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Red blood cells (RBCs), epoxyeicosatrienoic acids (EETs) and adenosine triphosphate (ATP)

Houli Jiang, Gail D. Anderson, and John C. McGiff

Department of Pharmacology, New York Medical College, Valhalla, NY 10595, USA

Abstract

In addition to serving as carriers of O₂, red blood cells (RBCs) regulate vascular resistance and the distribution of microvascular perfusion by liberating adenosine triphosphate (ATP) and epoxyeicosatrienoic acids (EETs) upon exposure to a low O₂ environment. Therefore, RBCs act as sensors that respond to low pO₂ by releasing millimolar amounts of ATP, a signaling molecule, and lipid mediators (EETs). The release of EETs occurs by a mechanism that is activated by ATP stimulation of P2X₇ receptors coupled to ATP transporters, which should greatly amplify the circulatory response to ATP. RBCs are reservoirs of EETs and the primary sources of plasma EETs, which are esterified to the phospholipids of lipoproteins. Levels of free EETs in plasma are low, about 3% of circulating EETs. RBC EETs are produced by direct oxidation of arachidonic acid (AA) esterified to glycerophospholipids and the monooxygenase-like activity of hemoglobin. On release, EETs affect vascular tone, produce profibrinolysis and dampen inflammation. A soluble epoxide hydrolase (sEH) regulates the concentrations of RBC and vascular EETs by metabolizing both *cis*- and *trans*-EETs to form dihydroxyeicosatrienoic acids (DHETs). The function and pathophysiological roles of *trans*-EETs and *erythro*-DHETs has yet to be integrated into a physiological and pathophysiological context.

Keywords

epoxyeicosatrienoic acids; red blood cells; arachidonic acid; soluble epoxide hydrolase; glycerophospholipids

Introduction

Epoxyeicosatrienoic acids (EETs) are arachidonic acid (AA)-derived lipid mediators that dilate arterioles by activating the large conductance Ca²⁺ activated K⁺ channel. EETs are opposed by 20-hydroxyeicosatetraenoic acid (20-HETE) at this site [24, 29]. EETs were first identified as products of the rat liver microsomal cytochrome P450 pathway of AA metabolism [8]. Red blood cells (RBCs) have been shown to serve as reservoirs for EETs, which are abundant and incorporated into the *sn*-2 position of the RBC membrane phospholipids [22]. Stored EETs can be released by activating phospholipases (Fig. 1) in response to neuronal, hormonal and chemical stimuli to function in phospholipid-mediated signal transduction [31, 33]. RBC EETs are a major source of plasma EETs that are

esterified to the phospholipids of lipoproteins [22]. Free EETs account for 3% of rat plasma EETs. RBC EETs can be produced by direct oxidation of AA that is esterified to glycerophospholipids [32]. Hemoglobin (Hb) has been reported to possess monooxygenase-like activity by Mieyal et al. [46]. The monooxygenase-like activity of Hb can be activated by nicotinic acid and riboflavin, which serve as electron-carrier cofactors and divert AA peroxidation to form EETs. This process obviates the need for NADPH reductase. The *de novo* production of EETs produced by RBCs occurs *via* AA transformation by the monooxygenase-like activity of Hb, which involves a calcium dependent cytoplasmic phospholipase A₂ (rPLA₂) [41] that cleaves free AA from phospholipids. In addition to their vasoactivity and effects on renal tubular salt and water metabolism [29], EETs exert potent anti-inflammatory effects by activating transcription of the endothelial peroxisome proliferator activated receptor (PPAR) [27, 29, 34]. The resulting inhibition of nuclear factor κ B-mediated expression of adhesion molecules and endothelin synthesis counters the development or progression of vascular wall damage and atherogenesis [4, 27]. The wide range of actions of EETs is further exhibited by their ability to activate fibrinolysis by inducing the expression of tissue plasminogen activator [34].

Metabolism of EETs by soluble epoxide hydrolase

Soluble epoxide hydrolase (sEH) regulates the concentration of vascular EETs by metabolizing them to form dihydroxyeicosatrienoic acids (DHETs) [13, 35]. sEH demonstrates regioselectivity and stereoselectivity because the rates of EET hydration are highest for 14,15-EET, and it prefers 14(R),15(S)-EET [49]. In contrast, hydration of 11,12-EET is not stereoselective. DHETs are considered to be inactive, although exceptions have been reported, such as the effect of sEH on metabolism of 11,12-EET, which did not lose its coronary arteriolar dilator activity upon hydrolysis by sEH to form 11,12-DHET [24]. In contrast, the coronary vasodilator actions of 8,9- and 14,15-EETs are eradicated upon their conversion to DHETs. EETs and DHETs reduce cellular cholesterol levels as effectively as simvastatin in the human hepatoma (HepG₂) cell line [11]. The cholesterol-lowering response was found only for 11,12- and 14,15-DHETs, which showed similar potency to 11,12- and 14,15-EETs. Inhibition of sEH also increases EET incorporation into phospholipids [48], which potentiates endothelium-dependent vasorelaxation to bradykinin mediated by 11,12-EET acting as an endothelial-derived hyperpolarizing factor (EDHF) [16]. Indeed, the renal vasodilator response to intra-arterial administration of bradykinin has been shown to produce a prostaglandin, a nitric oxide (NO) and a CYP450 AA metabolite [29]. The renal vasodilator response to bradykinin could be potentiated by increasing EET levels by either preventing their degradation through inhibiting sEH or by adding EETs to vascular tissues [48]. Increased sEH activity has been associated with diabetes [37, 47] and hypertension [1, 20], whereas elevated EET levels, in response to inhibition of sEH, lowered blood pressure in hypertensive states [9, 18, 20]. sEH has become a therapeutic target for controlling blood pressure [28, 42, 50], vascular inflammation [10, 38, 43] and cancer progression [30] as well as providing cardiac [25, 40] and renal protection [19] by raising tissue and plasma levels of EETs.

Due to the cardiovascular effects of substituted urea-derived sEH inhibitors (CUDA, AUDA) [12], it is possible that sEH inhibitors are not very specific and do more than inhibit

EET metabolism. CUDA and AUDA activate PPAR α directly by functioning as a PPAR α ligand, which is similar to 11,12-EET [29]. Thus, some of the actions of EETs, such as their ability to stimulate PPAR α can be duplicated by this class of sEH inhibitors, which activate PPAR α through an EET-independent mechanism.

***trans*-EETs and sEH**

RBC sEH prefers hydrolysis of *trans*-EETs to *cis*-EETs because the $V_{\max}$ of *trans*-EET hydrolysis by rat RBCs is about 3 times that of *cis*-EETs [22]. Therefore, inhibition of sEH will have a greater effect on *trans*-EET concentration in tissues and plasma than *cis*-EET concentration. On giving a sEH inhibitor, the increased levels of *trans*-EETs and *erythro*-DHETs are thought to account for the blood pressure-lowering effects of sEH inhibition in the spontaneously hypertensive rat (SHR), a rat genetic model of hypertension. Additionally, the greater vasodilator potency of *trans*-EETs with regard to *cis*-EETs and the retention of vasoactivity of the *erythro*-DHETs contribute to the antihypertensive effects of inhibition of sEH [21].

EETs formed by oxidation of AA esterified to glycerophospholipids

Isoprostanes and isoleukotrienes are produced by free radical oxidation of AA and demonstrate potent biological activity, which is the case with EETs arising from non-enzymatic oxidation of esterified AA [22]. Nakamura, Bratton and Murphy [32] have proposed a non-enzymatic pathway for generating EETs that is induced by free radical-based peroxidation of phospholipids, which is an alternative pathway to cytochrome P450 epoxygenases and activated Hb production of EETs. They developed a model system in which *tert*-butyl-hydroperoxide (*t*-BHP), a lipophilic agent, was used to increase hydroperoxide tone in cellular membranal glycerophospholipids. Thus, in response to the oxidizing agent, *t*-BHP, EETs were produced *in situ* by oxidizing AA that was already incorporated in the membranal phospholipid bilayer, not by re-acylation of lysophospholipid precursors. This process increased EETs esterified in glycerophosphoethanolamine, -serine and -choline by 30- to 60-fold.

EETs arising enzymatically or by an Hb-catalyzed mechanism can be esterified in the fatty acyl chains of glycerophospholipids *via* an acyl-CoA synthase (CoA)-dependent mechanism [48], which is the dominant mechanism responsible for incorporating EETs into human platelet phospholipids [51]. During activation of platelets with either thrombin or platelet activating factor, EETs were released from phospholipids and reached an estimated intracellular concentration of about 1 $\mu\text{mol/L}$, which has been shown to inhibit platelet aggregation by affecting platelet cyclooxygenase activity and calcium entry [51]. Cobra venom PLA₂ was also used to release AA metabolites esterified in the *sn*-2 position of platelet phospholipids. Gas chromatography/mass spectrometry (GC/MS) analyses showed endogenous EETs and 20-HETE. The amount of 20-HETE was 200-fold lower than the amount of EETs. Unbound EETs and 20-HETE were not detected. More than 60% of the EETs were esterified in platelet phosphatidylinositol, which suggests involvement in signal transduction. Platelet EETs were thought to be produced enzymatically by the cytochrome P450 system because chiral analysis showed only a single enantiomer, which is an indicator

of enzymatic origin. In a study of EETs extracted from hepatic phospholipids, Capdevila and colleagues [6] traced EET incorporation into phospholipid pools.

Given the magnitude of the EET reservoir capacity of RBCs and the RBC-EET releasing mechanism, which is activated by ATP stimulation of the erythrocyte P2X₇ receptor [23], RBC EETs are postulated to be the chief source of free EETs in plasma (3%) and EETs esterified in circulating phospholipids (95% of circulating EETs). The disposition of intracellular EETs and DHETs shows remarkable diversity [44]. Unlike EETs that can be released from RBCs by a purinoceptor mechanism, DHETs are extruded from cells. Cellular retention of EETs likely reflects binding to cytosolic fatty acid binding proteins (FABP), which possibly act as transport proteins for EETs. In contrast, DHETs show weak binding to FABP [44]. We have analyzed RBC EETs with electrospray ionization tandem mass spectrometry and found that both *cis*- and *trans*-EETs increase when rat RBCs are exposed to *t*-BHP, which oxidizes esterified AA[22]. Incorporation of newly formed free EETs into RBC phospholipids by an acyl CoA dependent mechanism also occurs [48]. The plasma levels of *cis*- and *trans*-EETs of RBC origin are similar in the rat model; *cis*-EETs, 21.4 vs. *trans*-EETs, 16.8 ng/ml [22] (Tab. 1)

ATP, a key signaling molecule

In addition to serving a primary role as carriers of O₂, RBCs also regulate microvascular resistance, thereby controlling the distribution of blood flow to the microvasculature by liberating ATP from RBCs upon exposure to a low O₂ environment [45]. The release of RBC EETs and ATP in response to RBC deformation and exposure to low oxygen levels can be prevented by inhibition of either cPLA₂ or the P2X₇-ATP-EET-releasing mechanism [23]. As noted, EETs are candidates for EDHF [5, 16, 17]. Renal EET synthesis also can be stimulated by an adenosine mechanism, which operates through a renal adenosine A_{2A} receptor linked to epoxigenase activity. Epoxigenase activity prevents hypertension induced by high salt intake [7, 26] and reduces elevation of blood pressure produced by infused angiotensin II (ANGII) [20].

In the context of coronary physiology, a prevailing question has addressed the mechanism linking coronary blood flow to myocardial O₂ consumption. In 1963, Berne proposed the adenosine hypothesis of coronary blood flow control [15] that was tested by blockading the coronary vascular adenosine receptor during exercise. Adenosine receptor blockade had no effect on coronary blood flow during exercise, although it decreased reactive hyperemia in response to coronary occlusion. The ATP hypothesis for the control of coronary blood flow during exercise was set in motion by demonstrating that ATP is released from RBCs during hypoxia [2]. The ATP hypothesis was tested in unanesthetized dogs by measuring arterial and coronary venous plasma ATP at rest and during treadmill exercise [14]. Coronary blood flow increased as coronary venous ATP concentration rose during exercise (Fig. 2). Furthermore, the increase in coronary venous plasma ATP concentration correlated with the decline in coronary venous O₂ tension during exercise.

RBCs, ATP and EETs

RBCs generate millimolar amounts of ATP, which exceeds the ATP-generating ability of most tissues [45]. Once released from the RBC, ATP acts on endothelial cell purinergic receptors [36] and activates a retrograde conducted response in arterioles. This activation dilates the upstream “feeder” artery, which increases oxygenated blood flow to sites in the microvasculature, where O₂ extraction is high [39]. As previously mentioned, ATP stimulates the P2X₇ RBC membranal purinoceptor, which releases EETs from RBCs [23]. We have shown that the release of ATP from RBCs is linked to generation and release of EETs from RBCs and, presumably, from the contiguous endothelium. EETs, in turn, affect vascular tone and produce profibrinolytic and anti-inflammatory effects [44]. ATP also functions as an extracellular signaling molecule by interacting with P2 receptors to mediate diverse responses in the vasculature [3]. The P2X₇ receptor is the major purinoceptor in human erythrocytes. Stimulation of the P2X₇ receptor elicits cytoskeletal rearrangement, pore formation and changes in permeability and trafficking, which results in the release of ATP and EET [23]. ATP also activates EET secretion from RBCs, which should greatly amplify the response to ATP in the microvasculature.

Conclusion

Based on the above studies, we hypothesize that hemoglobin is associated with transforming arachidonic acid to EETs. EETs occupy storage sites in RBCs by the following two mechanisms: peroxidation of phosphoglycerolipids and incorporation of EETs into phosphoglycerolipids. EET is released from RBCs by ATP stimulation of the P2X₇ receptor, which is linked to ATP transporters, and erythrocyte sEH regulates the concentration of free EETs in RBCs. Inhibition of sEH increases EET esterification in RBC phospholipids and EETs released from RBCs.

RBCs release EETs and ATP on their passage through the microcirculation in response to RBC deformation and hypoxemia, which suggests a functional role for EETs acting in concert with ATP that regulates the microcirculation and affects the rheological characteristics of blood. These findings on formation, release and hydrolysis of *cis*- and *trans*-EETs by RBCs should lead to the development of novel therapies for the management of circulatory diseases, particularly hypertension and diabetes and the associated development of atherosclerosis.

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Abbreviations

20-HETE	20-hydroxyeicosatetraenoic acid
AA	arachidonic acid
cPLA₂	cytoplasmic phospholipase A ₂

DHETs	dihydroxyeicosatrienoic acids
EETs	epoxyeicosatrienoic acids
PPAR	peroxisome proliferator-activated receptor
RBCs	red blood cells
sEH	soluble epoxide hydrolase
<i>t</i>-BHP	<i>tert</i> -butyl-hydroperoxide

References

1. Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C, Hammock BD, Shyy JY, Zhu Y. Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. *Proc Natl Acad Sci USA*. 2007; 104:9018–9023. [PubMed: 17495027]
2. Bergfeld GR, Forrester T. Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnea. *Cardiovasc Res*. 1992; 26:40–47. [PubMed: 1325292]
3. Burnstock G, Knight GE. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int Rev Cytol*. 2004; 240:31–304. [PubMed: 15548415]
4. Campbell WB. New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol Sci*. 2000; 21:125–127. [PubMed: 10740283]
5. Campbell WB, Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension*. 2007; 49:590–596. [PubMed: 17200437]
6. Capdevila JH, Kishore V, Dishman E, Blair IA. A novel pool of rat liver inositol and ethanolamine phospholipids contains epoxyeicosatrienoic acids (EETs). *Biochem Biophys Res Commun*. 1987; 146:638–644. [PubMed: 3619897]
7. Capdevila JH, Wei S, Yan J, Karara A, Jacobson HR, Falck JR, Guengerich FP, DuBois RN. Cytochrome P-450 arachidonic acid epoxidase. Regulatory control of the renal epoxidase by dietary salt loading. *J Biol Chem*. 1992; 267:21720–21726. [PubMed: 1400482]
8. Chacos N, Falck JR, Wixtrom C, Capdevila J. Novel epoxides formed during the liver cytochrome P-450 oxidation of arachidonic acid. *Biochem Biophys Res Commun*. 1982; 104:916–922. [PubMed: 6803794]
9. Chiamvimonvat N, Ho CM, Tsai HJ, Hammock BD. The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J Cardiovasc Pharmacol*. 2007; 50:225–237. [PubMed: 17878749]
10. Davis BB, Thompson DA, Howard LL, Morisseau C, Hammock BD, Weiss RH. Inhibitors of soluble epoxide hydrolase attenuate vascular smooth muscle cell proliferation. *Proc Natl Acad Sci USA*. 2002; 99:2222–2227. [PubMed: 11842228]
11. Enayetallah A, Cao L, Grant DF. Novel role of soluble epoxide hydrolase in regulating cholesterol in mammalian cells. *The Open Drug Metab J*. 2007; 1:1–6. [PubMed: 22582093]
12. Fang X, Hu S, Watanabe T, Weintraub NL, Snyder GD, Yao J, Liu Y, et al. Activation of peroxisome proliferator-activated receptor α by substituted urea-derived soluble epoxide hydrolase inhibitors. *J Pharmacol Exp Ther*. 2005; 314:260–270. [PubMed: 15798002]
13. Fang X, Weintraub NL, McCaw RB, Hu S, Harmon SD, Rice JB, Hammock BD, Spector AA. Effect of soluble epoxide hydrolase inhibition on epoxyeicosatrienoic acid metabolism in human blood vessels. *Am J Physiol Heart Circ Physiol*. 2004; 287:H2412–H2420. [PubMed: 15284062]
14. Farias M III, Gorman MW, Savage MV, Feigl EO. Plasma ATP during exercise: possible role in regulation of coronary blood flow. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1586–H1590. [PubMed: 15563530]
15. Feigl EO. Berne's adenosine hypothesis of coronary blood flow control. *Am J Physiol Heart Circ Physiol*. 2004; 287:H1891–H1894. [PubMed: 15475525]

16. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature*. 1999; 401:493–497. [PubMed: 10519554]
17. Fulton D, McGiff JC, Quilley J. Pharmacological evaluation of an epoxide as the putative hyperpolarizing factor mediating the nitric oxide-independent vasodilator effect of bradykinin in the rat heart. *J Pharmacol Exp Ther*. 1998; 287:497–503. [PubMed: 9808672]
18. Ghosh S, Chiang PC, Wahlstrom JL, Fujiwara H, Selbo JG, Roberds SL. Oral delivery of 1,3-dicyclohexylurea nanosuspension enhances exposure and lowers blood pressure in hypertensive rats. *Basic Clin Pharmacol Toxicol*. 2008; 102:453–458. [PubMed: 18312493]
19. Imig JD. Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases. *Am J Physiol Renal Physiol*. 2005; 289:F496–F503. [PubMed: 16093425]
20. Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension*. 2002; 39:690–694. [PubMed: 11882632]
21. Jiang H, McGiff JC, Quilley J, Sacerdoti D, Reddy LM, Falck JR, Zhang F, et al. Identification of 5,6-*trans*-epoxyeicosatrienoic acid in the phospholipids of red blood cells. *J Biol Chem*. 2004; 279:36412–36418. [PubMed: 15213230]
22. Jiang H, Quilley J, Manmohan-Reddy L, Falck JR, Wong PYK, McGiff JC. Red blood cells: reservoirs of *cis*- and *trans*-epoxyeicosatrienoic acids. *Prostaglandins Other Lipid Mediat*. 2005; 75:65–78. [PubMed: 15789616]
23. Jiang H, Zhu AG, Mamczur M, Falck JR, Lerea KM, McGiff JC. Stimulation of rat erythrocyte P2X₇ receptor induces the release of epoxyeicosatrienoic acids. *Br J Pharmacol*. 2007; 151:1033–1040. [PubMed: 17558440]
24. Larsen BT, Miura H, Hatoum OS, Campbell WB, Hammock BD, Zeldin DC, Falck JR, Gutterman DD. Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK_{Ca} channels: implications of soluble epoxide hydrolase inhibition. *Am J Physiol Heart Circ Physiol*. 2006; 290:H491–H499. [PubMed: 16258029]
25. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, et al. Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Hum Mol Genet*. 2006; 15:1640–1649. [PubMed: 16595607]
26. Liclican EL, McGiff JC, Falck JR, Carroll MA. Failure to upregulate the adenosine 2A receptor-epoxyeicosatrienoic acid pathway contributes to the development of hypertension in Dahl salt-sensitive rats. *Am J Physiol Renal Physiol*. 2008; 295:F1696–F1704. [PubMed: 18829737]
27. Liu Y, Zhang Y, Schmelzer K, Lee TS, Fang X, Zhu Y, Spector AA, et al. The antiinflammatory effect of laminar flow: the role of PPARG γ , epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc Natl Acad Sci USA*. 2005; 102:16747–16752. [PubMed: 16267130]
28. Loch D, Hoey A, Morisseau C, Hammock BO, Brown L. Prevention of hypertension in DOCA-salt rats by an inhibitor of soluble epoxide hydrolase. *Cell Biochem Biophys*. 2007; 47:87–98. [PubMed: 17406062]
29. McGiff, JC.; Ferreri, NF. Eicosanoid and the kidney. In: Alpern, RJ.; Hebert, SC., editors. *Seldin and Giebisch's The Kidney*. Boston: Elsevier; 2008. p. 359–384.
30. Morisseau C, Hammock BD. Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. *Annu Rev Pharmacol Toxicol*. 2005; 45:311–333. [PubMed: 15822179]
31. Munzenmaier DH, Harder DR. Cerebral microvascular endothelial cell tube formation: role of astrocytic epoxyeicosatrienoic acid release. *Am J Physiol Heart Circ Physiol*. 2000; 278:H1163–H1167. [PubMed: 10749710]
32. Nakamura T, Bratton DL, Murphy RC. Analysis of epoxyeicosatrienoic and monohydroxyeicosatetraenoic acids esterified to phospholipids in human red blood cells by electrospray tandem mass spectrometry. *J Mass Spectrom*. 1997; 32:888–896. [PubMed: 9269087]
33. Negro-Vilar A, Snyder GD, Falck JR, Manna S, Chacos N, Capdevila J. Involvement of eicosanoids in release of oxytocin and vasopressin from the neural lobe of the rat pituitary. *Endocrinology*. 1985; 116:2663–2668. [PubMed: 3922746]

34. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, Liao JK. Activation of Gα_s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J Biol Chem*. 2001; 276:15983–15989. [PubMed: 11279071]
35. Ota K, Hammock BD. Cytosolic and microsomal epoxide hydrolases: differential properties in mammalian liver. *Science*. 1980; 207:1479–1481. [PubMed: 7361100]
36. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998; 50:413–492. [PubMed: 9755289]
37. Rodriguez M, Clare-Salzler M. Eicosanoid imbalance in the NOD mouse is related to a dysregulation in soluble epoxide hydrolase and 15-PGDH expression. *Ann NY Acad Sci*. 2006; 1079:130–134. [PubMed: 17130543]
38. Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP, Hammock BD. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc Natl Acad Sci USA*. 2005; 102:9772–9777. [PubMed: 15994227]
39. Segal SS, Duling BR. Flow control among microvessels coordinated by intercellular conduction. *Science*. 1986; 234:868–870. [PubMed: 3775368]
40. Seubert JM, Sinal CJ, Graves J, Degraff LM, Bradbury JA, Lee CR, Goralski K, et al. Role of soluble epoxide hydrolase in postischemic recovery of heart contractile function. *Circ Res*. 2006; 99:442–450. [PubMed: 16857962]
41. Shin HS, Chin MR, Kim JS, Chung JH, Ryu CK, Jung SY, Kim DK. Purification and characterization of a cytosolic, 42-kDa and Ca²⁺-dependent phospholipase A2 from bovine red blood cells: its involvement in Ca²⁺-dependent release of arachidonic acid from mammalian red blood cells. *J Biol Chem*. 2002; 277:21086–21094. [PubMed: 11909855]
42. Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, Gonzalez FJ. Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem*. 2000; 275:40504–40510. [PubMed: 11001943]
43. Smith KR, Pinkerton KE, Watanabe T, Pedersen TL, Ma SJ, Hammock BD. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc Natl Acad Sci USA*. 2005; 102:2186–2191. [PubMed: 15684051]
44. Spector AA, Fang X, Snyder GD, Weintraub NL. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog Lipid Res*. 2004; 43:55–90. [PubMed: 14636671]
45. Sprague RS, Stephenson AH, Ellsworth ML. Red not dead: signaling in and from erythrocytes. *Trends Endocrinol Metab*. 2007; 18:350–355. [PubMed: 17959385]
46. Starke DW, Blisard KS, Miesal JJ. Substrate specificity of the monooxygenase activity of hemoglobin. *Mol Pharmacol*. 1984; 25:467–475. [PubMed: 6727868]
47. Thomas H, Schladt L, Knehr M, Oesch F. Effect of diabetes and starvation on the activity of rat liver epoxide hydrolases, glutathione S-transferases and peroxisomal beta-oxidation. *Biochem Pharmacol*. 1989; 38:4291–4297. [PubMed: 2688656]
48. Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P, Spector AA. Epoxide hydrolases regulate epoxyeicosatrienoic acid incorporation into coronary endothelial phospholipids. *Am J Physiol*. 1999; 277:H2098–H2108. [PubMed: 10564166]
49. Zeldin DC, Kobayashi J, Falck JR, Winder BS, Hammock BD, Snapper JR, Capdevila JH. Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *J Biol Chem*. 1993; 268:6402–6407. [PubMed: 8454612]
50. Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, Morisseau C, Luria A, et al. Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J Cereb Blood Flow Metab*. 2007; 27:1931–1940. [PubMed: 17440491]
51. Zhu Y, Brand-Schieber E, McGiff J, Balazy M. Identification of arachidonate P-450 metabolites in human platelet phospholipids. *Hypertension*. 1995; 25:854–859. [PubMed: 7721444]

Fig. 1. Release of free EETs from rat RBCs. RBCs were washed 3 times with buffer and then incubated with and without 2 units of PLA₂ at 37°C for 10 min ($n = 4$, $p < 0.05$). Inset, GC/MS analysis of the released EETs indicate that there are both 5,6-*cis*- and 5,6-*trans*-EETs [22, with permission]

Fig. 2.

(**A**) Linear regression lines for mean coronary blood flow on coronary venous plasma ATP concentration during rest and exercise. There was a significant increase in coronary blood flow with coronary venous plasma ATP concentration ($p < 0.01$). These findings indicate that ATP may contribute to the increase in coronary blood flow (p values are based on Draper slopes for 10 dogs). (**B**) Linear regression for mean coronary venous plasma ATP concentration on coronary venous oxygen tension during rest and exercise. There was a significant increase in coronary venous plasma ATP concentration as the coronary venous oxygen tension declined ($p < 0.01$). These findings indicate that the coronary venous plasma ATP concentration is sensitive to the oxygen level of the coronary venous blood (p values are based on Draper slopes for 10 dogs). [12, with permission]

Tab. 1

EETs present in 14-week old male SD rat plasma

EETs	<i>cis</i> -EET	<i>trans</i> -EET
14,15	6.4 ± 0.2	3.6 ± 0.4 *
11,12	3.6 ± 0.5	4.2 ± 0.3
8,9	5.7 ± 0.5	3.1 ± 0.4 *
5,6	5.7 ± 0.4	5.9 ± 0.5
Total	21.4 ± 0.4	16.8 ± 0.4 *

ng/ml, mean ± SE, n = 6, * p < 0.05 compared with corresponding *cis*-EET regioisomers or total, respectively