11(R),12(S),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-triienoic acid: an endothelium-derived 15-lipoxygenase metabolite that relaxes rabbit aorta

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Submitted 11 September 2007; accepted in final form 16 January 2008

Gauthier KM, Chawengsub Y, Goldman DH, Conrow RE, Anjaiah S, Falck JR, Campbell WB. 11(R),12(S),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-triienoic acid: an endothelium-derived 15-lipoxygenase metabolite that relaxes rabbit aorta. Am J Physiol Heart Circ Physiol 294: H1467–H1472, 2008. First published January 18, 2008; doi:10.1152/ajpheart.01052.2007.—Previous studies indicate that 11,12,15-trihydroxyeicosatetraenoic acid (11,12,15-THETA), an endothelium-derived hyperpolarizing factor in the rabbit aorta, mediates a portion of the relaxation response to acetylcholine by sequential metabolism of arachidonic acid by 15-lipoxygenase, hydroperoxide isomerase, and epoxide hydrolase. To determine the stereochemical configuration of the endothelial 11,12,15-THETA, its activity and chromatographic migration were compared with activity and migration of eight chemically synthesized stereoisomers of 11,12,15-THETA. Of the eight isomers, only 11(R),12(S),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-triienoic acid comigrated with the biological 11,12,15-THETA on reverse- and normal-phase HPLC and gas chromatography. The same THETA isomer (10−7 to 10−4 M) relaxed the rabbit aorta in a concentration-related manner (maximum relaxation = 69 ± 5%). These relaxations were blocked by apamin (10−7 M), an inhibitor of small-conductance Ca2+-activated K+ channels. In comparison, 11(S),12(R),15(S),5(Z),8(Z),13(E)-THETA (10−4 M) relaxed the aorta by 22%. The other six stereoisomers were inactive in this assay. With use of the whole cell patch-clamp technique, it was shown that 10−4 M 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA increased outward K+ current in isolated aortic smooth muscle cells by 119 ± 36% at +60 mV, whereas 10−4 M 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA increased outward K+ current by only 20 ± 2%. The 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA-stimulated increase in K+ current was blocked by pretreatment with apamin. These studies suggest that 11(R),12(S),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-triienoic acid is the active stereoisomer produced by the rabbit aorta. It relaxes smooth muscle by activating K+ channels. The specific structural and stereochemical requirements for K+ channel activation suggest that a specific binding site or receptor of 11,12,15-THETA is involved in these actions.

endothelium-derived hyperpolarizing factor; smooth muscle cells; potassium channels; arachidonic acid

ACETYLCHOLINE, BRADYKININ, and shear stress dilate vascular smooth muscle by stimulating the release of soluble mediators from the endothelium (5, 9, 11, 17). These mediators include nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). EDHF acts on vascular smooth muscle to open Ca2+-activated K+ (KCa) channels, hyperpolarize the cell membrane, and cause relaxation. Thus inhibitors of KCa channels or inhibition of membrane hyperpolarization with high extracellular K+ blocks the action of EDHF. Several substances, including epoxyeicosatrienoic acids, K+, H2O2, and C-type natriuretic peptide, exhibit EDHF activity (2, 4, 7, 16). In rabbit arteries, we found that EDHF activity is mediated by a 15-lipoxygenase (15-LO) metabolite of arachidonic acid (AA) (3, 23). The rabbit endothelium metabolizes AA to 15(S)-hydroperoxyeicosatetraenoic acid [15(S)-HPETE] through 15-LO (23, 24, 26). 15-HPETE is rearranged to 15-hydroxy-11,12-epoxyeicosatrienoic acid (15-H-11,12-EETA) by a hydroperoxide isomerase, possibly a cytochrome P-450. Epoxide hydrolase converts 15-H-11,12-EETA to 11,12,15-trihydroxyeicosatrienoic acid (11,12,15-THETA). In isolated aortic smooth muscle cells, 11,12,15-THETA activates small-conductance KCa (SKCa) channels, an effect blocked by apamin (3, 12, 27). In addition, 11,12,15-THETA hyperpolarizes smooth muscle cells and causes relaxation. These effects are also inhibited by apamin or an increase in extracellular K+ concentration. 11,12,15-THETA mediates endothelium-dependent relaxations to acetylcholine and AA in rabbit arteries.

Although 11,12,15-THETA has been identified as the vasodilatory 15-LO metabolite produced by rabbit endothelium, the stereochromical configuration of this THETA is not known. The rabbit endothelium contains 15-LO and produces the S isomer of 15-hydroxyeicosatetraenoic acid at 96% optical purity (21, 26). Thus the 15-hydroxy group has the S configuration. The amount of 11,12,15-THETA produced by the aorta is not adequate for stereochemical determination of the 11- and 12-hydroxyl groups. As a result, we have chemically synthesized eight stereoisomers of 11,12,15(S)-THETA and compared their biological activities and chromatographic migrations with activity and migration of the biologically produced 11,12,15-THETA. These studies indicate that only 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA comigrated with the biological THETA in several chromatographic systems, relaxes the rabbit aorta and activates K+ channels. These findings strongly suggest that the EDHF produced by the rabbit aorta is 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA.

METHODS

11,12,15-THETA isomers. Since rabbit endothelium contains 15-LO and synthesize 15(S)-HPETE at 96% optical purity, only isomers of 11,12,15(S)-THETA were studied (21, 26). The hydroxyl groups at carbons 11 and 12 can have the R or S configuration, and the
Δ3,14-olefin can have the cis (Z) or trans (E) configuration. These permutations give rise to eight stereoisomers of 11,12,15(5)-THETA, i.e., four with a cis-Δ3,14-olefin [13(Z) series] and four with a trans-Δ3,14-olefin [13(E) series]. These eight stereoisomers of 11,12,15(5)-THETA were prepared by total asymmetric synthesis as previously described (6, 8) and compared chromatographically and via bioassay with natural material. The structures of these eight stereoisomers are shown in Fig. 1.

Preparation of biological 11,12,15-THETA from rabbit aorta. The experimental protocol was approved by the Animal Care Committee of the Medical College of Wisconsin, and procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Aortas were isolated from 1- to 2-month-old New Zealand White rabbits and cleaned of adhering connective tissue and fat (3, 22, 23). The vessels were rinsed in HEPES buffer [in mM: 10 HEPES, 150 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, and 6 mM glucose (pH 7.4)] and cut into 3-mm-long rings. Vessels were incubated for 10 min at 37°C in HEPES buffer containing 10−5 M indomethacin and 0.05 μCi of 5×10−5 M [14C]AA. A-23187 (2 μM) was added, and the vessels were incubated for an additional 5 min. The reaction was stopped by addition of ethanol to a final concentration of 15%, and the HEPES buffer was collected, acidified (pH <3.5) with glacial acetic acid, and extracted on octadecylsilica solid-phase extraction columns as previously described (22, 23). The extracted metabolites were evaporated to dryness under a stream of nitrogen and stored at −40°C until analysis by HPLC.

Purification of aortic 11,12,15-THETA by HPLC. The biologically produced [14C]11,12,15-THETA was isolated by HPLC, and its migration time in three HPLC systems was compared with that of the synthetic 11,12,15-THETA stereoisomers (3, 23, 24). Elution times of the biological [14C]11,12,15-THETA were determined by measurement of the radioactivity in the column eluate, whereas elution time of the synthetic 11,12,15-THETA stereoisomers was determined by UV absorbance at 205 nm. The extracted aortic metabolites of [14C]AA were first resolved into their components by reverse-phase (RP) HPLC using a Nucleosil C18 column (5 μm, 4.6 × 250 mm) and solvent system I (23). Solvent A was water, and solvent B was acetonitrile containing 0.1% glacial acetic acid. The program was a 40-min linear gradient from 50% solvent B in solvent A to 100% solvent B. Flow rate was 1 ml/min. Column eluate was monitored for absorbance at 205 nm and collected in 0.2-ml fractions by a fraction collector. An aliquot of each fraction was removed, and radioactivity was determined by liquid scintillation spectrometry. Fractions corresponding to the THETAs (fractions 27–35, 5–7.5 min) were collected, acidified, and extracted with 50:50 cyclohexane-ethyl acetate. The solvent was removed under a stream of nitrogen, and the residue was redissolved in the HPLC mobile phase. The THETA fraction was rechromatographed on RP-HPLC using the same column with solvent system II (23). In solvent system II, solvent A was water containing 0.1% glacial acetic acid, and solvent B was acetonitrile. The program consisted of a 5-min isocratic phase with 35% solvent B in solvent A followed by a 35-min linear gradient to 85% solvent B. Flow rate was 1 ml/min. The column eluate was monitored for absorbance at 205 nm and collected in 0.2-ml aliquots, and radioactivity was determined as described above. Fractions containing the THETAs (15.0–17.6 min, fractions 75–88) were collected, acidified, and extracted with 50:50 cyclohexane-ethyl acetate. The THETA fraction was further purified by normal-phase (NP) HPLC using a Nucleosil silica column (5 μm, 4.6 × 250 mm) (3, 24). Solvent system III consisted of an isocratic separation for which 95.9:4.0:0.1 hexane-isopropanol-glacial acetic acid was used at a flow rate of 1 ml/min. The column eluate was monitored for absorbance at 205 nm and collected in 0.5-ml fractions, and the radioactivity was determined as described above. The radioactive peak containing 11,12,15-THETA (fractions 93–99, 46.5–49.5 min) was collected, dried under a stream of nitrogen, derivatized, and analyzed by gas chromatography (GC)–mass spectrometry (MS; see below). The GC-MS analysis confirmed that these fractions contained 11,12,15-THETA with major ions [mass-to-charge ratio (m/z)] of 569 (M-15, loss of CH3), 405 [M-179, loss of (CH3)2SiOH and (CH3)3SiO]+, 301 (M-283), 283 [M-301, [(CH3)3SiO]2-CH2-CH(CH2)2-COOCH3], and 173 [M-411, [(CH3)3SiO]2-CH2-CH(CH2)2-COOCH3]. The relative intensity of m/z 283 vs. 173 indicates the presence of 11,12,15-THETA (23). Mass spectra with the same major ions were obtained with synthetic 11,12,15-THETA standards.

GC-MS analysis of aortic and synthetic 11,12,15-THETAs. The pooled 11,12,15-THETA fractions isolated from solvent system III were evaporated to dryness using a nitrogen stream and derivatized for GC-MS as previously described (23). The biological sample and synthetic 11,12,15-THETA standards were dissolved in 120 μl of acetone and then treated with ethereal diazomethane for 6 min at 0°C to form the methyl ester. The reacted sample was evaporated to dryness under nitrogen, and the hydroxyl groups were converted to the trimethylsilyl (TMS) ethers by 60 min of incubation with 15 μl of bis-TMS-trifluoroacetamide at 37°C. GC-MS was performed with a Hewlett-Packard mass spectrometer (model 5989A) coupled with a gas chromatograph (model 5890, series 2). Chemical ionization of the

<table>
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<th>Biological sample</th>
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<th>NP-HPLC (min)</th>
<th>GC (min)</th>
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<td>19.1</td>
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<td>80.1</td>
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Fig. 1. Chromatographic elution times of 11,12,15(S)-trihydroxyicosatetraenoic acids (THETA) and THETA isolated from the biological sample. RP-HPLC and NP-HPLC, reverse-phase and normal-phase HPLC; GC, gas chromatography.
samples was performed at 65–70 eV with methane as the reagent gas. Ions were measured in the positive-ion mode as total ion current. The derivatized biological 11,12,15-THETA and synthetic THETA standards were resolved using a 14-m capillary DB-5 column with a linear gradient from 100 to 300°C. Migration times of the 11,12,15-THETA standards were compared with that of the biological 11,12,15-THETA.

Vascular reactivity of 11,12,15-THETA isomers. Thoracic aorta was obtained from 1- to 2-mo-old New Zealand White rabbits and placed in Krebs bicarbonate buffer (mM: 118 NaCl, 4 KCl, 3.3 CaCl2, 24 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, and 11 glucose) as previously described (3, 20, 22). The tissue was carefully cleaned of adhering fat and connective tissue and cut into 3-mm-long rings, with care taken not to damage the endothelium. Aortic rings were suspended in 6-ml tissue baths containing Krebs bicarbonate buffer maintained at 37°C and continuously bubbled with 95% O2/5% CO2, to prevent vessel desiccation. The rings were rinsed, and vessels returned to resting tension. Once the aortic rings had reached peak contraction, the tissue baths were rinsed, and vessels returned to resting tension. Aortic rings were suspended in 6-ml tissue baths containing Krebs bicarbonate buffer maintained at 37°C and continuously bubbled with 95% O2/5% CO2. Isometric tension was measured with force-displacement transducers (model PT-03C, Grass) and amplifiers (model ETH-400, ADInstruments) and recorded on a MacIntosh computer using MacLab software. Resting tension was adjusted to its length-tension maximum of 2 g, and vessels were allowed to equilibrate for 1 h. Constrictions were produced by increasing the KCl concentration of the Krebs buffer to 40 mM. After the vessels reached peak contraction, the tissue baths were rinsed, and vessels returned to resting tension. Once the aortic rings had reproduced, stable responses to KCl, the tissue was contracted with 10−8–10−7 M phenylephrine to 50–75% maximal KCl contraction. The 11,12,15(S)-THETA isomers were dissolved in ethanol and tested for activity. In other experiments, the vessels were pretreated with 100 nM apamin for 10 min before preconstriction with phenylephrine and addition of the 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA standard. Similar amounts of phenylephrine were used to contract the control and apamin-treated arterial rings. Relaxations are expressed as percent relaxation relative to phenylephrine precontraction, with 100% representing basal tension.

K+ channel activity of 11,12,15-THETA isomers. Aortic smooth muscle cells were enzymatically dispersed using published methods (12). Whole cell recordings of K+ currents were obtained in freshly isolated aortic smooth muscle cells using an amplifier (Axopatch 200B, Axon Instruments), pClamp 8 software (Axon Instruments), and standard methods, as previously described (12). Briefly, macroscopic K+ currents were generated by progressive 10-mV depolarizing steps (500-ms duration, 5-s intervals) from a constant holding potential of −60 to +60 mV. Currents were sampled at 3 kHz and filtered at 1 kHz. Trials were performed in triplicate and averaged to estimate K+ current density. Membrane capacitance of each cell was estimated by integration of the capacitive current generated by a 10-mV hyperpolarizing pulse after electronic cancellation of pipette-patch capacitance. Indomethacin (10−5 M) was present in all perfusate solutions. After control vehicle currents were recorded, increasing concentrations (10–100 μM) of 11(R),12(S),15(S),5(Z),8(Z),13(E)- or 11(R),12(R),15(S),5(Z),8(Z),13(E)-THETA were added, and currents were obtained. In a subset of experiments, cells were treated with 10−7 M apamin before addition of 11(R),12(S),15(S)-THETA. Results are shown as percent current density, with 100% equal to current density measured at +60 mV under control (vehicle) conditions.

Data analysis. Relaxation and patch-clamp results are presented as means ± SE. Significance of difference between mean values was evaluated by two-way ANOVA followed by the Student-Newman-Keuls multiple comparison test. P < 0.05 was considered statistically significant.

RESULTS

Structures of the 11,12,15-THETA stereoisomers are shown in Fig. 1. Migration times of the 11,12,15(S)-THETA stereoisomers were determined by RP-HPLC using solvent system II and by NP-HPLC using solvent system III and compared with the biological [14C]11,12,15-THETA produced by rabbit aorta. Similarly, GC elution times of the methyl ester, TMS ether derivatives of the 11,12,15(S)-THETA stereoisomers, and biological 11,12,15-THETA were compared. Figure 2 summarizes the sequential purification of the [14C]11,12,15-THETA produced by the rabbit aorta by RP-HPLC (systems I and II) and NP-HPLC (system III). The biological 11,12,15-THETA eluted at 15.5 min by RP-HPLC (system II, Fig. 2B), at 47.5 min by NP-HPLC (system III, Fig. 2C), and at 13.7 min on GC. The 11,12,15-THETA stereoisomers eluted between 15.2 and 15.9 min by RP-HPLC, 16.9 and 80.4 min by NP-HPLC, and 13.5 and 14.3 min by GC. Most of the stereoisomers were resolved by HPLC. Of the eight 11,12,15(S)-THETA isomers, only 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA comigrated with the biological 11,12,15-THETA in the three chromatographic
systems (15.5 min by RP-HPLC and 13.7 min by GC). By NP-HPLC, the biological 11,12,15-THETA coeluted at the same time as the 11(R),12(S),15(S)-E,7(Z),8(Z),13(E)-THETA standard (47.6 min). NP-HPLC elution times of the other 11,12,15-THETA stereoisomers differed by 4.2–32.8 min from the biological 11,12,15-THETA. Thus we conclude that the biological 11,12,15-THETA and the 11(R),12(S),15(S)-E,7(Z),8(Z),13(E)-THETA similarly comigrate when these HPLC methods are used.

The eight 11,12,15(S)-THETA stereoisomers were tested for their ability to relax phenylephrine-precontracted rabbit aorta. None of the four stereoisomers with the cis-Δ13,14-olefin [13(Z)] relaxed the aorta or contracted the rabbit aorta at 10−7 – 10−4 M (Fig. 3). Of the four isomers with the trans-Δ13,14-olefin [13(E)], 11(R),12(S),15(S)-THETA relaxed the rabbit aorta in a concentration-related manner, with significant relaxations at 10−6 M (Fig. 3A). Maximal relaxations to this isomer were 69 ± 5% (* P < 0.01). These relaxations were inhibited by pretreatment with the SKCa channel blocker apamin (100 nM). In comparison, 10−4 M 11(S),12(R),15(S)-THETA relaxed the aorta by only 22% (Fig. 3C). At 10−4 M, 11(R),12(R),15(S)-THETA contracted the aorta (Fig. 3B), whereas 11(S),12(S),15(S)-THETA was without effect (Fig. 3D). Ethanol vehicle used with the THETA standards was without effect (Fig. 3A).

Whole cell K+ currents of isolated rabbit aortic smooth muscle cells were evoked by 10-MV stepwise increases from a holding potential of −60 to +60 mV (Figs. 4 and 5). Outward currents increased with the depolarizing potentials. 11(R),12(S),15(S)-THETA caused concentration-related increases in the outward current. Maximum current density at +60 mV increased 39 ± 9% at 10−5 M and 119 ± 36% at 10−4 M. At 10−5 M, 11(R),12(R),15(S)-THETA was without effect and 10−4 M increased current 20 ± 2%. Cell capacitance averaged 11.2 ± 1.2 pA/pF. Pretreatment with 100 nM apamin inhibited the increase in outward current activated by 11(R),12(S),15(S)-THETA (Fig. 5).

DISCUSSION

In the rabbit aorta, acetylcholine causes endothelium-dependent relaxations that are partially inhibited by cyclooxygenase and nitric oxide synthase blockers (3,20,25). These relaxations were associated with endothelium-dependent hyperpolarization of the smooth muscle. These findings indicate that a portion of the endothelium-dependent relaxation is mediated by EDHF. The prostaglandin- and nitric oxide-independent relaxations to acetylcholine are inhibited by the SKCa channel blocker apamin, high extracellular K+, phospholipase inhibitors, and lipoxygenase inhibitors (3,12,20,27). They are also inhibited by antisense oligonucleotides against 15-LO, but not by scrambled oligonucleotides (26). These studies indicate that the EDHF is an AA metabolite of 15-LO. 11,12,15-THETA and its precursor 15-H-11,12-EETA are the major 15-LO metabolites produced by the rabbit aorta, rabbit mesenteric artery, and aortic endothelium. These studies have identified 11,12,15-THETA as the EDHF in rabbit arteries.

Very little is known about the mechanism of action of 11,12,15-THETA because of the lack of availability of the eicosanoid. Biosynthetic 11,12,15-THETA, produced by the rabbit aorta, relaxed precontracted rabbit aorta, hyperpolarized vascular smooth muscle, and activated SKCa channels in smooth muscle cells (3,12). The exact structure of the biologically active 11,12,15-THETA was not previously known. Since rabbit aorta contains 15-LO and synthesizes 15(S)-HPETE at 94% optical purity (21,26), the THETA must

![Fig. 3. Effect of 11,12,15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-THETA stereoisomers on preconstricted rabbit aorta. Aortic rings were preconstricted with phenylephrine to 50–75% of maximal KCl contraction. THETA stereoisomers were added in increasing concentrations, and changes in isometric tension were measured. A: 11(R),12(S),15(S)-THETA stereoisomer ([RSS]-THETA) relaxations. Relaxation to 13(E)-THETA was determined under control conditions and with aortic rings pretreated with the SKCa channel blocker apamin (100 nM). Relaxations to vehicle control are also shown. B: 11(R),12(R),15(S)-THETA stereoisomer ([RSS]-THETA) relaxations. C: 11(S),12(R),15(S)-THETA stereoisomer ([RSS]-THETA) relaxations. D: 11(S),12(S),15(S)-THETA stereoisomer ([SSS]-THETA) relaxations. Values are means ± SE. *Significantly different from vehicle control. **Significantly different from RSS-13(E)-THETA.](http://ajpheart.physiology.org/)

\[\text{RSS-13(E)-THETA} \quad \text{RSS-13(Z)-THETA} \quad \text{Vehicle} \]

\[\text{RSS-13(Z)-THETA} \quad \text{RSS-13(Z)-THETA} \quad \text{Vehicle} \]

\[\text{SSS-13(E)-THETA} \quad \text{SSS-13(Z)-THETA} \quad \text{Vehicle} \]

\[\text{SSS-13(Z)-THETA} \quad \text{SSS-13(Z)-THETA} \quad \text{Vehicle} \]
aortic smooth muscle cells. Outward K\textsuperscript{+}-hydroxyl. However, configurations of the 11(S),12(R),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-trienoic acid comigrated with the biological 11,12,15-THETA on RP-HPLC, NP-HPLC, and GC, relaxed the rabbit aorta, and activated SKCa channels. These studies suggest that 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA is the vasodilator produced by the rabbit aortic endothelium.

Although previous studies indicate that biological 11,12,15-THETA relaxes the rabbit aorta and activates SKCa channels (3, 12), the exact concentration of the biosynthesized THETA could not be determined. Thus it was important to determine the potency of the synthetic 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA. These studies indicate that 10\textsuperscript{-7}-10\textsuperscript{-4} M 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA relaxed the preconstricted aorta in a concentration-related manner. Maximal relaxation was 69%. High 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA concentrations (≥10\textsuperscript{-6} M) were required to cause relaxation. THETAs share lipophilic properties of other fatty acids: they are poorly soluble in aqueous buffers, stick to the bath surfaces, and form micelle aggregates. Therefore, free THETA concentrations available for the activation of relaxation are less than the added concentrations.

Nothing is known about the metabolism of 11,12,15-THETA by vascular cells. Since there are strict structural and stereocentric requirements for vascular activity, metabolism of 11,12,15-THETA would result in a loss of activity. Many eicosanoids and prostanooids with a 15-hydroxy group are inactivated by 15-hydroxyprostaglandin dehydrogenase, which converts the hydroxyl to a ketone, and subsequent reduction of the Δ\textsuperscript{13,14}-olefin by a reductase (1, 14). β- and ω-oxidation are other inactivation pathways. It is not known whether 11,12,15-THETA is a substrate for these enzymes.

In rabbit arteries, 11,12,15-THETA acts as an EDHF (3, 12). Relaxations to the biological 11,12,15-THETA are blocked by high extracellular K\textsuperscript{+} and apamin, indicating an action on K\textsubscript{Ca} channels. Similar to the biological 11,12,15-THETA, 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA activated SKCa channels, which will cause membrane hyperpolarization and vascular relaxation. Relaxations to 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA were inhibited by the SKCa channel blocker apamin. 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA also increased aortic smooth muscle cell outward K\textsuperscript{+} current,

contain a 15(S)-hydroxyl. However, configurations of the 11- and 12-hydroxyls were not known. Since the aorta does not produce an adequate mass of 11,12,15-THETA for analytic determination of the stereocentric structure, we compared chromatographic migration of the biological 11,12,15-THETA with that of eight synthetic stereoisomers (Fig. 1). Of these isomers, only 11(R),12(S),15(S)-trihydroxyeicosa-5(Z),8(Z),13(E)-trienoic acid comigrated with the biological 11,12,15-THETA. 11,12,15-THETA on RP-HPLC, NP-HPLC, and GC, relaxed the rabbit aorta, and activated SKCa channels. These studies suggest that 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA is the vasodilator produced by the rabbit aortic endothelium.

Although previous studies indicate that biological 11,12,15-THETA relaxes the rabbit aorta and activates SKCa channels (3, 12), the exact concentration of the biosynthesized THETA could not be determined. Thus it was important to determine the potency of the synthetic 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA. These studies indicate that 10\textsuperscript{-7}-10\textsuperscript{-4} M 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA relaxed the preconstricted aorta in a concentration-related manner. Maximal relaxation was 69%. High 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA concentrations (≥10\textsuperscript{-6} M) were required to cause relaxation. THETAs share lipophilic properties of other fatty acids: they are poorly soluble in aqueous buffers, stick to the bath surfaces, and form micelle aggregates. Therefore, free THETA concentrations available for the activation of relaxation are less than the added concentrations.

Nothing is known about the metabolism of 11,12,15-THETA by vascular cells. Since there are strict structural and stereocentric requirements for vascular activity, metabolism of 11,12,15-THETA would result in a loss of activity. Many eicosanoids and prostanooids with a 15-hydroxy group are inactivated by 15-hydroxyprostaglandin dehydrogenase, which converts the hydroxyl to a ketone, and subsequent reduction of the Δ\textsuperscript{13,14}-olefin by a reductase (1, 14). β- and ω-oxidation are other inactivation pathways. It is not known whether 11,12,15-THETA is a substrate for these enzymes.

In rabbit arteries, 11,12,15-THETA acts as an EDHF (3, 12). Relaxations to the biological 11,12,15-THETA are blocked by high extracellular K\textsuperscript{+} and apamin, indicating an action on K\textsubscript{Ca} channels. Similar to the biological 11,12,15-THETA, 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA activated SKCa channels, which will cause membrane hyperpolarization and vascular relaxation. Relaxations to 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA were inhibited by the SKCa channel blocker apamin. 11(R),12(S),15(S),5(Z),8(Z),13(E)-THETA also increased aortic smooth muscle cell outward K\textsuperscript{+} current,
which was blocked by apamin. Other 11,12,15(S)-THETA isomers did not display this vascular activity. Interestingly, $10^{-4} \text{M} 11(R),12(R),15(S),5(Z),8(Z),13(E)$-THETA caused a small but significant increase in outward $K^+$ current but caused vascular constriction. The reason for this discrepancy is not known, but it suggests that the mechanism of the vascular constriction is unrelated to $K^+$ channel activation.

These studies indicate that an exact stereochemical configuration of 11,12,15-THETA is needed for biological activity. The configuration of the hydroxys and olefin between carbons 13 and 14 is critical for full activity. All THETAs with the cis-$\Delta^{13,14}$,olefin [13(Z) series] were inactive. Of the 11,12,15-THETAs with the trans-$\Delta^{13,14}$-olefin [13(E) series], 11(R), 12(S), 15(S)-THETA was a potent agonist, whereas the other stereoisomers were weak agonists or were without activity. The requirements for a specific structure and stereochemical configuration suggest that a specific binding site or receptor is involved in mediating the $K_{\text{ca}}$ channel activation and vasorelaxations. There are examples of membrane receptors for trihydroxylated AA metabolites. Lipoxin A4, a trihydroxy

**REFERENCES**


