahydro-14,15-EET) was prepared as follows. The alkylation of epoxide-1 to give bis-acetylene 33 (90%) was carried out as described for the conversion of bromide 16 to 17 (see scheme 2). The properties of the product are as follows. TLC: EtOAc-hexane (1:9), Rf 0.91 (t, J = 7.2 Hz, 3 H), 1.26–1.61 (m, 18 H), 2.01–2.06 (m, 2 H), 2.13–2.22 (m, 1 H), 2.34–2.39 (m, 1 H), 2.92–2.96 (m, 2 H), 3.45–3.52 (m, 4 H), 3.69 (t, J = 6.1 Hz, 2 H), 3.76–3.84 (m, 6 H), 7.67–7.69 (m, 4 H).

The P-2 Ni hydrogenation of 2-acetylene 33 to give cis-cis-diene 34 (80%) was carried out as described for the conversion of diene 6 to alcohol 7 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (1:9), Rf 0.24; 1H NMR (400 MHz, CDCl3-TMS): δ 0.91 (t, J = 7.3 Hz, 3 H), 0.98 (s, 9 H), 1.33–1.36 (m, 4 H), 1.49–1.67 (m, 14 H), 2.15–2.22 (m, 2 H), 2.34–2.39 (m, 1 H), 2.92–2.96 (m, 2 H), 3.45–3.52 (m, 4 H), 3.69 (t, J = 6.1 Hz, 2 H), 3.76–3.84 (m, 6 H), 7.67–7.69 (m, 4 H).

The desilylation of 27 to alcohol 28 (64%) was carried out as described for the conversion of diene 6 to alcohol 7 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.38; 1H NMR (400 MHz, CDCl3-TMS): δ 0.91 (t, J = 7.3 Hz, 3 H), 1.28–1.64 (m, 22 H), 2.01–2.06 (m, 2 H), 2.13–2.22 (m, 1 H), 2.32–2.41 (m, 1 H), 2.91–2.94 (m, 2 H), 3.64 (t, J = 8.0 Hz, 2 H), 5.40–5.54 (m, 2 H); 13C NMR (75 MHz, CDCl3-TMS): δ 14.10, 22.27, 24.88, 26.38, 26.46, 27.62, 27.91, 29.24, 29.42, 29.43, 29.57, 29.64, 29.74, 31.93, 34.30, 56.64, 57.52, 123.98, 132.88, 180.20.

Preparation of cis-14,15-oxidoicosa-5(Z),11(Z)-dienoic acid (8,9-tetrahydro-14,15-EET). cis-14,15-Oxidoicosa-5(Z),11(Z)-dienoic acid (8,9-tetrahydro-14,15-EET) (14,15-EE-5,11-ZD; acid 36) was prepared as follows. The alkylation of epoxy-1 to give bis-acetylene 33 (90%) was carried out as described for the conversion of bromide 16 to 17 (see scheme 2). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.24; 1H NMR (400 MHz, CDCl3-TMS): δ 0.91 (t, J = 7.2 Hz, 3 H), 1.26–1.61 (m, 18 H), 2.01–2.06 (m, 6 H), 2.15–2.22 (m, 1 H), 2.34–2.39 (m, 1 H), 2.92–2.96 (m, 2 H), 3.45–3.52 (m, 4 H), 3.69 (t, J = 6.1 Hz, 2 H), 3.76–3.84 (m, 6 H), 7.67–7.69 (m, 4 H).

The desilylation of cis-cis-diene 34 to alcohol 35 (80%) was carried out as described for the conversion of diene 6 to alcohol 7 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (1:4), Rf 0.24; 1H NMR (400 MHz, CDCl3-TMS): δ 0.90 (t, J = 7.3 Hz, 3 H), 1.06 (s, 9 H), 1.33–1.36 (m, 4 H), 1.49–1.67 (m, 14 H), 2.14–2.27 (m, 7 H), 2.53–2.58 (m, 1 H), 2.94–2.97 (m, 1 H), 3.09–3.14 (m, 1 H), 3.69 (t, J = 6.1 Hz, 2 H), 3.76–3.84 (m, 6 H), 7.67–7.69 (m, 4 H).
Preparation of trans-14,15-EET. trans-14,15-EET (38) was prepared as follows. n-BuLi (0.1 ml, 2.5 M solution in THF) was added to a solution of diphenylphosphine (46 mg, 0.25 mmol) in THF (3 ml) at 0°C and stirred for 2 h at 23°C (scheme 5; Fig. 6). A solution of 14,15-EET (20 mg, 0.0625 mmol) in THF (3 ml) was added over 5 min (7). After 2 h, freshly distilled MeI (35 mg, 0.25 mmol) was added to the reaction mixture, which was then allowed to stand for 30 min (the color of the reaction mixture turns white from red). The contents were diluted with EtOAc (10 ml), washed with H2O (10 ml) and brine (10 ml), dried (Na2SO4), and concentrated in vacuo. The residue was purified by SiO2 PTLC to give 37 (14 mg, 74%) as a colorless oil. The properties of the product are as follows. TLC: EtOAc-hexane (3:7), Rf ~ 0.31; 1H NMR (400 MHz, CDCl3-TMS): δ 0.88 (t, J = 6.4 Hz, 3 H), 1.22–1.38 (m, 6 H), 1.71 (quintet, J = 7.4 Hz, 2 H), 1.98 (dd, J = 7.0, 7.0 Hz, 2 H), 2.13 (dd, J = 7.0, 7.0 Hz, 2 H), 2.36 (apparent t, J = 7.4 Hz, 2 H), 2.74–2.82 (m, 6 H), 5.32–5.44 (m, 8 H).

Product 38 was prepared from 37 in 55% yield as described for the conversion of methyl ester 8 to acid 10 (see scheme 1). The properties of the product are as follows. TLC: EtOAc-hexane (1:2), Rf ~ 0.36; 1H NMR (400 MHz, CDCl3-TMS): δ 0.92 (t, J = 7.0 Hz, 3 H), 1.26–1.57 (m, 8 H), 1.71 (apparent quintet, J = 7.3 Hz, 2 H), 2.11 (dd, J = 7.2, 7.2 Hz, 2 H), 2.25–2.31 (m, 1 H), 2.37 (dd, J = 7.2, 7.2 Hz, 2 H), 2.42–2.49 (m, 1 H), 2.75–2.84 (m, 6 H), 5.32–5.58 (m, 6 H).

Preparation of cis-10,11-oxidoheptadeca-4(Z),7(Z)-dienoic acid (10,11-epoxyheptadecadienoic acid). cis-10,11-Oxidoheptadeca-4(Z),7(Z)-dienoic acid (10,11-epoxyheptadecadienoic acid) (10,11-EHDD: 42) was prepared as follows. Pb(OAc)4 (1.06 g, 5.4 mmol) was added in three portions over 15 min to a stirring –40°C solution of diol 39 (Ref. 17; 450 mg, 1.2 mmol) in CH2Cl2 (10 ml) (scheme 6; Fig. 7). After 0.5 h, the reaction mixture was warmed to room temperature and then passed through a small pad of silica gel with CH2Cl2 (250 ml) as eluent. The eluent was dried over Na2SO4 and concentrated in vacuo to give the corresponding aldehyde as a labile, colorless liquid (350 mg) that was used immediately without further purification.

NaBH4 (44 mg, 1.2 mmol) was added to a 0°C solution of the preceding crude aldehyde (350 mg) in MeOH-CH2Cl2 (30 ml, 2:1). After 0.5 h, the solvent was evaporated in vacuo and the residue was purified by SiO2 chromatography (EtOAc-hexanes, 1:9) to give alcohol 40 (240 mg, 80%). The properties of the product are as follows. 1H NMR (CDCl3, 400 MHz): δ 0.89 (t, J = 6.8 Hz, 3 H), 1.25–1.39 (m, 6 H), 2.05 (q, J = 7.2 Hz, 2 H), 2.36 (q, J = 8 Hz, 2 H), 2.79–2.87 (m, 4 H), 3.66 (q, J = 6.4 Hz, 3 H), 5.30–5.44 (m, 5 H), 5.52–5.58 (m, 1 H).

Methanesulfonyl(mesityl)chloride (154 mg, 1.35 mmol) was added dropwise with stirring to alcohol 40 (230 mg, 0.96 mmol) in CH2Cl2 (10 ml) followed by triethylamine (202 mg, 2.0 mmol). After 6 h, the reaction mixture was washed with water (2 × 50 ml) and brine (50 ml) and dried, and all volatiles were removed in vacuo. SiO2 chromatography (EtOAc-hexanes, 5:95) of the residue afforded the corresponding mesylate (290 mg, 91%) as a colorless oil. The properties of the product are as follows. 1H NMR (CDCl3, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3 H), 1.27–1.37 (m, 6 H), 2.05 (q, J = 6.8 Hz, 2 H) 2.54 (q, J = 6.8 Hz, 2 H), 2.79–2.85 (m, 4 H), 4.22 (t, J = 6.8 Hz, 2 H), 5.30–5.44 (m, 5 H), 5.33–5.60 (m, 1 H). The preceding mesylate (290 mg, 0.96 mmol) was stirred with KCN (56 mg, 1.1 mmol) in DMSO (10 ml) at room temperature for 12 h. The reaction mixture was diluted with water (20 ml) and extracted with ether (2 × 50 ml). The combined organic extracts were washed with water (2 × 10 ml) and brine (10 ml) and dried over Na2SO4, and the solvent was evaporated in vacuo. SiO2 chromatography (EtOAc-hexanes, 1:99) of the residue afforded the corresponding cyanide (240 mg, 90%) as a colorless oil. The properties of the product are as follows. 1H NMR (CDCl3, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3 H), 1.25–1.38 (m, 6 H), 2.05 (q, J = 8.0 Hz, 2 H), 2.36–2.46 (m, 4 H), 2.79–2.86 (m, 4 H), 5.32–5.41 (m, 5 H), 5.52–5.06 (m, 1 H); 13C NMR (CDCl3, 75 MHz): δ 14.15, 17.56, 22.65, 23.36, 25.71, 27.30, 29.37, 31.58, 119.36, 125.53, 127.21, 127.37, 129.12, 130.76, 131.57.

Disobutyraluminum hydride (DIBAL-H; 980 μl, 1.0 M solution in toluene, 1.5 mmol) was added to a –78°C solution of the above cyanide (240 mg, 1.00 mmol) in CH2Cl2 (10 ml). The reaction mixture was slowly warmed to –40°C. After 0.5 h, the reaction was quenched with MeOH (100 μl, 3.0 mmol) and then warmed to room temperature. The mixture was diluted with ether (150 ml), washed with water (2 × 50 ml) and brine (50 ml), dried over Na2SO4, and concentrated in vacuo. SiO2 chromatography (EtOAc-hexanes, 5:95) of the residue afforded aldehyde 41 (187 mg, 83%) as a colorless oil. The properties of the product are as follows. 1H NMR (CDCl3, 400 MHz): δ 0.88 (t, J = 7.2 Hz, 3 H), 1.26–1.39 (m, 6 H), 2.05–2.12 (m, 2 H), 2.41–2.43 (m, 2 H), 2.45–2.57 (m, 2 H), 2.81–2.93 (m, 4 H), 5.31–5.45 (m, 4 H).

Aldehyde 41 (185 mg, 0.80 mmol) in acetonitrile (20 ml) was slowly added to a 20°C solution of Jones reagent (2.4 ml of 1.0 M solution, 2.4 mmol). After 0.5 h, the reaction was quenched with isopropanol (2 ml, 2.4 mmol), concentrated in vacuo, and extracted with ether (2 × 100 ml). The combined ethereal extracts were washed with water (2 × 50 ml) and brine (50 ml)
ml), dried over Na₂SO₄, and concentrated in vacuo. SiO₂ chromatography (EtOAc-hexanes, 5:95) of the residue afforded the corresponding acid (165 mg, 84%) as a colorless oil. The properties of the product are as follows. 1H NMR (400 MHz, CDCl₃): δ 0.88 (t, J = 7.2 Hz, 3 H), 1.25–1.36 (m, 6 H), 2.02–2.07 (m, 1 H), 2.17–2.34 (m, 7 H), 2.82–2.84 (m, 2 H), 2.91–2.96 (m, 2 H), 3.67 (s, 3 H), 5.37–5.52 (m, 4 H), mass spectrometry [matrix-assisted laser desorption ionization (MALDI), α-cyano-4-hydroxycinnamic acid matrix]: M⁺ (280.25), M + Na⁺ (300.37).

Preparation of 12,13-epoxy-octadec-6(Z),9(Z)-dienoic acid. 12,13-Epoxy-octadec-6(Z),9(Z)-dienoic acid (12,13-EODD) was made from γ-linolenic acid (Nu Chek Prep, Elysian, MN) by internal epoxidation as described for the conversion of 37 to 38. The epoxide was obtained in 70% overall yield as a colorless oil. The properties of the product are as follows. 1H NMR (CDCl₃, 400 MHz): δ 0.90 (t, J = 7.0 Hz, 3 H), 1.24–1.58 (m, 10 H), 1.62–1.74 (m, 2 H), 2.02–2.14 (m, 2 H), 2.16–2.28 (m, 2 H), 2.32–2.46 (m, 2 H), 2.80 (t, J = 6.4 Hz, 2 H), 2.90–3.00 (m, 2 H), 5.32–5.56 (m, 4 H).

RESULTS

Vascular Relaxations to 14,15-EET and 14,15-EET Analogs

The structures and names of the 19 analogs of 14,15-EET that were tested for agonist activity are shown in Fig. 1. 14,15-EET relaxed the U-46619-precontracted bovine coronary artery in a concentration-related manner as previously described (Figs. 8–10). Similar relaxation responses were obtained with 14,15-, 11,12-, 8,9-, and 5,6-EET in the bovine and canine coronary arteries (3, 39, 41). The four regioisomers were equipotent. The sensitivity of the vessels to 14,15-EET varied slightly from heart to heart over the 3-yr period of the study. For this reason, analogs were compared with 14,15-EET in each experiment. We initially compared the effect of analogs with changes in the carbon-1 carboxyl group, 14,15-EET-SI, 14,15-epoxyeicosatrienio14,15-EET-OH), 14,15-epoxycosa-11(Z)-enol (14,15-EE-11-ZE-OH), and 14,15-EET methyl ester (14,15-EET-Me). The distance between the carboxyl and epoxy groups was reduced by two carbons with 12,13-epoxyoctadecadienoic acid (12,13-EODD), four carbons with 10,11-epoxydodecadienoic acid (10,11-EHD), and six carbons with 8,9-EBDE. 14,15-EET-SI relaxed the coronary artery and was equipotent and equally active with 14,15-EET (Fig. 5A). 14,15-EET-Me also relaxed the coronary artery and was equipotent with 14,15-EET (Fig. 5C). The maximal activity of 14,15-EET-Me was slightly greater than that of 14,15-EET. In contrast, 14,15-EET-OH and 14,15-EE-11-ZE-OH were less active and less potent than 14,15-EET (Fig. 5B). They relaxed the vessels slightly at the highest concentration tested, 10⁻⁵ M. Shortening the carbon chain length to 14 (8,9-EBDE), 16 (10,11-EHD), or 18 (12,13-EODD) carbons resulted in a loss of potency and maximal activity (Fig. 5D). The sulfonimide group is commonly used as a substitute for carboxyl groups because it has a similar pKa (1). Thus it is not surprising that 14,15-EET-SI had activity and potency similar to those of 14,15-EET. The conversion of the carboxyl to an alcohol resulted in a large loss in activity, indicating the importance of an acid group at carbon-1. The potency of the methyl ester was surprising but may represent conversion of 14,15-EET-Me to 14,15-EET by vascular cells (2, 42). Changing the relationship between the epoxy and carboxyl group by shortening the carbon chain also decreased the activity, indicating the importance of the distance of 12 carbons between these groups.

In the second series of analogs, one, two, or three of the double bonds were eliminated. EET analogs with a single double bond varied in activity (Fig. 9A). The 14,15-EEZE with a Δ8 double bond was as potent and as active as 14,15-EET. In contrast, 14,15-EEZE analogs with the Δ5 or Δ11 double bonds were less potent than 14,15-EET or 14,15-EE-5ZE. The concentration-response curve for 14,15-EE-5ZE was shifted to the right ~10-fold compared with 14,15-EET. These data indicate that the Δ8 double bond is essential for full agonist potency, whereas the Δ5 and Δ11 double bonds are not as critical for potency. The concentration-response curve for 14,15-EE-5,ZD was shifted significantly to the right of the curve for 14,15-EET but retained the same maximal activity (Fig. 9B). This further indicates the importance of the Δ8 double bond. The saturated analog without double bonds (14,15-EEA) relaxed the vessels in a concentration-related manner, but the concentration-response curves were shifted to the right of the curve for 14,15-EET (Fig. 9C). Thus a loss of double bonds decreased the potency of the EET but did not eliminate activity. The presence of
the Δ8 double bond is necessary for full agonist potency.

The third series of analogs changed the epoxy group. The oxygen of the epoxy group was changed to a sulfur (14,15-EET-thiirane) or nitrogen (14,15-EET-aziridine). Neither analog caused relaxation, indicating the hydrolysis product of 14,15-EET, has two adjacent alcohols. 14,15-DHET relaxed the bovine coronary artery as previously indicated in the canine coronary artery (Fig. 10A; Refs. 33, 45). However, in our studies, the concentration-response curve for 14,15-DHET was shifted approximately fivefold to the right of the curve for 14,15-EET with no change in maximal effect. Thus the hydrolysis of the epoxy to a vicinal diol results in loss of potency but retention of full agonist activity. The 14(S),15(R)-EET isomer was more potent than the 14(R),15(S)-EET isomer (Fig. 10B). The concentration-response curve for 14(R),15(S)-EET was shifted to the right ~10-fold of the curve for 14(S),15(R)-EET. Thus the response is stereoselective. Finally, the 14,15-(cis)-isomer was more potent than the 14,15-(trans)-isomer (Fig. 10C). These studies indicate that a 14(S),15(R)-epoxy oxygen in the cis configuration is required for full agonist potency.

Figure 11 shows the chemical structure and ball and stick molecular model of the active vasodilator. It contains the carbon-1 carboxyl, the Δ8 double bond, 20 carbons, and the 14(S),15(R)-(cis)-epoxide that are required for full agonist potency and activity. It is 14(S),15(R)-(cis)-epoxyeicosa-Δ8-enoic acid.

**DISCUSSION**

EETs are synthesized by the vascular endothelium and are participants in the endothelium-dependent relaxations to bradykinin and acetylcholine (3, 15, 18, 20, 23, 39). They open KCa channels on vascular smooth muscle. This results in membrane hyperpolarization and vasodilation. The activation of KCa channels by EETs requires a G protein (16, 21, 27); however, it is not known whether a receptor is involved. Binding sites for 14,15-EET have been described in macrophages but not in smooth muscle (47, 48). Our laboratory (28) showed that 11,12-EET stimulates the endogenous ADP-ribosylation of the G protein Gα, resulting in activation of KCa channels. These findings may indicate that EETs can increase KCa channel activity, but it is unclear whether this involves a receptor-mediated mechanism. Because the EETs relax coronary arteries and open KCa channels in nanomolar concentrations, it is likely that the EETs have a binding site(s) and the binding event is amplified to produce the biological response. In the present study, we examined the structure-activity relationships between a number of 14,15-EET analogs to determine which portion of the 14,15-EET molecule was necessary for vasodilation.

All four regioisomeric EETs relax bovine and canine coronary arteries, indicating that the position of the epoxy group on the arachidonic acid backbone does not influence this action (3, 39, 41). Arteries from different vascular beds differ in the EET regioisomer that causes relaxation. For example, 11,12-EET, but not 14,15-EET, relaxed the rat renal artery (52). Only 5,6-EET relaxed the rat tail artery (4). At the present time, it is not clear whether these observations in the coronary artery indicate that vascular smooth muscle cells have four EET, regiosomer-specific receptors, four binding proteins that activate endogenous ADP ribosylation, or that the position of the epoxy group is not a structural
requirement for activity. Whether the EETs act through G protein-coupled receptors, directly on a G protein, or through endogenous ADP ribosylation, the present study indicates that there are specific structural requirements for 14,15-EET to cause vasorelaxation of bovine coronary arteries. Changing the carboxy group at carbon-1 to an alcohol, shortening the distance between the carboxyl and epoxy groups, or conversion of the oxygen of the 14,15-epoxide to a thiirane sulfur or aziridine nitrogen results in loss of activity. For full agonist potency, the epoxide must be a \(S,R\)-stereoisomer in the \(cis\)-epoxide configuration. Removal of the double bonds decreases the potency but does not necessarily result in complete loss of activity. Some double bonds were more critical than others. There was little difference between the loss of the \(\Delta 8\) double bonds and the loss of the \(\Delta 5\), \(\Delta 8\), and \(\Delta 11\) double bonds. 14,15-EE-5-ZE, 14,15-EE-11-ZE, and 14,15-EEA had reduced activity. The \(\Delta 8\) double bond is required for full agonist potency. Thus the \(\Delta 5\) and \(\Delta 11\) double bonds do not seem as important as the \(\Delta 8\) double bond. These data indicate that two double bonds can be removed

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**Fig. 9.** Effect of EET analogs on vascular tone in precontracted bovine coronary arteries. The number of double bonds in 14,15-EET was changed from 3 (\(\Delta 5\), \(\Delta 8\), and \(\Delta 11\)) double bonds in 14,15-EET to a single double bond in the three 14,15-EEZE analogs (A), to 2 double bonds in 8,9-tetrahydro-14,15-EET (14,15-EE-5,11-ZD; B), and to no double bonds in 14,15-EEA (C). Each value represents the mean ± SE for the \(N\) indicated. *\(P < 0.001\) compared with 14,15-EET control.

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**Fig. 10.** Effect of EET analogs on vascular tone in precontracted bovine coronary arteries. The structure of the epoxy group in 14,15-EET was changed to a sulfur in 14,15-EET-thiirane and a nitrogen in 14,15-EET-aziridine and hydrolyzed to a vicinal diol in 14,15-dihydroxyeicosatrienoic acid (DHET) (A). The stereochemistry of the epoxy group was either 14(\(S\)),15(\(R\))-EET or 14(\(R\)),15(\(S\))-EET in B and 14,15-(\(cis\))-EET or 14,15-(\(trans\))-EET in C. Each value represents the mean ± SE for the \(N\) indicated. *\(P < 0.05\) and **\(P < 0.001\) compared with 14,15-EET control. 

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Fig. 11. Chemical structure and ball and stick molecular model of the active vasodilator 14(S),15(R)(-cis)-epoxyeicosa-Δ8-enoic acid.

without affecting potency or activity. Interestingly, the EEZE and EEZD analogs would be less susceptible to autoxidation and therefore more stable for physiological studies. These findings indicate that the presence of the negatively charged carboxyl and oxygen of the cis-epoxide are essential for activity. The cis-double bond must convey rigidity and a specific conformation in the carbon backbone that is required for full potency.

Fang and co-workers (14) showed that 11,12-EET is metabolized in porcine aortic smooth muscle cells by a combination of β-oxidation and hydrolysis of the epoxide by epoxide hydrolase. The major metabolites were 11,12-DHET and 7,8-dihydroxy-hexadecadienoic acid (7,8-DHHD). Human skin fibroblast metabolized 11,12-EET by β-oxidation to 9,10-EODD and 7,8-EHDD and 14,15-EET to 10,11-EHDD and 12,13-EODD (13). 11,12-DHET and 7,8-DHHD relaxed the porcine coronary artery, but a quantitative comparison to 11,12-EET was not made. The other metabolites were not tested for activity. We found that 10,11-EHDD and 12,13-EODD were less potent than 14,15-EET, indicating that β-oxidation represents a pathway of 14,15-EET inactivation.

Interestingly, 14,15-DHET relaxed the bovine coronary artery; however, the DHET was fivefold less potent than 14,15-EET. This finding indicates that the vicinal diol can partially substitute for the epoxide group. This finding confirms published studies that 14,15-DHET is a potent vasorelaxant in coronary arteries and microvessels (14, 33). In recent studies, we (2, 3) and others (23, 33) reported that the relaxations to 14,15-DHET were blocked by increasing the extracellular potassium concentration from 4.8 to 20 mM and by inhibitors of KCa channels. 14,15-DHET activated KCa channels in cell-attached patches but was 10-fold less potent than 14,15-EET (2, 3). Like 14,15-

EET, 14,15-DHET failed to open KCa channels in inside-out patches unless GTP was added to the bathing solution (16, 21, 27). Thus 14,15-DHET appears to have the same mechanism of action as 14,15-EET, with both requiring a G protein for KCa channel activation.

Interestingly, 14,15-EET-Me was as active as 14,15-EET in relaxing coronary arteries. This finding was surprising because results with other analogs suggested the need for a negatively charged group at carbon-1. These differences could be reconciled by conversion of the methyl ester to the free acid. Previous studies indicate that long-chain fatty acid methyl esters are taken up by cells and hydrolyzed to their fatty acids (25, 42). In support of this possibility, we have found that [14C]14,15-EET-Me is metabolized to 14,15-

DHET and, to a lesser extent, 14,15-EET by coronary arteries (2). These biochemical studies indicate that the coronary artery contains an epoxide hydrolase to convert the EET to DHET and esterases to convert the methyl esters to the free acids. These data are consistent with the free acid of 14,15-DHET and, to a lesser extent, 14,15-EET mediating the action of 14,15-EET-Me. Because the 14,15-DHET was fivefold less potent than 14,15-EET in causing relaxation, it is surprising that 14,15-EET-Me was as active as 14,15-EET if 14,15-DHET mediates its effect. This discrepancy is understandable if EETs and DHETs act intracellularly. The EETs and DHETs would not enter the cell as easily as the EET-Me, so less would get to the site of action. In addition, the free acids of the EET and DHET are incorporated into membrane phospholipids and may reduce the amount of EET/DHET reaching the site of action (44). Previous studies from our lab support this possibility. When [14C]EETs were incubated with coronary arteries for 30 min, <10% of the EET was converted to the DHET (39). This finding suggests that the EET does not have access to the intracellular soluble epoxide hydrolase. In contrast, 14,15-[14C]EET-Me was almost completely converted to 14,15-DHET or 14,15-DHET-Me after 10 min (2). These findings support an intracellular site of action of the 14,15-EET and 14,15-DHET.

In summary, there are specific structural requirements for 14,15-EET to cause relaxation of the bovine coronary artery. These requirements include a negatively charged carboxyl group at carbon-1 separated by 12 carbons from a 14(S),15(R)-cis-epoxy oxygen. The Δ8 cis-double bond is necessary for full agonist potency. The vicinal diol of 14,15-DHET may partially substitute for the 14,15-epoxy group. From these data, inferences can be made about the essential elements for the 14,15-EET receptor/binding site in the bovine coronary artery. There must be 1) an ionic binding site that interacts with the carboxylic acid that is ionized at physiological pH; 2) p-p bonding between the Δ8 double bond and aromatic amino acids such as a phenylalanine or tyrosine; 3) hydrogen bonding with the epoxide in which the oxygen functions as an acceptor; and 4) a shape-specific channel or cleft for the epoxide that best accommodates the bend or kink formed by the cis-
epoxide rather than the more linear chain formed by the trans-epoxide.

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