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Pathways of Epoxyeicosatrienoic Acid Metabolism in Endothelial Cells

IMPLICATIONS FOR THE VASCULAR EFFECTS OF SOLUBLE EPOXIDE HYDROLASE INHIBITION*

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Epoxyeicosatrienoic acids (EETs) are products of cytochrome P-450 epoxygenase that possess important vasodilating and anti-inflammatory properties. EETs are converted to the corresponding dihydroxyeicosatrienoic acid (DHET) by soluble epoxide hydrolase (sEH) in mammalian tissues, and inhibition of sEH has been proposed as a novel approach for the treatment of hypertension. We observed that sEH is present in porcine coronary endothelial cells (PCEC), and we found that low concentrations of *N,N′*-dicyclohexylurea (DCU), a selective sEH inhibitor, have profound effects on EET metabolism in PCEC cultures. Treatment with 3 μM DCU reduced cellular conversion of 14,15-EET to 14,15-DHET by 3-fold after 4 h of incubation, with a concomitant increase in the formation of the novel β-oxidation products 10,11-epoxy-16:2 and 8,9-epoxy-14:1. DCU also markedly enhanced the incorporation of 14,15-EET and its metabolites into PCEC lipids. The most abundant product in DCU-treated cells was 16,17-epoxy-22:3, the elongation product of 14,15-EET. Another novel metabolite, 14,15-epoxy-20:2, was present in DCU-treated cells. DCU also caused a 4-fold increase in release of 14,15-EET when the cells were stimulated with a calcium ionophore. Furthermore, DCU decreased the conversion of [^{3}H]11,12-EET to 11,12-DHET, increased 11,12-EET retention in PCEC lipids, and produced an accumulation of the partial β-oxidation product 7,8-epoxy-16:2 in the medium. These findings suggest that in addition to being metabolized by sEH, EETs are substrates for β-oxidation and chain elongation in endothelial cells and that there is considerable interaction among the three pathways. The modulation of EET metabolism by DCU provides novel insight into the mechanisms by which pharmacological or molecular inhibition of sEH effectively treats hypertension.

Epoxyeicosatrienoic acids (EETs)[1] are arachidonic acid metabolites produced by cytochrome P-450 epoxygenases (1, 2). EETs are formed by endothelial cells and produce vasodilation in a number of vascular beds, including the coronary circulation (2–4). This occurs through hyperpolarization of the vascular smooth muscle cells by activation of Ca^{2+}-activated K^+ channels, suggesting that EETs function as endothelium-derived hyperpolarizing factors (4–7). In addition, EETs are taken up and incorporated into phospholipids by endothelial cells (8), a process that may contribute to the endothelium-dependent vascular relaxation response (9). Incorporation of EETs into phospholipids also may play a role in their effects on cytokine-induced expression of adhesion molecules (10), Ca^{2+} signaling (11), and tyrosine kinase activity in the endothelium (12). Although a great deal is known about the biological actions of EETs in the vasculature, relatively little is known about the biochemical pathways that mediate EET uptake and metabolism in endothelial cells.

A major pathway for EET metabolism in many tissues is hydration of the epoxide group to a diol, forming the corresponding dihydroxyeicosatetraenoic acid (DHET), a reaction mediated by an epoxide hydrolase (13, 14). The two most studied forms of epoxide hydrolase and the two that have been shown to metabolize arachidonic acid epoxides in mammals are a soluble epoxide hydrolase (sEH) and a microsomal epoxide hydrolase (14–16). Considerable interest has recently centered on sEH because of results suggesting that it is a potential target for the treatment of hypertension. Disruption of the sEH gene was found to lower systolic blood pressure in male mice (17). Likewise, *N,N′*-dicyclohexylurea (DCU), a recently discovered selective sEH inhibitor that has a K_i = 30 nM for recombinant human sEH (18), reduced blood pressure in the spontaneously hypertensive rat (19). Both of these studies demonstrated that the anti-hypertensive effects were associated with reduced conversion of EETs to DHETs in the kidney (17, 19).

One potential mechanism whereby inhibition of sEH could reduce blood pressure is by enhancing the amount of EET available for incorporation into endothelial phospholipids. Thus, whereas EETs were shown to be avidly taken up and

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[1] The abbreviations used are: EETs, epoxyeicosatrienoic acid(s); DHETs, dihydroxyeicosatrienoic acid(s); sEH, soluble epoxide hydrolase; 4-PCO, 4-phenylchalcone oxide; PCEC, porcine coronary artery endothelial cells; DCU, *N,N′*-dicyclohexylurea; AA, arachidonic acid; HPLC, high performance liquid chromatography; LC/MS, liquid chromatography combined with mass spectrometry; TLC, thin layer chromatography; CID, collision-induced decompositions; [$^2H_8$]14,15-EET, [5,6,8,9,11,12,14,15-$^2H_8$]14,15-EET; tDPPO, *trans*-1,3-diphenylpropene oxide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

incorporated into endothelial cell phospholipids, DHETs appeared to be released preferentially from the cells (8). In keeping with this notion, we have previously observed that 4-phenylchalcone oxide (4-PCO), a nonspecific inhibitor of epoxide hydrolases (20), blocked conversion of 14,15-EET to 14,15-DHET and increased 14,15-EET incorporation in porcine coronary artery endothelial cell (PCEC) phospholipids (21). Consequently, endothelium-dependent relaxation responses were augmented in coronary arteries. Taken together, these findings suggest that epoxide hydrolase inhibition might beneficially affect vascular function by enhancing the amount of EET incorporated into endothelial cells. Moreover, these reports suggest that sEH functions to enzymatically degrade EETs and thereby terminate their biological activity.

On the other hand, several recent studies have challenged the view that sEH is simply a degradation pathway for EETs. For example, whereas DHETs were originally believed to be devoid of vasoactive properties (22–24), more recent reports indicate that they are capable of dilating conduit coronary arteries and coronary microvessels (9, 25, 26). Also, DHETs are incorporated into endothelial cell phospholipids, albeit less avidly than the precursor EETs (27). Finally, porcine coronary artery smooth muscle cells were found to metabolize 11,12-DHET through a β-oxidation pathway, thereby generating a 16-carbon diol that also possessed vasodilating activity (25). These reports indicate that epoxide hydrolase can, in some instances, generate biologically active EET metabolites. Moreover, the latter study raised the possibility that enzymes other than epoxide hydrolases might be capable of metabolizing EETs in vascular cells.

The purpose of the present study was to delineate the pathways of EET uptake and metabolism in endothelial cells during sEH inhibition. We have identified two novel pathways of EET metabolism in endothelial cells that could have important implications regarding the mechanisms by which sEH inhibition lowers blood pressure. Moreover, elucidation of these pathways provides important new insight into the mechanisms by which cytochrome P-450 epoxygenase products may regulate vascular function.

EXPERIMENTAL PROCEDURES

Synthesis of [^{3}H]EET—[5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid ([^{3}H]AA, 55 Ci/mmol, American Radiolabeled Chemicals, St. Louis, MO) was mixed with AA (Cayman Chemical, Ann Arbor, MI) at a final concentration of 0.2 μCi/nmol. After removing the solvent, the fatty acid was methylated with 12% BF_3 in methanol for 45 min at 50 °C. Following extraction, the AA methyl ester was incubated with 3-chloroperoxybenzoic acid in methylene chloride for 1.5 h at 0 °C (28). The reaction was stopped by addition of 50 μl of methyl sulfide, and the mixture was allowed to stand at room temperature for 20 min. The products were resuspended in methylene chloride and washed first with NaCl-saturated water and then water. The [^{3}H]EET methyl esters were isolated and purified by reverse-phase high performance liquid chromatography (HPLC) using a gradient that started at 65% acetonitrile, 35% water, was ramped to 85% acetonitrile, 15% water over 55 min, and then was taken to 100% acetonitrile at 60 min and held constant for 5 min. Fractions containing the purified [^{3}H]11,12- and 14,15-EET[2] methyl esters were hydrolyzed with 50 μl of 2 N NaOH in 500 μl of methanol at 50 °C for 1 h, and the [^{3}H]EETs were extracted from the mixture with water-saturated ethyl acetate (28). After drying under N_2, the [^{3}H]EETs were suspended in 95% ethanol and stored at −80 °C. Purity was checked by isocratic normal phase HPLC with a solvent consisting of 90% *n*-heptane and 10% of a mixture of *n*-heptane, isopropyl alcohol, and acetic acid (100:5:0.2, v/v/v). Only samples of >98% purity were used for the cell studies.

Cell Culture and Incubation—PCEC were isolated and grown in modified Medium 199 supplemented with 10% fetal bovine serum as described previously (9, 21). The cultures were maintained until confluent at 37 °C in a humidified atmosphere containing 5% CO_2. Stocks were subcultured weekly by trypsinization, and cultures were used for experiments between passages 3 and 7. Experiments were carried out with confluent monolayers using modified Medium 199 containing 0.1 μM bovine serum albumin.

Prior to incubation, the radiolabeled EET or AA was mixed with the corresponding non-radiolabeled compound obtained from Cayman Chemical to obtain the substrate concentration necessary for each experiment and a specific activity of 0.1 μCi/nmol. The PCEC were incubated initially in 1 ml of medium containing either DCU dissolved in dimethyl sulfoxide or the same amount of dimethyl sulfoxide alone as a control. The DCU was purchased from Aldrich, and a liquid chromatography combined with mass spectrometry (LC/MS) analysis and melting point measurement verified the purity. After 45 min, the radiolabeled substrate was added and the incubation continued. The medium was removed at the end of the incubation period, and the cells were washed twice and harvested by scraping into methanol. In some studies, the initial incubation medium was removed, and after washing, the cells were incubated for an additional 20 min in 1 ml of fresh medium containing either 3 μM DCU and 2 μM calcium ionophore A23187 or the ionophore alone.

Assay of Incubation Medium—The radioactivity remaining in the medium after the incubation was measured by liquid scintillation counting. Lipids contained in the medium were extracted twice with 4 ml of ice-cold ethyl acetate saturated with water; the extracts were combined; the solvent was evaporated under N_2; and the residue was dissolved in acetonitrile. The lipids were separated by reverse-phase HPLC using a Gilson dual pump gradient system equipped with model 306 pumps, a model 117 Dual wavelength UV detector, a model 231 XL automatic sample injector (Gilson Medical Electronics, Inc., Middleton, WI), and a 3 μm 4.6 × 150-mm Spherisorb C_{18} column obtained from Alltech (Deerfield, IL). The elution profile consisted of water adjusted to pH 3.4 with phosphoric acid and an acetonitrile gradient that increased from 27.5 to 100% over 60 min at a flow rate of 0.7 ml/min (29). The distribution of radioactivity was measured by combining the column with scintillator solution and passing the mixture through an on-line flow detector (IN/US Systems, Inc., Tampa, FL).

Analyses of Cell Lipids—The lipids were extracted from the PCEC with a 2:1 mixture of chloroform/methanol. After the phases were separated and the solvent removed under N_2, the lipids were dissolved in 200 μl of chloroform/methanol, and an aliquot of this mixture was dried under N_2 and assayed for radioactivity in a liquid scintillation spectrometer (25). To determine the distribution of the radioactivity in the cell lipids, aliquots of the extract were separated by TLC on Whatman LK5D silica gel plates obtained from Alltech Associates (Deerfield, IL) with a solvent system of chloroform, methanol, 40% methylamine (65:35:5, v/v/v). The radioactivity contained in the separated lipids was assayed using a Radiomatic gas flow proportional scanner with automatic peak search and integration (25, 29). Authentic phospholipid standards purchased from Avanti Polar Lipids (Naperville, IL) were added to each chromatogram and visualized by staining (30).

To separate and identify the radiolabeled fatty acid derivatives incorporated in the PCEC lipids, additional aliquots of the extract were hydrolyzed for 1 h at 50 °C in 0.5 ml of methanol containing 50 μl of 0.2 N NaOH and 10% H_2O. After the pH was adjusted to 7.2 with ice-cold H_3PO_4/H_2O (1:50, v/v), the free fatty acids were extracted twice with 5 ml of ice-cold ethyl acetate saturated with H_2O. The solvent was removed under N_2, and the lipids were dissolved in acetonitrile and separated by reverse-phase HPLC (29).

Identification of EET Metabolites—A Hewlett-Packard 1100 MSD LC/MS system was used to separate and identify the metabolites (31). HPLC was carried out on a Supelco C_{18} 5 μm 4.6 × 150 mm Discovery™ column with mobile phase solvents consisting of water/formic acid (100:0.03 v/v) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.7 ml/min. The gradient was maintained at 30% solvent B for the first 2 min and then linearly ramped to 57% solvent B at 20 min, 65% solvent B at 40 min, 70% solvent B at 45 min, and 95% solvent B at 50 min. Negative ion electrospray was used with the fragmentor voltage set at 140 V in order to produce in-source collision-induced decompositions (CID). N_2 nebulizing gas was maintained at 60 bar, whereas the N_2 drying gas was set to a flow rate of 10 liters/min at 350 °C. Data were processed with the Hewlett-Packard Chemstation software program.

To confirm the identification of the metabolites, additional studies were done with [5,6,8,9,11,12,14,15-$^2H_8$]14,15-EET ([$^2H_8$]14,15-EET, Biomol Research Laboratories, Inc., Plymouth Meeting, PA). The PCEC cultures were incubated initially with 10 μM DCU for 45 min, and then 5 μM [$^2H_8$]14,15-EET was added and the incubation continued for 5 h.

[2] The ^{3}H is present at carbon 5,6,8,9,11,12,14, and 15. The 11,12- and 14,15- refer to the location of the epoxide group.

After the media and cells were separated, the lipids were extracted from each and were analyzed by LC/MS as indicated above.

Epoxide Hydrolase Activity—Harvested PCEC were suspended in 1 ml of chilled 0.1 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, phenylmethylsulfonyl fluoride, and dithiothreitol. The cells were disrupted using a Polytron homogenizer at 9,000 rpm for 30 s; the homogenate was centrifuged at 9,000 × *g* for 10 min at 4 °C; and the supernatant solution was used as the enzyme extract. Protein concentration was measured using the Pierce BCA assay. Epoxide hydrolase activity was measured using racemic [^{3}H]*trans*-1,3-diphenylpropene oxide (tDPPO), a selective substrate for the enzyme (32). The tDPPO was synthesized by reducing the chemical precursor with [^{3}H]sodium borohydride (PerkinElmer Life Sciences) to give a final specific activity of 50 mCi/mmol (32). After the tDPPO was diluted with unlabeled material, 1 μl of a 5 mM solution of [^{3}H]tDPPO in dimethylformamide was added to 100 μl of enzyme preparation in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.1 mg/ml albumin. The final tDPPO concentration was 50 μM. After incubating at 30 °C for 15 min, the reaction was quenched by addition of 60 μl of methanol and 200 μl of isooctane. These solvents extracted the remaining epoxide from the aqueous phase, and the quantity of radioactive diol present in the aqueous phase was measured by liquid scintillation counting. Each assay was performed in triplicate.

Statistical Analysis—The data are expressed as means ± S.E. Values were analyzed by Student's *t* test for unpaired data. Probability values of 0.05 or less were considered to be statistically significant.

RESULTS

Determination of Epoxide Hydrolase Activity—The 9000 × *g* supernatant solution from the PCEC homogenate was incubated with [^{3}H]tDPPO, a substrate that is specific for sEH (32). The preparation produced 2.1 nmol min^{-1} mg protein^{-1} of radiolabeled diol metabolite, demonstrating that the coronary artery endothelial cells contain sEH activity. This finding is consistent with previous studies indicating that PCEC cultures convert 14,15-EET and 8,9-EET to the corresponding DHETs (21).

Effect of sEH Inhibition on 14,15-EET Metabolism—To investigate the role of sEH in endothelial EET metabolism, we determined whether DCU, a potent and selective inhibitor of recombinant sEH (18), would inhibit the conversion of EET to DHET in intact PCEC. The cultures were incubated for either 75 min or 4 h with 2 μM [^{3}H]14,15-EET, with or without 3 μM DCU, and the distribution of radiolabeled products in the culture medium was determined by reverse-phase HPLC. Representative chromatograms are shown in Fig. 1. 14,15-DHET accounted for 85% of the radioactivity contained in the medium of control cultures after a 75-min incubation, and less than 10% remained as EET (Fig. 1*A*). By contrast, when DCU was added to the medium, most of the radioactivity remained as 14,15-EET and only 13% was converted to 14,15-DHET after 75 min (Fig. 1*B*). Small amounts of two additional radiolabeled products, designated X and Y with retention times of 25.6 and 31 min, respectively, were detected in the medium of the cultures treated with DCU. When the incubation was extended to 4 h, 14,15-DHET accounted for >95% of the radioactivity in the medium (Fig. 1*C*). However, when DCU was present during the 4-h incubation, EET remained the most abundant radiolabeled compound in the medium, X and Y increased, and DHET accounted for only 35% of the radioactivity (Fig. 1*D*).

Additional experiments further investigated the time-dependent effect of DCU and the effect of increasing DCU concentrations on [^{3}H]14,15-EET metabolism. After incubation, the medium obtained from each culture was assayed by HPLC, and the differences in radiolabeled metabolite production are illustrated in Fig. 2. The inhibition of 14,15-DHET formation approached a maximum at 3 μM DCU when the PCEC were incubated with 2 μM [^{3}H]14,15-EET for 75 min (Fig. 2*A*), and the calculated IC_{50} of DCU was 0.23 μM. Radiolabeled DHET accumulation reached a maximum in 1–2 h when no inhibitor was present. However, it increased much more slowly when 3 μM DCU was present, and 50% less 14,15-DHET was formed at the end of the 8-h incubation (Fig. 2*B*). The amounts of radiolabeled metabolites X and Y in the medium during the 8-h incubation with DCU are shown in Fig. 2*C*. Product Y formed more rapidly, reached a maximum at 4 h, and then declined, whereas

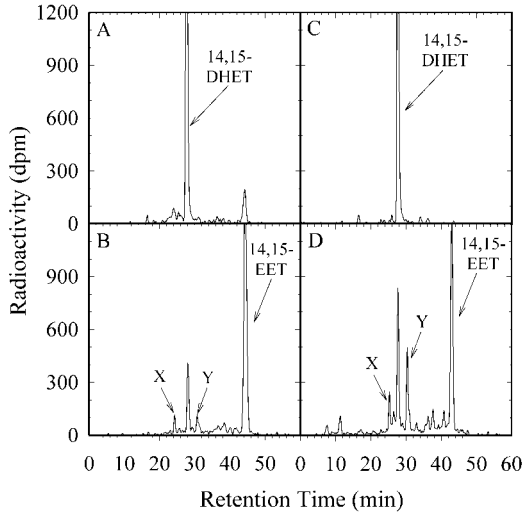

FIG. 1. **Effect of DCU on [^{3}H]14,15-EET products present in the culture medium.** PCEC were incubated with either 3 μM DCU dissolved in dimethyl sulfoxide or a corresponding amount of dimethyl sulfoxide for 45 min. This medium was removed and replaced with fresh medium containing 3 μM DCU in dimethyl sulfoxide and 2 μM [^{3}H]14,15-EET or dimethyl sulfoxide and 2 μM [^{3}H]14,15-EET (control), and the incubation was continued for either 75 min (*left panel*) or 4 h (*right panels*). After incubation, the medium was removed and lipids extracted and analyzed for radioactivity by HPLC with an on-line flow scintillation counter. Radiochromatograms from a single culture are shown, but similar results were obtained from two additional cultures in each case: ***A***, 75 min control; ***B***, 75 min with 3 μM DCU; ***C***, 4 h control; ***D***, 4 h with 3 μM DCU. The identifications of compounds X and Y are described in the text, and mass spectra of the compounds are shown in Fig. 3.

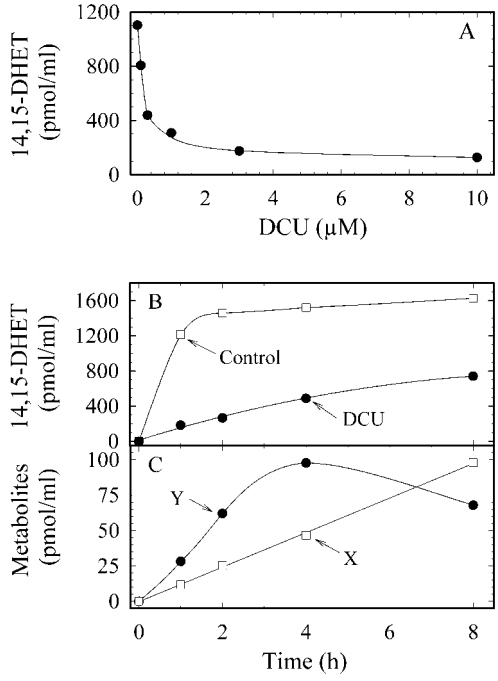

FIG. 2. **Time- and concentration-dependent formation of radiolabeled 14,15-EET products in the culture medium.** PCEC were incubated with 2 μM [^{3}H]14,15-EET and DCU where indicated, and the medium was analyzed for radioactivity as described in Fig. 1. The time of incubation in *A* was 75 min. *B* and *C*, the DCU concentration was 3 μM. The picomole values are calculated from the specific activity of [^{3}H]14,15-EET added to the cultures. Each point is the average of results obtained from two separate cultures, and both values were within 10% agreement.

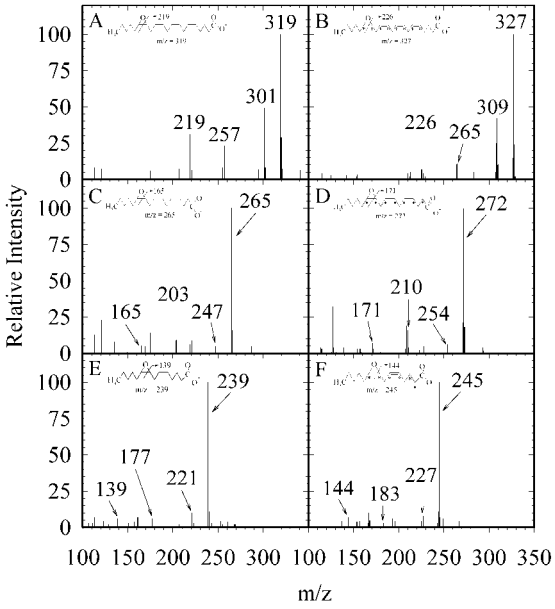

FIG. 3. **Mass spectra of the main products and their deuterated analogs contained in the incubation medium.** The spectra were obtained by LC/MS with in-source CID. In order to obtain sufficient quantities of products for structural identification, it was necessary to utilize 75-cm^2 PCEC cultures. The time of incubation was 4 h, and DCU was present in the culture medium. A similar incubation was done with [$^2H_8$]14,15-EET to obtain the corresponding deuterated compounds. In both cases, HPLC analysis indicated a pattern of metabolites similar to that shown in Fig. 1*D*. The mass spectra correspond to the following: *A*, the 14,15-EET present in the culture medium at the end of the incubation; *B*, [$^2H_8$]14,15-EET present in the culture medium at the end of the incubation; *C*, compound Y; *D*, the deuterated analog of compound Y; *E*, compound X; *F*, the deuterated analog of compound X. The * in the structural diagrams of the deuterated compounds indicate the carbons that contain ^{2}H.

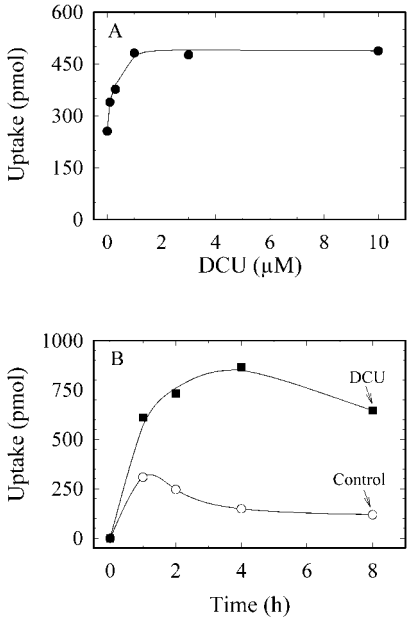

FIG. 4. **Time- and concentration-dependent effects of DCU on 14,15-EET uptake by PCEC.** The cultures were incubated with 2 μM [^{3}H]14,15-EET for various times as described in Fig. 1. The time of incubation in the DCU concentration study was 75 min (*A*), and the DCU concentration in the time-dependent study was 3 μM (*B*). After removal of medium and washing the cells with buffer, the cell lipids were extracted, and an aliquot was assayed for radioactivity. The picomole values are calculated from specific activity of [^{3}H]14,15-EET added to the cultures. Each point is the average of results obtained from two separate cultures, and both values were within 10% agreement.

product X increased progressively and was more abundant at the end of the 8-h incubation.

Identification of Metabolites—The three main radiolabeled compounds that accumulated in media containing DCU were identified by LC/MS (Fig. 3). In-source CID was utilized to generate additional structural information. The mass spectra of deuterium-labeled metabolites isolated from the medium following incubation of PCEC cultures with [$^2H_8$]14,15-EET also were obtained to confirm the assignments to the fragment ions in the spectra of the metabolites.

The mass spectrum of 14,15-EET contains a $(M - H)^-$ ion of m/z 319, an ion of m/z 301 due to loss of one water molecule, and an ion of m/z 219 from fragmentation of the carbon chain through the oxirane ring (31, 33). The fragment ion of m/z 257, which is of lesser importance, could be the result of loss of water and either loss of CO_2 or loss of a neutral alkene and H_2 via charge remote fragmentation (31). The identity of the compound, which appeared to be 14,15-EET based on HPLC retention time (Fig. 1), was confirmed by the mass spectrum (Fig. 3*A*) and that of the corresponding deuterated analog (Fig. 3*B*).

The $(M - H)^-$ and fragment ions that are analogous to those of 14,15-EET are labeled in the mass spectra of metabolites Y and X. These ions were utilized to determine the molecular weight, if an epoxide is present, and the location of the epoxide ring in the carbon chain. Comparison of the in-source CID mass spectra of metabolite Y (Fig. 3*C*) and its deuterated analog (Fig. 3D) indicates that the masses of the $(M - H)^-$ ions (m/z 265 and 272) differ by 7 daltons. The fragment ions corresponding to the loss of water (m/z 247 and 254) also differ by 7 Da, but the fragment ions that are the result of carbon chain cleavage at the epoxide ring (m/z 165 and 171) differ by only 6 Da. This confirms that due to the in-source CID the $(M - H)^-$ ions are fragmenting via cleavage of the carbon chain at the epoxide ring, and one deuterium is retained by the neutral loss. Thus, the structure of metabolite Y is consistent with a structure of 10,11-epoxyhexadecadienoic acid (10,11-epoxy-16:2).

Both the in-source CID mass spectra of metabolite X (Fig. 3*E*) and its deuterated analog (Fig. 3*F*) show abundant $(M - H)^-$ ions of m/z 239 and 245, ions due to loss of water (m/z 221 and 227), and ions due to carbon chain cleavage at the oxirane ring (m/z 139 and 144). The data suggest a structure of 8,9-epoxytetradecaneoic acid (8,9-epoxy-14:1).

These findings indicate that in addition to being converted to diols by sEH, 14,15-EET can undergo β-oxidation by endothelial cells, with each cycle removing two carbons from the carboxyl end of the molecule.

Effect of sEH Inhibition on 14,15-EET Incorporation into Cells—A series of experiments was done to determine whether inhibition of sEH would affect the amount of 14,15-EET taken up and retained by the PCEC. The effects of DCU concentration and time of incubation on the total amount of radiolabeled 14,15-EET present in the cell lipids are shown in Fig. 4. DCU produced a dose-dependent increase in uptake of 2 μM [^{3}H]14,15-EET, with the maximum accumulation occurring at 1 μM DCU in a 75-min incubation (Fig. 4*A*). During the course of an 8-h incubation, the amount incorporated by the control cells reached a maximum at 1 h and then declined (Fig. 4*B*). The amount contained in the cells was greater when 3 μM DCU was present throughout the 8-h incubation; the maximum accumulation did not occur until 4 h, and the incorporation at 8 h was 5 times greater than in the control cultures.

Analysis by TLC indicated that the endothelial phospholipids contained more than 90% of the radioactivity incorporated by the PCEC (Fig. 5). The phospholipid fractions containing the radioactivity were phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Similar distri-

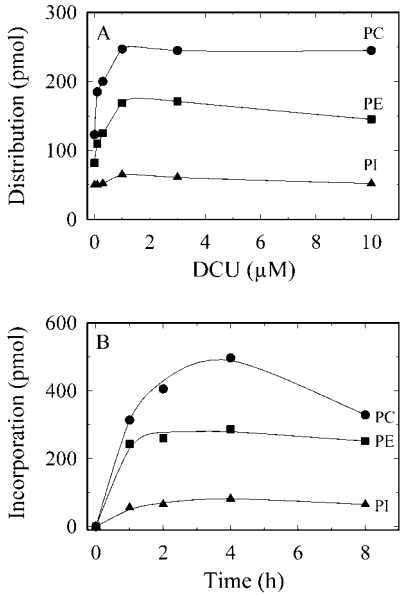

FIG. 5. **Time- and concentration-dependent effects of DCU on 14,15-EET incorporation into PCEC phospholipids.** The incubation and analysis was done as described in Fig. 4, except that the cell lipid extracts were separated by TLC, and the radioactivity contained in each of the phospholipid fractions was determined. The picomole values are calculated from specific activity of [^{3}H]14,15-EET added to the cultures. Each point is the average of results obtained from two separate cultures, and both values were within 10% agreement.

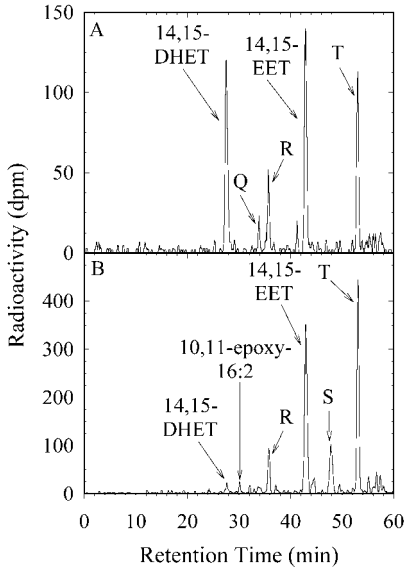

FIG. 6. **Radiolabeled 14,15-EET products present in the cell lipids.** The PCEC were incubated with 2 μM [^{3}H]-14,15-EET for 4 h in the absence (*A*) or presence (*B*) of 3 μM DCU. After incubation, the cell lipids were extracted, hydrolyzed by saponification, and analyzed for radioactivity by reverse-phase HPLC as described in Fig. 1. Radiochromatograms from a single culture are shown, but similar results were obtained from two additional cultures in both cases. The identifications of compounds Q, S, and T are described in the text, and mass spectra of these compounds are shown in Fig. 7. Compound R was not been identified.

butions, PC > PE > PI, occurred in the control and DCU-treated cultures. When the DCU concentration was raised, however, the amounts of radioactivity in PC and PE increased, whereas the amount in PI did not change appreciably (Fig. 5*A*). The time dependence of the distribution was examined when the PCEC cultures were incubated with 3 μM DCU. The incorporation of 14,15-EET into PE and PI reached a steady state level within 1–2 h, whereas the amount in PC continued to increase during the first 4 h and then declined (Fig. 5*B*).

Radiolabeled Compounds in Cell Lipids—The extent to which the 14,15-EET incorporated by the PCEC was chemically modified was determined by HPLC analysis of the hydrolyzed cell lipid extract. The results of an experiment in which the PCEC were incubated with 2 μM [^{3}H]14,15-EET for 4 h are shown in Fig. 6. In the control cultures, only 32% of the radioactivity contained in the PCEC lipids remained as 14,15-EET, whereas 30% was converted to 14,15-DHET (Fig. 6*A*). A compound containing 24% of radioactivity, designated T, eluted with a retention time of 53 min, and two other compounds with retention times of 33 and 35.4 min, designated Q and R, respectively, contained smaller amounts of radioactivity.

The distribution when 3 μM DCU was present during the incubation is shown in Fig. 6*B*. As indicated by the difference in the scale of the *y* axis, much more radiolabeled material was contained in the hydrolyzed cell lipid extract when DCU was present. Metabolite T was the most abundant product and accounted for 40% of the incorporated radioactivity, and 36% was present as 14,15-EET, 7% as metabolite R, and less than 2% as 14,15-DHET. A metabolite with a retention time of 48 min, designated S, accounted for 14% of the radioactivity. This product was not detected in the control cultures. In addition, 10,11-epoxy-16:2, a metabolite that accumulated in the medium (designated as product Y in Figs. 1*D* and 2*C*), accounted for 2% of the radioactivity in the cells. Product Q, which was present in the control cells, was not detected in the cells incubated with DCU. Thus, taken together, these results indicate that after a 4-h incubation of endothelial cells with 14,15-EET, most of the incorporated radioactivity is in the form of metabolites of 14,15-EET, and the profile of incorporated metabolites is altered by inhibition of sEH.

Identification of Metabolites in Cells—The in-source CID mass spectra of metabolites T, S, and Q and their deuterated analogs obtained from the PCEC lipids in a corresponding incubation with [$^2H_8$]14,15-EET are shown in Fig. 7. Although an in-source CID mass spectrum of metabolite R also was obtained, we have not been able to assign a structure for this compound.

The fragmentations of the $^2H_8$-labeled products confirm the assignments to the fragment ions in the spectra of the 14,15-EET metabolites. Specifically, a comparison of the in-source CID mass spectrum of metabolite T (Fig. 7*A*) and its deuterated counterpart (Fig. 7*B*) indicates that the masses of the $(M - H)^-$ ions (*m*/*z* 347 and 355) differ by 8 Da. The fragment ions corresponding to the loss of water (*m*/*z* 329 and 337) also differ by 8 Da, but the fragment ions that are the result of carbon chain cleavage at the epoxide ring (*m*/*z* 247 and 254) differ by only 7 Da. Thus, the structure of metabolite T is consistent with 16,17-epoxydocosatrienoic acid (16,17-epoxy-22:3), the chain elongation product of 14,15-EET. Both the in-source CID mass spectra of metabolite S (Fig. 7*C*) and its deuterated analog (Fig. 7*D*) show abundant $(M - H)^-$ ions of *m*/*z* 321 and 329, ions due to loss of water (*m*/*z* 303 and 311), and ions due to carbon chain cleavage at the oxirane ring (*m*/*z* 221 and 228). These data suggest a structure of 14,15-epoxyeicosadienoic acid (14,15-epoxy-20:2).

The fragmentation pattern of metabolite Q differs from that of the other products. The $(M - H)^-$ ions (*m*/*z* 365 and 373) fragment to lose first one molecule of water and then another (*m*/*z* 347 and 329 in Fig. 7*E*; *m*/*z* 355 and 337 in Fig. 7*F*, the corresponding deuterated derivative). The mass difference between fragment ions, which in the previous spectra was thought to indicate epoxide groups, is only 6 Da (*m*/*z* 235 and 241 in Fig. 7, *E* and *F*, respectively), indicating that the metabolite is not an epoxy fatty acid. However, it is consistent

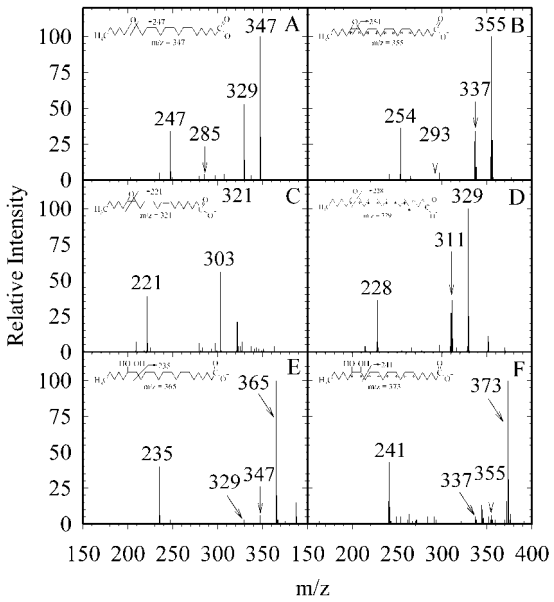

FIG. 7. **Mass spectra of 14,15-EET metabolites and their deuterated analogs contained in the cell lipids.** The incubation conditions and lipid extraction from the PCEC were the same as described in Fig. 6, and the mass spectra were obtained by LC/MS with in-source CID. Deuterated compounds were obtained from the hydrolyzed cell lipid extract following incubation of PCEC cultures with [$^2H_8$]14,15-EET. The mass spectra correspond to the following: *A*, compound T; *B*, the deuterated analog of compound T; *C*, compound S; *D*, the deuterated analog of compound S; *E*, compound Q; and *F*, the deuterated analog of compound Q (the compounds are designated as indicated in Fig. 6). The * in the structural diagrams of the deuterated compounds indicates the carbons that contain ^2H.

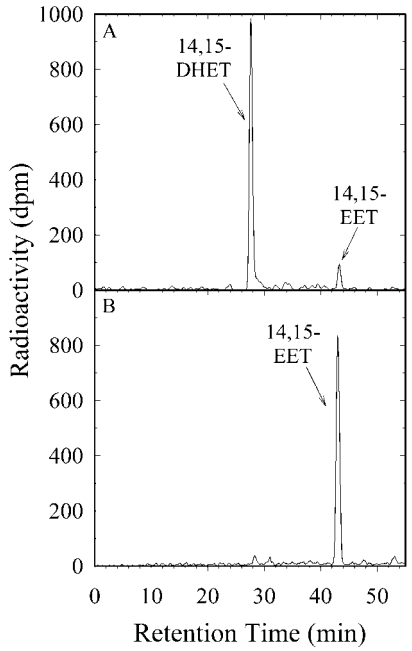

FIG. 8. **Effect of DCU on radiolabeled products released into the medium in response to calcium ionophore A23187.** The PCEC were pretreated with vehicle or 3 μM DCU for 45 min and then incubated with 2 μM [^3H]14,15-EET for 4.5 h as described in Fig. 1. After washing, the radiolabeled cells were incubated for an additional 20 min with 2 μM A23187, without (*A*) or with (*B*) 3 μM DCU. The medium was collected and assayed for radiolabeled products as described in Fig. 1. Radiochromatograms from a single culture are shown, but similar results were obtained from two additional cultures in each case.

with a structure of 16,17-dihydroxydocosatrienoic acid (16,17-diOH-22:3) (34), the chain elongation product of 14,15-DHET.

These findings show that 14,15-EET can be elongated by endothelial cells and that the process is augmented in the presence of sEH inhibition. Moreover, the results indicate that elongation products of 14,15-EET and its metabolites can be incorporated into endothelial phospholipids.

Effect of sEH Inhibition on EET Release from Cells—Previous studies indicated that PCEC rapidly release some of the EET incorporated in cell phospholipids if the cultures are incubated with a calcium ionophore (9). The material is released into the medium as the free acid (9, 21). Since sEH inhibition increased the incorporation of 14,15-EET and altered the distribution of 14,15-EET metabolites contained in the PCEC lipids, we investigated whether the amount or type of products released would be affected by the presence of DCU. PCEC cultures were incubated for 4.5 h with 2 μM [^3H]14,15-EET, with or without 3 μM DCU. After the medium was removed, the cultures were washed and incubated for 20 min in medium containing 2 μM ionophore A23187. The PCEC treated with DCU released 4.1 times more radiolabeled products (128 $\pm$ 5 pmol as compared with 31 $\pm$ 2 pmol, $n = 3$, $p < 0.05$; the pmol values are calculated from the specific radioactivity of the [^3H]14,15-EET used to load the cells). The radiolabeled material was present in the medium as the free acid. The greater release is consistent with the fact that the PCEC treated with DCU contained 4.8 times more radiolabeled material in phospholipids at the start of the incubation with the calcium ionophore.

HPLC analysis of the medium was done to determine whether DCU also affected the composition of the radiolabeled material released from the cells (Fig. 8). Only 5% of the radioactivity released by the control cultures remained as 14,15-EET during the 20 min of incubation with the ionophore, and 90% was converted to DHET (Fig. 8*A*). By contrast, 95% of the radioactivity released by the PCEC treated with 3 μM DCU remained as EET (Fig. 8*B*).

Effect of DCU on 11,12-EET Metabolism—Additional studies were done with 11,12-EET to determine whether DCU would produce similar effects with another EET regioisomer. HPLC data showing the effects of 3 μM DCU on the radiolabeled compounds present in the medium during incubation of PCEC with 2 μM [^3H]11,12-EET are shown in Fig. 9. 11,12-DHET accounted for 21% of the radioactivity contained in the medium of control cultures after 75 min of incubation, and 65% remained as EET (Fig. 9*A*). DCU reduced the conversion of 11,12-EET to 11,12-DHET. After 75 min, DHET accounted for only 5% of the radiolabeled material in the medium when DCU was present (Fig. 9*B*). 11,12-DHET accounted for 60% of the radioactivity in the medium when the incubation was extended to 4.5 h, and only 3% remained as 11,12-EET (Fig. 9*C*). When DCU was present throughout the 4.5-h incubation, 30% of the medium radioactivity remained as 11,12-EET, and only 20% was converted to 11,12-DHET (Fig. 9*D*). In addition, a metabolite designated J with a retention time of 32 min accounted for 5% of the radioactivity in the 75 min of incubation with DCU. The amount of metabolite J increased to 20% after 4.5 h. LC/MS analysis of compound J with in-source CID indicated a structure of 7,8-epoxyhexadecadienoic acid (7,8-epoxy-16:2) (data not shown).

HPLC analysis of the hydrolyzed cell lipids at the end of the 4-h incubation indicated that the control cultures contained 440 $\pm$ 8 pmol of [^3H]11,12-EET ($n = 3$), whereas the cultures incubated with 3 μM DCU contained 640 $\pm$ 13 pmol, an increase of 45% ($p < 0.05$).

Effect of DCU on AA Metabolism—We also examined the effect of DCU on AA uptake to determine whether the increased incorporation of EETs into PCEC lipids might be due to an effect of DCU unrelated to sEH inhibition. PCEC cultures

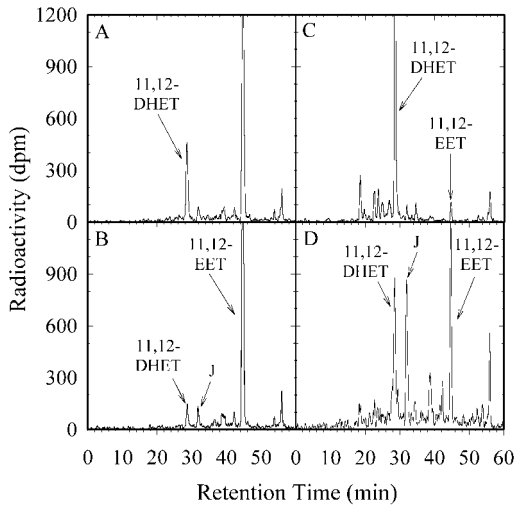

FIG. 9. **Effect of DCU on [^{3}H]11,12-EET products present in the culture medium.** PCEC were incubated as described in Fig. 1, except that the radiolabeled substrate was 2 μM [^{3}H]11,12-EET. The incubations were for either 75 min (*left panels*) or 4.5 h (*right panels*), and the medium was analyzed for radioactivity by HPLC with an on-line flow scintillation counter. Radiochromatograms from a single culture are shown, but similar results were obtained from two additional cultures in each case: *A*, 75 min control; *B*, 75 min with 3 μM DCU; *C*, 4.5 h control; *D*, 4.5 h with 3 μM DCU.

were incubated for 4 h with 2 μM [^{3}H]AA and 3 μM DCU, and control cultures were similarly incubated without DCU. Treatment with DCU did not appreciably alter the amount of [^{3}H]AA incorporated into the cells (1090 $\pm$ 54 pmol in the control cultures as compared with 1260 $\pm$ 51 pmol in those treated with DCU, $n = 3$, $p > 0.05$). Furthermore, about 45% of radioactivity remaining in the medium of both cultures was converted to prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$, indicating that DCU did not affect the production or release of AA metabolites from the cells.

DISCUSSION

In the present study, we identified three distinct but interactive pathways of EET metabolism in coronary endothelial cells: (*a*) conversion of 14,15-EET to 14,15-DHET by sEH is the prevailing pathway; (*b*) both 14,15-EET and 14,15-DHET can be incorporated into membrane phospholipids and converted to elongation products; (*c*) when the sEH is inhibited, 14,15-EET is converted to chain-shortened epoxide metabolites through a β-oxidation process, and the β-oxidation products can be further metabolized to elongation products.

In a previous study, we showed that treatment of endothelial cells with 4-PCO blocked conversion of 14,15-EET to 14,15-DHET but did not stimulate the formation of β-oxidation products in the medium or elongation products in the cells (21). The most likely reason that these novel pathways of 14,15-EET metabolism were detected in the present study is that DCU is a more effective and longer lasting inhibitor of sEH than 4-PCO. This permitted the incubations to be carried out for up to 8 h in the present study, as compared with 1 h in the study with 4-PCO. Because 4-PCO produces transient inhibition of epoxide hydrolase, and as a benzylic epoxide 4-PCO is somewhat unstable, especially in the presence of glutathione (18, 35), the compound proved to be of limited utility in elucidating the effects of epoxide hydrolase inhibition on EET metabolism and incorporation. In contrast, DCU, a substituted urea derivative, is a potent, selective inhibitor of recombinant human and murine sEH (18). Also, DCU is a slowly reversible sEH inhibitor that has a duration of action long enough to be useful *in vivo*. Therefore, DCU was suitable to perform a detailed analysis of the effects of sEH inhibition on endothelial EET metabolism.

When the endothelial sEH was inhibited by DCU, 14,15- and 11,12-EET were converted to chain-shortened epoxy-fatty acids. These products accumulated primarily in the extracellular fluid. No such products have been identified in any previous studies in which endothelial cells were incubated with EETs. Similar EET metabolites were, however, observed in a previous study with human skin fibroblasts, and data with mutant fibroblasts indicated that they are formed by peroxisomal β-oxidation (29). The initial metabolites that accumulated in the medium, 10,11-epoxy-16:2 from 14,15-EET and 7,8-epoxy-16:2 from 11,12-EET, are formed after two β-oxidation cycles (36, 37). These compounds contain a $\Delta^{4,5}$ double bond that must be removed through the action of 2,4-dienoyl CoA reductase before β-oxidation can continue (37). This is presumed to be a slow step that allows some of the 16-carbon CoA intermediates to accumulate, be hydrolyzed by a thiolase, and be released from the cells as the fatty acid (29, 38, 39). β-Oxidation eventually continued as indicated by the subsequent decline of 10,11-epoxy-16:2 and accumulation of 8,9-epoxy-14:1, a process that also occurs during the β-oxidation of arachidonic acid (38, 40). Although the β-oxidation should proceed further (37), we could not detect any additional chain-shortened metabolites. This may be because the incubations were not carried out for a long enough period or these products are degraded too rapidly to accumulate.

Cultured human skin fibroblasts contain very little epoxide hydrolase activity and convert only trace amounts of EETs to DHETs (29). Based on this, we conclude that β-oxidation is an alternative pathway for EET metabolism that occurs when conversion to DHET is limited. Peroxisomal β-oxidation is the main metabolic pathway in endothelial cells for other types of fatty acid biomediators, including 15- and 12-hydroxyeicosatetraenoic acid (HETE) and 13-hydroxyoctadecadienoic acid (41–43). However, the present results indicate that this is not the primary pathway for EET metabolism in the coronary endothelium, since appreciable amounts of EET are channeled into the β-oxidation pathway only when DHET formation is inhibited. Channeling of 14,15-EET into the β-oxidation pathway also occurred with *N*-cyclohexyl-*N*′-dodecylurea, a urea derivative with different substituents than DCU that is approximately ten times more potent against recombinant human and mouse sEH (data not shown). Although EET β-oxidation might function as a degradative pathway, it is distinctly possible that one or more of the epoxy-fatty acid products could have metabolic or functional effects. In support of this possibility, the β-oxidation product of 11,12-DHET was found to produce vasodilation of porcine coronary arteries (25).

The present findings also demonstrate for the first time that 14,15-EET and its derivatives can undergo chain elongation by endothelial cells. Three of the products formed by chain elongation were identified by LC/MS. 16,17-Epoxy-22:3, the elongation product of 14,15-EET, was formed by the control cultures but accumulated in increased amounts in PCEC treated with DCU. Thus, as observed regarding β-oxidation, more 14,15-EET is channeled into the elongation pathway when sEH is inhibited, and conversion to 14,15-DHET is decreased. A small amount of 14,15-epoxy-20:2 also accumulated when the cultures were treated with DCU. This product probably was formed by elongation of 10,11-epoxy-18:2, the 18-carbon intermediate produced by β-oxidation. Although not observed by HPLC, we detected the formation of very small amounts of 12,13-epoxy-18:2 and deuterated 12,13-epoxy-18:2 by LC/MS.[3] Like the mass spectrum of deuterated 14,15-epoxy-20:2 (Fig. 7*D*), the mass spectrum of the deuterated 10,11-epoxy-18:2

[3] T. L. Kaduce, L. M. Teesch, X. Fang, and A. A. Spector, unpublished observations.

contained a $(M - H)^-$ ion with 8 deuterium atoms, whereas the $(M - H)^-$ ion of deuterated 8,9-epoxy-16:2 contains only 7 deuterium atoms (Fig. 3*D*). Therefore, analogous to the formation of eicosatrienoic acid from arachidonic acid (44), 14,15-epoxy-20:2 appears to be formed primarily by conversion of 14,15-EET to an 18-carbon intermediate that is subsequently elongated. The 14,15-DHET elongation product, 16,17-dihydroxy-22:3, also was detected in small amounts in the control cells, demonstrating that the coronary endothelium is capable of elongating DHETs when they accumulate. No information is presently available as to whether any of these products have biological activity.

In addition to blocking conversion to 14,15-DHET and enhancing the formation of β-oxidation and elongation products of 14,15-EET, treatment with DCU resulted in a marked and sustained accumulation of 14,15-EET and its metabolites in endothelial phospholipids. Such findings likely help to explain recent reports that targeted disruption of the sEH gene lowers systolic blood pressure in male mice (17), and that a substantial decrease in blood pressure occurs when spontaneously hypertensive rats are treated with DCU (19). For example, incorporation of EETs may alter the membrane properties of the endothelium or, because the increase occurs in PC and PI, affect signaling pathways within the endothelium. Furthermore, the augmented release of EETs following stimulation with calcium ionophore in DCU-treated cells may prolong smooth muscle hyperpolarization and thereby enhance endothelium-dependent vasorelaxation (9). These findings may provide novel insight into the cellular mechanisms by which pharmacological or molecular inhibition of sEH can effectively treat hypertension (17, 19).

The novel metabolites that were incorporated into the cells treated with DCU, including 16,17-epoxy-22:3 which was the most abundant product, were not released from the cells in response to stimulation with a calcium ionophore. Thus, 95% of the radioactivity released when the DCU-treated cells were incubated with the calcium ionophore was in the form of 14,15-EET. The reason that the EET metabolites were not released from the cells is unclear. However, given their apparent propensity to be retained in endothelial phospholipids, accumulation of these compounds could have an important modulating effect on cell signaling pathways.

As noted with 14,15-EET, the conversion of 11,12-EET to 11,12-DHET was reduced when DCU was present. More 11,12-EET accumulated in the PCEC, but the magnitude of the increase in intracellular 11,12-EET content was considerably smaller than that with 14,15-EET under these conditions. This probably is due to the fact that the endothelial sEH was less active against 11,12-EET than with 14,15-EET, a finding consistent with enzymatic results and data from the spontaneously hypertensive rat indicating that 14,15-EET is a better substrate than 11,12-EET for sEH (13, 19). The reduced metabolism of 11,12-EET by endothelial sEH may account for the fact that it has more potent anti-inflammatory properties in the vascular wall than 14,15-EET (10).

In summary, these findings suggest that in addition to being metabolized by sEH, EETs are substrates for β-oxidation and chain elongation pathways present in endothelial cells. The observation that diol metabolites and β-oxidation products of the epoxides also can undergo chain elongation suggests that there is considerable interaction among the three metabolic pathways. The emergence of β-oxidation and chain elongation when sEH is inhibited suggests that these processes function primarily as alternative pathways of EET metabolism. Whereas the β-oxidation products are largely excreted from the cells, the elongation products are predominantly incorporated into endothelial phospholipids. The modulation of EET metabolism, incorporation, and release by sEH inhibition in the endothelium could help to explain the reductions in blood pressure produced by DCU in spontaneously hypertensive rats and by sEH-knockout in male mice (17, 19).

REFERENCES

1. Capdevila, J. H., Falck, J. R., and Harris, R. C. (2000) *J. Lipid Res.* **41,** 163–181
2. Harder, D. R., Campbell, W. B., and Roman, R. J. (1995) *J. Vasc. Res.* **32,** 79–92
3. Rosolowsky, M., and Campbell, W. B. (1996) *Biochim. Biophys. Acta* **1299,** 267–277
4. Campbell, W. B., Gebremedhin, D., Pratt, P. F., and Harder, D. R. (1996) *Circ. Res.* **78,** 415–423
5. Hu, S., and Kim, H. S. (1993) *Eur. J. Pharmacol.* **230,** 215–221
6. Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D. R., Fleming, I., and Busse, R. (1999) *Nature* **401,** 493–497
7. Quilley, J., and McGiff, J. (2000) *Trends Pharmacol. Sci.* **21,** 121–124
8. VanRollins, M., Kaduce, T. L., Knapp, H. R., and Spector, A. A. (1993) *J. Lipid Res.* **34,** 1931–1942
9. Weintraub, N. L., Fang, X., Kaduce, T. L., VanRollins, M., Chartterjee, P., and Spector, A. A. (1997) *Circ. Res.* **81,** 258–267
10. Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., Zeldin, D. C., and Liao, J. K. (1999) *Science* **285,** 1276–1279
11. Mombouli, J. V., Holzmann, S., Kostner, G. M., and Graier, W. F. (1999) *J. Cardiovasc. Pharmacol.* **33,** 779–784
12. Hoebel, B. G., and Graier, W. F. (1998) *Eur. J. Pharmacol.* **346,** 115–117
13. Chacos, N., Capdevila, J., Falck, J. R., Manna, S., Martin-Wixtrom, C., Gill, S. S., Hammock, B. D., and Estabrook, R. W. (1983) *Arch. Biochem. Biophys.* **223,** 639–648
14. Zeldin, D. C., Kobayashi, J., Falck, J. R., Winder, B. S., Hammock, B. D., Snapper, J. R., and Capdevila, J. H. (1993) *J. Biol. Chem.* **268,** 6402–6407
15. Guenthner, T. M., and Karnezis, T. A. (1986) *Drug Metab. Dispos.* **14,** 208–213
16. Meijer, J., and DePierre, J. W. (1988) *Chem. Biol. Interact.* **64,** 207–249
17. Sinal, C. J., Miyata, M., Tohkin, M., Nagata, K., Bend, J. R., and Gonzalez, F. J. (2000) *J. Biol. Chem.* **275,** 40504–40510
18. Morisseau, C., Goodrow, M. H., Dowdy, D., Zheng, J., Greene, J. F., Sanborn, J. R., and Hammock, B. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 8849–8854
19. Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A. J., Newman, J. W., Parker, C., Graham, L., Engler, M. M., Hammock, B. D., Zeldin, D. C., and Kroetz, D. L. (2000) *Circ. Res.* **87,** 992–998
20. Guenthner, T. M. (1986) *Biochem. Pharmacol.* **35,** 839–845
21. Weintraub, N. L., Fang, X., Kaduce, T. L., VanRollins, M., Chatterjee, P., and Spector, A. A. (1999) *Am. J. Physiol.* **277,** H2098–H2108
22. Carroll, M. A., Schwartzman, M., Capdevila, J., Falck, J. R., and McGiff, J. C. (1987) *Eur. J. Pharmacol.* **138,** 281–283
23. Proctor, K. G., Falck, J. R., and Capdevila, J. (1987) *Circ. Res.* **60,** 50–59
24. Pfister, S. L., Falck, J. R., and Campbell, W. B. (1991) *Am. J. Physiol.* **261,** H843–H852
25. Fang, X., Kaduce, T. L., Weintraub, N. L., VanRollins, M., and Spector, A. A. (1996) *Circ. Res.* **79,** 784–793
26. Oltman, C. L., Weintraub, N. L., VanRollins, M., and Dellsperger, K. C. (1998) *Circ. Res.* **83,** 932–939
27. VanRollins, M., Kaduce, T. L., Fang, X., Knapp, H. R., and Spector, A. A. (1996) *J. Biol. Chem.* **271,** 14001–14009
28. Falck, J. R., Yadagiri, P., and Capdevila, J. (1990) *Methods Enzymol.* **187,** 357–365
29. Fang, X., Kaduce, T. L., VanRollins, M., Weintraub, N. L., and Spector, A. A. (2000) *J. Lipid Res.* **41,** 66–74
30. Fang, X., VanRollins, M., Kaduce, T. L., and Spector, A. A. (1995) *J. Lipid Res.* **36,** 1236–1246
31. Bernstrom, K., Kayganich, K., and Murphy, R. C. (1991) *Anal. Biochem.* **198,** 203–211
32. Borhan, B., Mebrahtu, T., Nazarian, S., Kurth, M. J., and Hammock, B. D. (1995) *Anal. Biochem.* **231,** 188–200
33. Adams, J., and Gross, M. L. (1988) *Org. Mass Spectrom.* **23,** 307–316
34. Bylund, J., Ericsson, J., and Oliw, E. H. (1998) *Anal. Biochem.* **265,** 55–68
35. Mullin, C. A., and Hammock, B. D. (1982) *Arch. Biochem. Biophys.* **216,** 423–439
36. Osmundsen, H., Bremer, J., and Pedersen, J. I. (1991) *Biochim. Biophys. Acta* **1085,** 141–158
37. Kunau, W.-H., Dommes, V., and Schulz, H. (1995) *Prog. Lipid Res.* **34,** 267–342
38. Gordon, J. S., Heller, S. K., Kaduce, T. L., and Spector, A. A. (1994) *J. Biol. Chem.* **269,** 4103–4109
39. Williard, D. E., Kaduce, T. L., Harmon, S. D., and Spector, A. A. (1998) *J. Lipid Res.* **39,** 978–986
40. Spector, A. A., Williard, D. E., Kaduce, T. L., and Gordon, J. A. (1997) *Prostaglandins Leukot. Essent. Fatty Acids* **57,** 101–105
41. Shen, X. Y., Figard, P. H., Kaduce, T. L., and Spector, A. A. (1988) *Biochemistry* **27,** 996–1004
42. Spector, A. A., Gordon, J. A., and Moore, S. A. (1988) *Prog. Lipid Res.* **27,** 271–323
43. Fang, X., Kaduce, T. L., and Spector, A. A. (1999) *J. Lipid Res.* **40,** 699–707
44. Spector, A. A., Williard, D. E., Kaduce, T. L., and Widstrom, R. L. (1999) *Prostaglandins Leukot. Essent. Fatty Acids* **60,** 377–382