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The Red Blood Cell Participates in Regulation of the Circulation by Producing and Releasing Epoxyeicosatrienoic Acids

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

Department of Pharmacology, New York Medical College, Valhalla, New York

Abstract

Red blood cells (RBCs) have an important function in regulation of the circulation by producing and releasing epoxyeicosatrienoic acids (EETs) in response to a low O_2 environment such as encountered in the cardiac microcirculation during exercise. RBCs, in their role as sensors of low pO_2, release ATP and critical lipid mediators, the EETs. Both *cis*- and *trans*-EETs are synthesized and stored in RBCs and are hydrolyzed by soluble epoxide hydrolases (sEH). The *trans*-EETs differ from *cis*-EETs in their higher vascular potencies and more rapid metabolism by sEH. Thus, inhibition of sEH results in greater *trans*-EET levels and increased positive vascular effects of *trans*-EETs vs *cis*-EETs. The *trans*-EETs are responsible for a significant decline in the elevated blood pressure in the spontaneously hypertensive rat on treatment with a sEH inhibitor to raise EET levels. We predict that *trans*-EETs and *cis*-EETs will occupy important therapeutic roles in a broad spectrum of diseases and abnormal physiological conditions such as that resulting from high salt intake and hypertension.

Keywords

epoxyeicosatrienoic acids; red blood cells

1. Introduction

In 1981, Capdevila *et al* [1] reported that a hepatic microsomal monooxygenase metabolized arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs). A key paper by Nakamura, Bralton and Murphy [2] that facilitated our entry into studies on the rat and human red blood cell (RBC) as a reservoir for EETs raised the concept that EETs on release from RBCs act as vasoregulators of the microcirculation. Thus, erythrocytes can serve “as a circulating reservoir from which EETs can be released” [2]. EETs possess notable properties that impact beneficially on the vasculature and retard progression of vascular disease as they enhance blood flow, diminish mitogenesis and inhibit platelet aggregation. In addition, EETs are anti-inflammatory, inhibit cell migration, promote fibrinolysis and influence

Correspondence to: John C. McGiff, M.D., Department of Pharmacology, New York Medical College, Basic Science Building, Rm. 514, Valhalla, NY 10595, Tel: 914-594-4075, Fax: 914-347-4956, john_mcgiff@nymc.edu.

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steroidogenesis [3-6]. Our initial study [7] on identification of *trans*-EETs recognized that all EETs produced by cytochrome P450 epoxygenases were of the *cis*-configuration [8,9]; however, formation of *trans*-EETs is possible through radical driven reactions as demonstrated for human RBCs by Nakamura *et al* and Jiang *et al* [2,7] (Fig. 1).

2. RBCs contain both *cis*- and *trans*-EETs

An additional key finding in our study of rat RBCs identified a 5,6-*trans*-EET with GC/MS and LC/MS/MS analyses [7]. This study produced direct evidence for the presence of 5,6-*trans*-EET in RBCs. As lipid peroxidation in RBCs can elevate esterified EETs by more than 30-fold [2], butylated hydroxytoluene and triphenylphosphine were used to quench free radical formation of EETs in blood samples to prevent free radical-mediated transformation of AA to EETs during analysis of RBC EET concentrations that was intended to reflect EET levels in circulating RBCs [7,10]. The *trans*-EETs occupied the *sn*-2 position of the RBC phospholipids from which they were released by phospholipase A_2-mediated hydrolysis to achieve transient free levels in the RBC cytosol where they were released into the plasma by an ATP-dependent mechanism or subject to rapid hydrolysis forming *erythro*-dihydroxyeicosatrienoic acids (DHETs) [11]. Indeed, the rate of release of 14,15-EET from phosphatidylcholine and phosphatidylinositol surpassed that for AA [12].

Rat RBCs contained *cis*- and *trans*-EETs of *ca* 20 ng/10^9 RBCs, quantitated by LC/MS analysis, which indicated that *cis*- and *trans*-EETs are present in similar amounts in normal Sprague-Dawley rat RBCs [10]. Rat plasma EETs have been reported at *ca* 10 ng/ml, primarily esterified to phospholipids of circulating lipoproteins [13]. The incorporation of EETs into phospholipids of cells and their binding to fatty acid binding proteins [14] suggest long lasting effects of EETs on release by hormonal stimulation [15]. Levels of 14,15-*trans*-EET are higher than those of 11,12-*trans*- and 8,9-*trans*-EET which were similar to plasma concentrations of 5,6-*trans*-EET [10].

3. Biological activities of 5,6-EETs

The *trans*-EET exhibited much greater vascular relaxation responses on rat renal interlobar arteries than those of 5,6-*cis*-EET (Fig 2). The 5,6-*erythro*-DHET metabolite of 5,6-*trans*-EET via the soluble epoxide hydrolase (sEH), also evoked vasodilation, an unexpected response as the DHET metabolite of EETs are usually inactive (Fig. 3). However, 5,6-*cis*-EET had similar effects to those of 5,6-*trans*-EET on collagen-induced platelet aggregation [9] (Fig 3).

4. sEH of rat RBCs hydrolyzes *cis*- and *trans*-EETs

Our study on RBC-EETs also disclosed hydrolysis of *cis*-/*trans*-EETs by rat RBCs [11]. We found a typical sEH in rat RBC cytosol indistinguishable from that in hepatocytes that preferred hydrolysis of *trans*- over *cis*-EETs in a decreasing order for both *cis*- and *trans*-EETs from 14,15-, 11,12-, 8.9- to 5,6-EETs [11]. The V_{MAX} of *trans*-EET hydrolysis by RBCs approaches 3 times that of the corresponding *cis*-EET. sEH activity is increased in diabetes [16] and in hypertension [17-20]. Increasing tissue and circulating EET levels are a therapeutic objective achieved by inhibition of sEH, which has been shown in experimental

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animals to control elevated blood pressure [18,19,21-24], reduce renal damage [25] and prevent strokes and moderate inflammation [26].

5. Inhibition of sEH lowers blood pressure in the spontaneously hypertensive rat (SHR)

As sEH inhibition should increase plasma *trans*-EETs to higher levels than *cis*-EETs based on preferential metabolism of *trans*-EETs, a study was designed in the SHR to characterize the relative effects of sEH inhibition on *cis*- and *trans*-EETs in lowering blood pressure in the SHR [24]. Control Wistar-Kyoto (WKY) rats demonstrated a manifestly higher total plasma EETs than the SHR (26 vs 16 ng/ml). However, sEH inhibition produced a significant elevation of plasma EETs in the SHR that involved only *trans*-EETs. Several factors have been shown to determine suppression of *trans*-EET activity in the SHR, the most important being the three-fold higher velocity of *trans*-EET hydrolysis by sEH compared to hydrolysis of *cis*-EETs, coupled to elevated sEH activity in the SHR [11]. Inhibition of sEH activity in the SHR produced elevated plasma EETs that lowered blood pressure via two-fold increase in plasma *trans*-EETs in contrast to the failure of sEH inhibition to increase total plasma *cis*-EET concentrations in either the WKY or the SHR [24]. Blood pressure was unaffected in control WKY rats. Additionally, the greater vasodilator potency of *trans*-EETs *vis a vis cis*-EETs also contributed to the blood pressure lowering response to sEH inhibition in the SHR.

6. The RBC secretes ATP that stimulates RBC EET release by activating P_2 receptors

The production of ATP by RBCs has many ramifications as a critical determinant of microvascular function [27, 28]. RBCs exceed the ATP synthesizing capacity of most tissues as they produce millimolar amounts of ATP [29]. Indeed, an area of intensive studies, the regulation of coronary blood flow during exercise, has yielded to a mechanism centered in RBCs that secretes ATP which acts as an extracellular signaling molecule by interacting with specific P_2 receptors to activate vasodilatation in the microcirculation [29]. Phospholipase stimulation of RBCs releases EETs stored in RBC membrane phospholipids [27]. AA also esterified to RBC glycerol phospholipids (10) are simultaneously cleaved by phospholipase activity and converted to EETs by monooxygenase-like activity [46] of hemoglobin (Hb). Then, EET release from rat RBCs follows in decreasing order: 14,15-, 11,12-, 8,9- and 5,6-EETs which exit from RBC via ATP transporters coupled to RBC membrane $P2X_7$ receptors that respond, presumably, to both plasma and RBC cytosolic ATP to enhance EET release from RBCs [27].

ATP stimulation of erythrocyte $P2X_7$ receptors releases both *cis*- and *trans*-EETs that are subject to hydrolysis by sEH in the erythrocyte cytosol, usually with loss of biological activity [27]. As noted, the sEH prefers hydrolysis of *trans*-EETs by a factor of three-fold [11]. Thus, inhibition of RBC sEH results in increased plasma *trans*-EETs, having greater vasodilator and anti-inflammatory potencies than *cis*-EETs and, thereby, offering therapeutic advantages superior to *cis*-EETs as demonstrated by Jiang *et al* [24]. Moreover, on passage

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of RBCs through the microcirculation, a positive synergistic effect of ATP and EETs on microvessels occurs by activating a retrograde dilator response in upstream “feeder” arteries [30] that increase blood flow to the microcirculatory sites exhibiting high O_2 extraction. These findings are therefore critical to another aspect of the contributions of the RBC to the microcirculation in addition to O_2 delivery; namely, allocation of blood flow distribution mediated by RBC production of ATP and EETs via a mechanism involving the RBC membrane $P2X_7$ receptor [27].

Acknowledgements

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Abbreviations

RBC	red blood cell
EET	epoxyeicosatrienoic acid
DHET	dihydroxyeicosatrienoic acid
SHR	spontaneously hypertensive rat
WKY	Wistar-Kyoto rat
sEH	soluble epoxide hydrolase

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Highlights

- Epoxyeicosatrienoic acids (EETs) are significant lipid mediators in circulatory regulation exerted by red blood cells (RBCs).
- Both *cis*- and *trans*-EETs are synthesized, stored, released and hydrolyzed by RBCs.
- The *trans*-EETs exhibit more potent vascular activities and are metabolized more rapidly by soluble epoxide hydrolase (sEH) than *cis*-EETs.
- Inhibition of sEH results in greater *trans*-EET levels and vascular effects of *trans*- vs *cis*-EETs.
- The *trans*- and *cis*-EETs have important therapeutic effects in hypertension and cardiovascular diseases.

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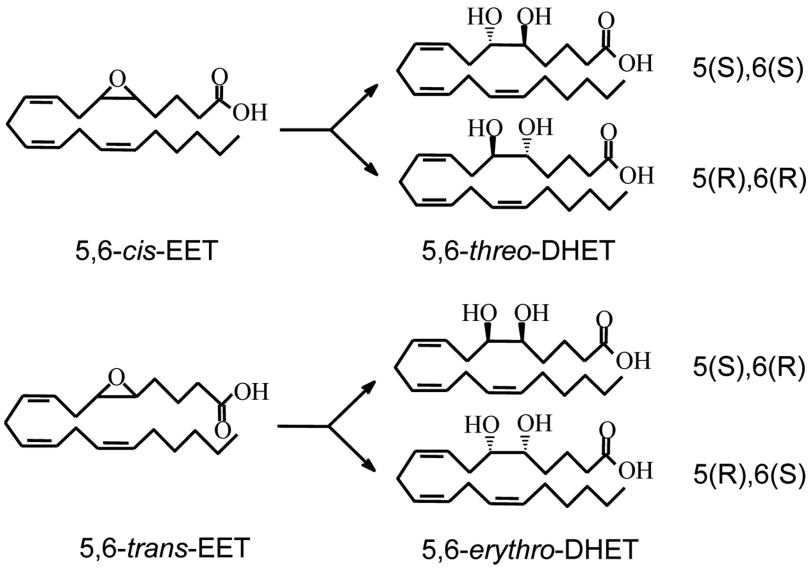

Fig. 1.

Structures of the 5,6-*cis*-EET, 5,6-*trans*-EET and their corresponding DHET enantiomers.

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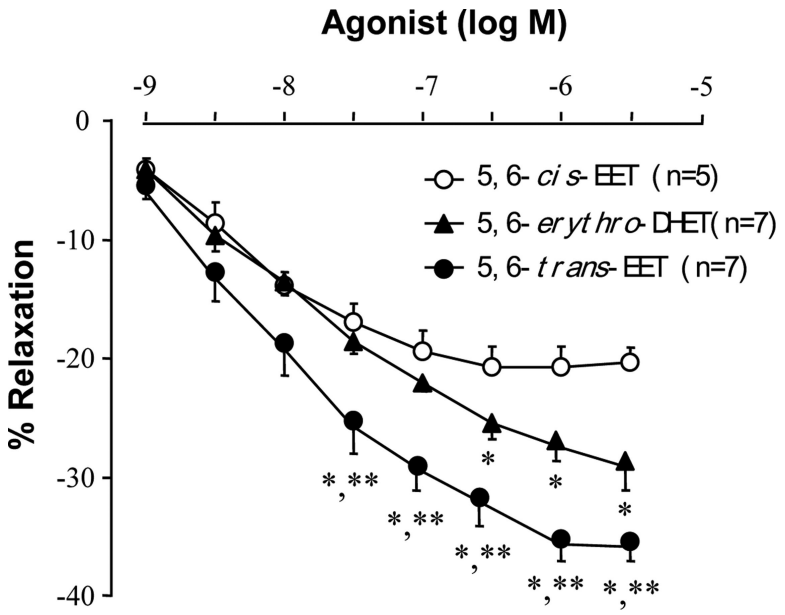

Fig. 2.

Vasorelaxation effects of 5,6-*trans*-EET and 5,6-*erythro*-DHET compared to 5,6-*cis*-EET. Renal interlobar artery rings of Sprague-Dawley rats were preconstricted by phenylephrine (1 μM) and isometric tension was measured. (*P<0.05, comparing to 5,6-*cis*-EET; **P<0.05 comparing to 5,6-*erythro*-DHET.)

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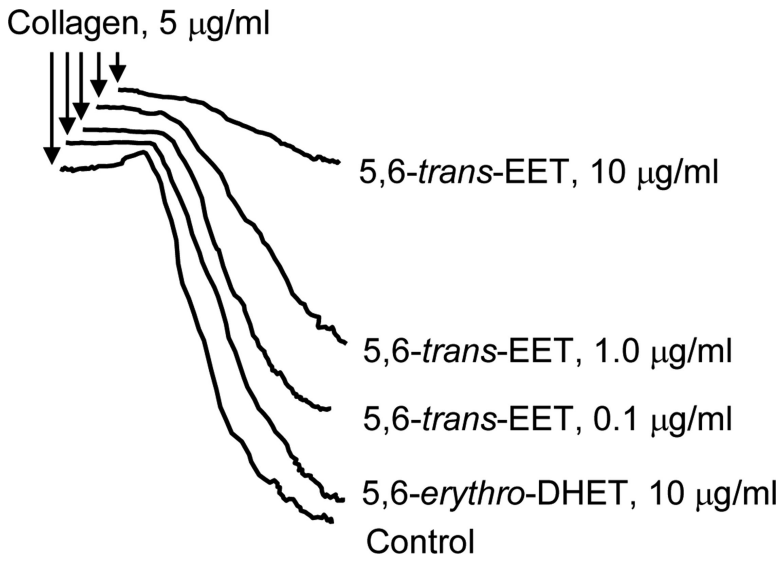

Fig. 3.

5,6-*trans*-EET inhibits Sprague-Dawley rat platelet aggregation. Platelets were preincubated with buffer (control), 5,6-*erythro*-DHET, or 5,6-*trans*-EET at the indicated concentrations for 2 min at 37°C. Collagen was added and platelet aggregation was allowed to proceed for 4 min, n=4-6.