Improved bioavailability of epoxyeicosatrienoic acids reduces TP-receptor agonist-induced tension in human bronchi

Farid Yannick Senouvo,1 Yacine Tabet,1 Caroline Morin,1 Roula Albadine,2 Chantal Sirois,3 and Eric Rousseau1

1Le Bilarium, Department of Physiology and Biophysics, 2Department of Pathology, and 3Service of Thoracic Surgery; Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada

Submitted 30 November 2010; accepted in final form 2 August 2011

INTRODUCTION

It has been well established that arachidonic acid (AA) produced from phospholipids by the cytoplasmic phospholipase A2 (PLA2) is metabolized by cyclooxygenases and lipoxigenases into biologically active mediators (11, 16). AA, however, can also be metabolized into epoxy-derivatives by cytochrome P-450 (CYP450) epoxygenase. Epoxyeicosatrienoic acid (EET) and thromboxane A2 are arachidonic acid derivatives. The former has initially been defined as an epithelium-derived hyperpolarizing factor displaying broncho-relaxing (4) and anti-inflammatory, vasodilating properties, as recently demonstrated (25), whereas thromboxane A2 induces vaso- and bronchoconstriction upon binding to thromboxane-prostanoid (TP)-receptor. EETs, however, are quickly degraded by the soluble epoxide hydrolase (sEH), which transforms EET into dihydroxy-eicosatrienoic acid (DHET) followed by 10.220.32.246 on June 12, 2017 http://ajplung.physiology.org/ Downloaded from

Am J Physiol Lung Cell Mol Physiol 301: L675–L682, 2011. First published August 5, 2011; doi:10.1152/ajplung.00427.2010.—Epoxyeicosatrienoic acid (EET) and thromboxane A2 are arachidonic acid derivatives. The former has initially been defined as an epithelium-derived hyperpolarizing factor displaying broncho-relaxing (4) and anti-inflammatory properties, as recently demonstrated (25), whereas thromboxane A2 induces vaso- and bronchoconstriction upon binding to thromboxane-prostanoid (TP)-receptor. EETs, however, are quickly degraded by the soluble epoxide hydrolase (sEH), which transforms EET into dihydroxy-eicosatrienoic acid (DHET) followed by addition of a water (H2O) molecule (5, 6, 11). The aim of this study was to investigate the effects of 14,15-EET on TP-receptor activation in human bronchi. Tension measurements performed on native bronchi from various species, acutely treated with increasing 14,15-EET concentrations, revealed specific and concentration-dependent relationships as well as a decrease in the tension induced by 30 nM U-46619, used as a synthetic TP-receptor agonist. Interestingly, acute treatments with 3 μM N-(methylsulfonyl)-2-(2-propynyloxy)-benzenethanamide, an epoxyenase inhibitor, which minimizes endogenous production of EET, resulted in an increased reactivity to U-46619. Furthermore, we demonstrated that chronic treatments with trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB), a sEH inhibitor, reduced human bronchi reactivity to U-46619. During our tension measurements, we also observed that human bronchi generated small-amplitude contractions; these spontaneous activities were reduced upon acute 14,15-EET treatments in the presence of t-AUCB. Altogether, these data demonstrate that endogenous and exogenous 14,15-EET could interfere with the activation of TP-receptors as well as with spontaneous oscillations in human airway smooth muscle tissues.

airway smooth muscle; N-(methylsulfonyl)-2-(2-propynyloxy)-benzenethanamide; U-46619; thromboxane A2; trans-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid; thromboxane-prostanoid-receptor

IT HAS BEEN WELL ESTABLISHED that arachidonic acid (AA) produced from phospholipids by the cytoplasmic phospholipase A2 (PLA2) is metabolized by cyclooxygenases and lipoxigenases into biologically active mediators (11, 16). AA, however, can also be metabolized into epoxy-derivatives by cytochrome P-450 (CYP450) epoxygenase. Epoxyeicosanoids have important roles in the regulation of vascular, pulmonary, cardiac, and renal functions (5, 6, 12, 16). The mechanisms behind these effects are still unclear. Nevertheless, the mode of action of epoxyeicosanoids, and other congeners, may involve the activation of surface membrane K+ channels and interaction with intracellular processes, which result in membrane hyperpolarization and relaxation (24).

Among the four existing regioisomers (5,6-; 8,9-; 11,12-; and 14,15-EET) produced by CYP450 isoforms 2C9 and 2J2, 14,15 epoxyeicosatrienoic acid (14,15-EET) is the most abundant in lung tissues (6, 28, 38). 14,15-EET was shown to trigger bronchorelaxing responses because its mode of action involves the activation of large-conductance, calcium-sensitive potassium channels (BKCa), which hyperpolarizes airway smooth muscle (ASM) cell membranes, thus promoting relaxation (2, 4, 24, 36, 38). 14,15-EET also displayed anti-inflammatory effects, which were likely attributable to specific interaction with peroxisome proliferated activated receptor-γ, a pathway known to reduce the proinflammatory cytokines and tissue responses (6, 18, 20, 26).

The beneficial effects of EET regioisomers are regulated by the action of a soluble epoxy hydrolase (sEH), which transforms EET into dihydroxy-eicosatrienoic acid (DHET) following the hydroxylation of the epoxy group into diol upon dissociation and addition of a water (H2O) molecule (5, 6, 11). It has been shown that DHET compounds are rather inactive metabolites compared with their corresponding epoxy-congeners (38).

sEH is a 55-kDa homodimer protein, expressed in liver, endothelium, vascular, and ASM cells (8). This enzyme displays two catalytic sites: one on its NH2-terminal side, displaying a lipid phosphatase activity, and the other on its COOH-terminal site responsible for the epoxy hydrolase activity (17). Presently available sEH inhibitors target the COOH-terminal site (13, 34). Actually, this soluble epoxy hydrolase isoform, which limits the bioavailability of epoxy compounds derived from ω6 AA, could also inactivate epoxy-derivatives from ω3 polysaturated fatty acids (10).

As mentioned earlier, AA can be used as a substrate by cyclooxygenase for prostaglandin synthesis. Thromboxane A2 (TXA2) synthase can then use these prostaglandins to produce TXA2, a high-affinity lipid mediator that triggers potent vaso- and broncho-constrictions (3, 33). These effects are directly mediated by the activation of the thromboxane-prostanoid receptor (TP-receptor), a G protein-coupled receptor (27). TXA2-binding to its receptor will trigger the activation of PLC, which will promote Ca2+ release from the endoplasmic reticulum Ca2+ stores. Ca2+ binding to calmodulin will, in turn, promote activation of the myosin light chain (MLC) kinase and phosphorylation of MLC, which will result in tension increases. Activation of TP-receptor will also promote RhoA-GTP activation of Rho-kinase. This will lead to the
inhibition of the MLC phosphatase, allowing long-lasting contractions (21).

A single study has pointed out putative interactions between 14,15-EET and TP-receptor activation in the vascular system (3). Because of their structural similarities, EET regioisomers and TXA2 agonists might bind to TP-receptors (3). Alternatively, 14,15-EET could interact downstream of the TP-receptor complex. The exact mechanism is still unknown and has never been assessed in lung tissues. We hypothesized that increasing bioavailability of 14,15-EET could prevent TP-receptor activation. In this study, we assessed whether 14,15-EET was able to decrease the tension induced by TP-receptor activation. Complementary approaches were used to perform 1) tension measurements on human, guinea pig, and rat bronchi as well as mice trachea and 2) immunodetection of human sEH with Western blot analysis and immunofluorescence. Herein, we report evidence that the beneficial effects of 14,15-EET on lung tissues involve interactions on TP receptors.

MATERIALS AND METHODS

Tissue preparation and culture. The study was approved by the institutional ethics committee at the University of Sherbrooke (protocol number 05 088 S1-M2). Human lung tissues were obtained from patients undergoing surgery for lung carcinoma who provided informed written consent. Inclusion criteria allow recruitment of patients undergoing a lobectomy for a single adenocarcinoma, whereas patients with any type of infectious disease were excluded by the medical team. The lung specimens were collected in the pathology department and transported in sterile saline solution, and a rest section distant from the carcinoma was dissected by the pathologist. Tissues samples were placed in a cold Krebs solution containing (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, and 11.1 dextrose, at pH 7.4 and transported to a level two culture room. Bronchial rings (inner diameter of 0.5-0.8 mm) were microdissected from the same bronchial segment and placed in individual wells of 24-well culture plates containing DMEM-F12 culture medium (1 mL/well) with 1% penicillin (10 IU/ml) and streptomycin (100 µg/ml). Culture plates were kept in a humidified incubator at 37°C under 5% CO2. Bronchial explants were untreated (control) or treated every 12 h during 48 h with either 10 nM trans-4-[4-(3-adamantan-1-yl-ureido)cyclohexyloxy]-benzoic acid (t-AUCB) alone or 10 nM t-AUCB combined with 300 nM 14,15-EET before pharmacological challenges. Some bronchi were used as fresh tissues for acute pharmacological experiments.

Mechanical tension measurements. Mechanical effects induced by specific agonists on human, guinea pig, and rat bronchial rings as well as on mice tracheal rings were measured as previously described (24). Rings were mounted in isolated organ baths containing Krebs solution thermostated at 37°C and bubbled continuously with carbogen (95% O2-5% CO2). An initial load of 0.8 g was applied to each ring during a 30-min equilibration period with three washouts. Mechanical tensions were assessed using transducer systems (Radnoti Glass Technology, Monrovia, CA) coupled to Polyview software (Grass-AstroMed, West Warwick, RI) to facilitate data acquisition and analysis. Protein sample preparation. Mice tracheas and parenchyma, rat tracheas, as well as human bronchial rings and pulmonary arteries were transferred separately to a buffer A solution containing: 0.3 M sucrose, 20 mM K-PIPES, 4 mM EGTA, and a cocktail of protease inhibitors. Cytosolic and microsomal fractions were prepared from human lung parenchyma (10 g/50 ml of buffer A), as well as guinea pig tracheas homogenized and ultracentrifuged at 85,000 g in the same buffer A solution as mentioned above. Microsomal fractions were kept in a cryoprotective buffer containing 0.3 M sucrose, 5 mM K-PIPES, and protease inhibitors. Cytosolic and microsomal fractions were frozen in liquid nitrogen as well and stored at −80°C.

SDS-PAGE and Western blot analysis. Protein samples (25 µg of protein/well) were dissolved in 2% SDS and separated by 10% SDS-PAGE with a 4% stacking gel. Gels were cast into a Mini-PROTEAN III dual cell (Bio-Rad, Mississauga, ON, Canada). The separated proteins from SDS-PAGE were electrophoretically transferred at 70 V onto nitrocellulose membranes (Bio-Rad) for 1 h at 4°C using the Mini-PROTEAN III system (Bio-Rad). Membranes were blocked with Tris-buff ered solution containing 0.1% Tween 20 (TBS-T) and 5% nonfat milk powder for 2 h at room temperature and incubated overnight with a specific primary antibody against human sEH raised from rabbits diluted in TBS-T + 5% milk. After three washings, membranes were incubated with peroxidase-conjugated donkey anti-rabbit IgG1 antiserum (Amersham, Baie d’Urfé, PQ, Canada) for 1 h at room temperature. Immunoblotting was revealed using an enhanced chemiluminescence kit (Roche).

Immunofluorescence. Thin sections from normal human bronchi were cryoprotected after snap freeze in optimal cutting temperature compound. Tissues were fixed in 10% formaldehyde for 30 min before being treated with 100 mM Glycine for 45 min to quench signal saturation. Tissue permeabilization was performed with 0.2% Triton X-100 in PBS. Thin sections were blocked in PBS + 0.5% goat serum for 1 h at 37°C. Slides were incubated for 1 h with rabbit anti-sEH antibody diluted in PBS + 0.5% goat serum, washed for 5 min, and incubated for another hour with mouse anti-α-actin diluted in PBS + 0.5% goat serum. After two washings, slides were incubated with Alexa-Fluor 488-conjugated anti-mouse IgG1 antiserum and Alexa-Fluor 647-conjugated anti-rabbit IgG1 antiserum. Nuclei were stained with DAPI diluted 1:1,000 in water, washed, and mounted with Vectashield. The slides were then viewed with an inverted microscope IX81 coupled to the FV1000 confocal from Olympus.

Immunoblotting. Tissue permeabilization was performed with 0.2% Triton X-100 in PBS. Thin sections were blocked in PBS + 0.5% goat serum for 1 h at 37°C. Slides were incubated for 1 h with rabbit anti-sEH antibody diluted in PBS + 0.5% goat serum, washed for 5 min, and incubated for another hour with mouse anti-α-actin diluted in PBS + 0.5% goat serum. After two washings, slides were incubated with Alexa-Fluor 488-conjugated anti-mouse IgG1 antiserum and Alexa-Fluor 647-conjugated anti-rabbit IgG1 antiserum. Nuclei were stained with DAPI diluted 1:1,000 in water, washed, and mounted with Vectashield. The slides were then viewed with an inverted microscope IX81 coupled to the FV1000 confocal from Olympus.

Results are expressed as means ± SE, with n indicating the number of experiments. Statistical analyses were performed with the Student’s t-test or one-way ANOVA. Differences were considered significant when P < 0.05. Data curve fittings were performed with Sigma Plot 9.0 (SPSS-Science, Chicago, IL) to determine IC50 values, whenever possible.

RESULTS

Sample population. Forty-two patients with pulmonary adenocarcinomas were met with and recruited for the present study. Among the 42 lung resections, 30 tissue samples (specimens) were suitable for tension measurements and used in the experiments reported of the present study.

14,15-EET relaxing effects in bronchi are different among mammalian species. Tension measurements were performed on human, rat, and guinea pig bronchi as well as on mice tracheas to assess their relative sensitivity to 14,15-EET. An initial load of 0.8 g was applied to each tissue as resting tone. All tissues were precontracted with 1-µM MCh, and concentration-response curves to 14,15-EET were then performed, resulting in concentration-dependant relaxations of various amplitudes. IC50 values of 0.45, 1.13, and 6.7 µM for 14,15-EET were obtained on humans, mice, and guinea pigs, respec-
tively (Fig. 1). Although the IC$_{50}$ for 14,15-EET could not be determined on rat bronchi, the data showed a low sensitivity of rat bronchi to 14,15-EET. These results suggest that 14,15-EET is a bronchodilating agent whose effectiveness varies among species.

Expression of sEH is endogenous in mammalian species. Figure 2A displays the Western blot analysis of sEH performed on protein samples prepared from human, rat, mouse, and guinea pig pulmonary tissues. Protein samples were migrated as follows: cytosolic and microsomal fractions derived from human lung parenchyma, bronchi, and pulmonary artery homogenates, rat and mouse trachea homogenates as well as guinea pig parenchyma cytosolic and microsomal fractions. Anti-sEH immunoreactive bands were detected at 55 kDa in each sample, except in rat trachea homogenate. The absence of immunoreactive bands in rat tissue was ascribed to a lack of crossreactivity with the specific primary antibody used (22). Note that the sEH immunostaining was higher in the cytosolic fraction from human parenchyma than in the corresponding microsomal fraction (Fig. 2, lane 1 vs. lane 2). Immunoreactive bands of identical relative molecular weight were detected in human bronchi as well as pulmonary artery homogenates. Some homogenates display immunoreactive bands of lower relative molecular weight, which might result from alternative splicing, as suggested by Przybyla-Zawislak in 2003 (30). Immunolocalization of the human sEH was performed on thin sections from human bronchi (Fig. 2B). sEH was detected in smooth muscle cell cytoplasm and adventitia along with α-actin. Together, these results suggest that the sEH is highly expressed in the cytoplasm of human and mouse ASM tissues.

Effects of 14,15-EET on TP-receptor activation. Tension measurements were performed on human bronchi (Fig. 3A) and mouse trachea (Fig. 3B). Preincubations with increasing 14,15-EET and 8,9-EET concentrations (ranging from 10 nM to 1 µM) were performed 10 min before challenges with a single concentration of U-46619 (30 nM) to assess the effects of the epoxyeicosanoid regioisomers on the tonic responses induced by TP-receptor activation. Preincubation with 1 µM 14,15-EET resulted in a 51% inhibition of the tension induced by U-46619 in human and mouse ASM tissues (Fig. 3, A and B, respectively). This inhibition was dependent on 14,15-EET concentration; hence 1 µM 8,9-EET regioisomer displayed a similar inhibitory effect (47%) on tone developed by 30 nM U-46619 on human bronchi (Fig. 3A). Together, these data suggest that TP-receptor activation is impaired in the presence of submicromolar concentrations of 8,9- and 14,15-EET. Thus inhibition of endogenous EET production may enhance the tonic response to TP-receptor agonists.

Acute epoxygenase inhibition. Human bronchi subjected to a basal tone of 0.8 g were sequentially challenged with 1 µM MCh and 30 nM U-46619 and then acutely treated with 3-µM methylsulfonyl-propargyloxy-phenylhexanamide (MS-PPOH), a CYP450 epoxygenase inhibitor, for 15 min before a final challenge with 30 nM U-46619. As shown in the bar histograms (Fig. 4B), the mean tension induced by U-46619 increased to 0.45 ± 0.07 g in the presence of MS-PPOH compared with the mean tension (0.35 ± 0.07 g) measured under control conditions. This suggests that the inhibition of CYP450 epoxygenase results in lower endogenous EET production, which, in turn, leads to larger tonic responses upon TP-receptor activation.

Effects of acute sEH inhibition on bronchospasms. Figure 6A displays a typical recording of spontaneous contractile activities from human bronchi. Acute treatment with 10 nM t-AUCB (Fig. 6A) slightly modified the frequency of these spontaneous contractile activities, whereas the addition of 1 µM 14,15-EET consistently abolished the spasmogenic activities. These spontaneous activities were usually overridden during pharmacological challenges with either 1 µM MCh, 1 µM histamine, or 30 nM U-46619 but systematically reappeared upon washouts with pharmacological agonists (data not shown).

Figure 6B illustrates that, in the presence of 10 µM 14,15 epoxyeicosanoic acid (14,15-EEZE), a 14,15-EET antagonist (25), and 10 nM t-AUCB, a higher concentration of 14,15-EET (3 µM) is required to block the oscillation pattern but resulted in a transient increase of the resting tone. Quantitative analyses of the area under the curve (AUC) are summarized in Fig. 6C (n > 10). Mean values demonstrate a slight decrease in spontaneous activity in the presence of t-AUCB, whereas exogenous addition of 10 µM 14,15-EEZE in the...
presence of t-AUCB enhanced the mean AUC attributable to an increase in oscillation frequency. Addition of 3 μM 14,15-EET reduced or abolished the spasmogenic oscillations. Note that spasmogenic activities were observed in small distal human bronchi (lower than 0.8 mm in diameter). Thus exogenous addition of 14,15-EET reduced the spasmogenic activities in human bronchi (Fig. 6A), whereas 14,15-EEZE antagonized the effects of exogenously added 14,15 EET and t-AUCB, a specific sEH inhibitor that would prevent endogenous epoxyeicosanoid degradation. The mechanism leading to the inhibition of spasmogenic activity by 14,15 EET is not yet resolved.

**DISCUSSION**

In this study, we examined the effects of 14,15-EET on the TP-receptor-induced tension on ASM from various mammalian lung tissues. However, this study has mainly been performed on fresh and 48-h cultured and pretreated human ASM tissues. We found that pretreatments with increasing concentrations of 14,15-EET or 8,9-EET could reduce U-46619-induced tension on both human bronchi and mouse trachea.

The most important finding of this study was that chronic treatments of human bronchi with t-AUCB for 48 h greatly reduced TP-receptor-induced tension. Hence, we demonstrated that reduced production (lower bioavailability) of endogenous EET following epoxygenase inhibition yielded larger tonic responses to TP-receptor challenges. Thus we propose that EET regioisomers, which are derived from AA metabolism, could play an important role in regulating TP-receptor responses from ASM. These epoxyeicosanoids could also abolish the spontaneous contractile activities.

**Human sample.** Bronchial rings were dissected from resection areas away from the tumor and were considered healthy because these areas were devoid of adenocarcinoma. Despite the scattering of the pharmacomechanical responses triggered on human bronchi, we were still able to obtain convincing data during the course of our investigations.

**Species-dependant variability in EET sensitivity.** It has previously been shown that 14,15-EET displays relaxing effects through activation of BK<sub>Ca</sub> channels and reduction of Ca<sup>2+</sup>-sensitivity (2, 25, 36, 38) on smooth muscle tissues from...
different mammals. According to the mean IC$_{50}$ values obtained in this study, however, we delineated major interspecies differences in 14,15-EET sensitivity in human, mice, and guinea pig pulmonary tissues. Mean IC$_{50}$ values derived from cumulative concentration-response curves suggest putative differences in receptor homology and affinity from one species to the other, which would translate into differences in signal transduction and tissue reactivity. Our present data, obtained under identical experimental conditions, would partially explain the discrepancy in EET potency in relaxing mammalian tissues found in the literature (9, 35, 37).

Concentration-dependent inhibitory effects of EET. As described in the literature, sEH can degrade epoxyeicosatrienoic acids, which are AA metabolites (31). TXA$_2$ is another AA metabolism produced by platelets, monocytes, macrophages, neutrophils, and lung parenchyma (32), whose constricting effects occur upon binding to its TP-receptor. A single study reported the ability of 14,15-EET to interact with the TP receptor in the vascular system (3). In this study, we investigated the concentration-dependent inhibitory effects of EET on human bronchi reactivity to a TP-receptor agonist.

Fig. 3. Inhibitory effects of 14,15-EET on U-46619 tonic responses in pulmonary tissues. A: quantitative analysis of the inhibitory effects induced by increasing concentrations of 14,15-EET and 8,9-EET preincubation on 30 nM of U-46619 challenges [thromboxane-prostanoid (TP)-receptor agonist] in human bronchi. B: same experiment performed on mouse tracheas using 14,15-EET. Each bar represents the mean ± SE (*P < 0.05, n = 5). Note that preincubation with 1 μM 14,15-EET and 8,9-EET prevented 51 and 47% of the tension induced by 30 nM U-46619 in human bronchi and 14,15-EET had basically the same effect on mouse tracheas.

$sEH$ detection and location. It is well known that the catalytic activity of sEH enzyme occurs through dissociation of a water molecule and concomitant dihydroxylation of the targeted substrate (8). sEH hydrolytic actions are selective to free fatty acid epoxides, such as epoxyeicosatrienoic acids (5, 14). In this study, we examined sEH expression in human, rat, mice, and guinea pig homogenate and subcellular fractions. sEH immunoreactive bands were detected in almost all tested samples. Human parenchymal cytosolic fraction as well as human bronchial homogenates displayed stronger sEH immunostaining bands. Other studies have reported sEH RNA expression in different tissues, including adipocytes (7, 29). Our data suggest that sEH is constitutively expressed in mammalian lung tissues. Moreover, we assessed the sEH location in human bronchi using an immunohistochemistry assay, which attested that sEH staining appeared mostly in smooth muscle cell cytoplasm. These data and the stronger sEH band in human parenchymal cytosolic fraction confirm that sEH is a soluble enzyme consistently found in lung tissues, including smooth muscle cells.

Fig. 4. 3-μM Methylsulfonyl-propargyloxy-phenylhexanamide (MS-PPOH) acute treatment modified the human bronchi reactivity to a TP-receptor agonist. A: illustration of human bronchi reactivity to 1 μM MCh and 30 nM U-46619 in the absence and presence of 3 μM MS-PPOH (a cytochrome P-450 epoxygenase inhibitor). B: quantitative analysis of human bronchi reactivity to 30 nM U-46619 in the presence of the epoxygenase inhibitor (MS-PPOH), induced a larger tension (n = 10). These results suggest that the production of endogenous EET reduced the active tone induced by a low concentration of U-46619.
gated the interaction of 14,15-EET with bronchial and tracheal smooth muscle TP-receptors in human and mice, respectively. In Keserü at al. (17), rat and mice smooth muscle conduits (thoracic aorta, tertiary bronchi) were bathed in a Krebs physiological solution and tested with 1 M U-46619 and 0.1–10 M 14,15-EET. Our study used 30 nM U-46619 in the same Krebs physiological solution. We demonstrated that pretreatments with 1 M 14,15-EET halved the U-46619-induced tension triggered by TP-receptor activation in both human bronchi and mice tracheas. This interaction could either be a competitive orthosteric binding, binding on an allosteric site, or even an interaction downstream of the receptor (27). Even if the precise mechanism is not yet known, many studies, including ours, have shown that 14,15-EET acts by interacting with the TP-receptor signaling in both vascular and pulmonary systems. Thus our data are consistent with the literature (3). Complementary 14,15-EET and U-46619 binding analyses on TP receptors would warrant a better definition of the apparent affinity constant.

**Dual effects of CYP-450 and sEH inhibitors.** CYP450 epoxygenase is the enzyme responsible for EET production. In human bronchi, the CYP450 2C9 isoform produces EET regioisomer, whereas, in mice tracheas, the CYP450 2J2 isoform appears to be responsible (1). Therefore, we assessed the effects of a treatment with MS-PPOH, a CYP450 epoxygenase inhibitor, on human bronchi. We demonstrated that bronchial reactivity to U-46619 was largely increased in the presence of MS-PPOH, compared with the responses with U-46619 alone as demonstrated in Fig. 4.

**Fig. 5. Effects of 48-h trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]benzoic acid (t-AUCB) pretreatments on tonic responses triggered by U-46619 in human bronchi.** Quantitative analysis of the effects of chronic treatments with or without 10 nM t-AUCB, a highly specific sEH inhibitor (H. J. Tsai, unpublished observations), in the absence or presence of 300 nM 14,15-EET on the tonic responses induced by 30 nM U-46619. All tensions were normalized to the responses induced by 1 M MCh (100% n = 16). Note that challenges with the TP-receptor agonist were reduced by 31.3% in the presence of t-AUCB. Addition of exogenous 14,15-EET during the culture period had no additive effect on bronchial reactivity.

**Fig. 6. Spasmolytic effect of exogenous 14,15-EET and 14,15 epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) on human bronchi.** A: spontaneous steady-state contractile activities recorded under control conditions in the presence of 10 nM t-AUCB and upon addition of 1 μM 14,15-EET. B: similar experiment performed in the presence of 10 μM 14,15-EEZE and 10 nM t-AUCB followed by the sequential addition of 1 and 3 μM 14,15-EET. C: quantitative analysis of the mean values of the area under the curve (AUC) under the same experimental conditions as reported above. Compared with control, spontaneous contractile activities were increased in the presence of 14,15-EEZE (middle bar), whereas they were decreased in the presence of exogenous 14,15-EET (right bar). */P < 0.05; **P < 0.01.
As previously reported, sEH degrades EET into DHET, which reduces their beneficial effects by decreasing their bioavailability. Earlier studies have assessed the effectiveness of sEH inhibitors such as t-AUCB and AUDA in preventing EET degradation (14, 18). Few, however, have used chronic treated pulmonary tissues with sEH inhibitors to test putative variations in ASM reactivity (19). Our study tested the effects of 48-h chronic treatment on human bronchi with t-AUCB in the absence or presence of exogenous 14,15-EET. The results obtained demonstrate that chronic treatments with the sEH inhibitor alone could reduce human bronchi reactivity to the TXA2 analog. The addition of exogenous 14,15-EET in chronic treatments did not potentiate the effects of the sEH inhibitors. Together, these data suggest that the modulation of endogenous EET production by CYP450 inhibitors and degradation by sEH inhibitors greatly affects human bronchi reactivity to TP-receptor activation.

Spasmolytic actions of exogenous EET on intracellular mechanisms. Distal human bronchi generate spasmogenic activities when mounted in isolated organ-bath systems. These spontaneous contractile activities, usually of low amplitude, are likely induced by variations in Ca2+ release from the intracellular Ca2+ pool (15). Hence, increases in free intracellular Ca2+ would, in turn, activate surface membrane Cl– and K+ currents, supporting spontaneously transient inward and outward currents, which would trigger changes in membrane voltage and low-amplitude contractions. Alternatively, they could also be explained by variations in myofilament Ca2+ sensitivity (24). We observed, however, no such changes in oscillation amplitude. It has been reported that EET and epoxy eicosatrienoic acid can modulate membrane conductance and Ca2+ sensitivity of ASM cells (24). In this study, we demonstrated the spasmolytic effects of exogenous 14,15-EET in the presence of sEH inhibitors. The specific sEH inhibitor (t-AUCB) was used to minimize degradation of endogenous epoxy derivatives, so the slight increase in oscillation frequency induced by t-AUCB was not expected. Nevertheless, exogenously added 14,15-EET abolished spontaneous bronchial oscillations, so our data further support the view that 14,15-EET modulates membrane conductance, which would minimize free intracellular Ca2+ changes (24, 25). Interestingly, in the presence of 14,15-EEZE, which antagonized the effects of 14,15 EET, we have consistently observed an increase in the oscillation pattern (Fig. 6C). This observation is consistent with an endogenous production of EET, which would result in a reduction of the spasmodic activity in human bronchi.

Conclusion. In summary, this study provided additional evidence that 14,15-EET interacts with TP-receptor activation to reduce the tonic responses of mammalian ASM tissues. We also demonstrated for the first time that 48-h treatment with a specific sEH inhibitor reduced human bronchi reactivity to a TXA2 analog. Our findings demonstrate that CYP450 inhibition reduces endogenous EET production and therefore increases TP-receptor responses, whereas inhibition of sEH increases endogenous EET bioavailability and therefore reduces reactivity to U-46619.

ACKNOWLEDGMENTS

We thank Dr. J. R. Falck for the gift of MS-PPOH, Dr. B. D. Hammock for AUDA, t-AUCB, and sEH antibodies, and Dr. Edmond Rizcallah and the members of the pathology laboratory for technical support. Ms. Stéphanie Corriveau helped us with patient recruitment.

GRANTS

This work was supported by a grant from the Faculty of Medicine and Health Sciences and the CRC E-LeBel of the CHUS to E. Rousseau.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

19. Liu JY, Yang J, Inceoglu B, Qiu H, Ulu A, Hwang SH, Chiamvimonvat N, Hammock BD. Inhibition of soluble epoxide hydrolase enhances the anti-inflammatory effects of aspirin and 5-lipooxygenase activation...