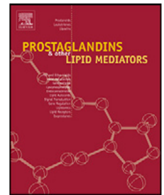
Prostaglandins & other Lipid Mediators 93 (2010) 84–92

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

journal homepage: www.elsevier.com/locate/prostaglandins



Increased expression and altered subcellular distribution of PKC-δ and PKC-ε in pulmonary arteries exposed to hypoxia and 15-HETE

Xiaohong Li [a,1], Cui Ma [b,1], Daling Zhu [b], Liwei Meng [a], Lei Guo [b], Yali Wang [b], Lei Zhang [b], Zhixue Li [a], Enyou Li [a,*]

[a] Department of Anaesthesiology, The First Affiliated Hospital of Harbin Medical University, 23 Youzheng Street, Nangang District, Harbin, Heilongjiang 150001, PR China
[b] Department of Biopharmaceutical Sciences, College of Pharmacy, Harbin Medical University, 157 Baojian Road, Nangang District, Harbin, Heilongjiang 150081, PR China

ARTICLE INFO

Article history:
Received 25 February 2010
Received in revised form 24 June 2010
Accepted 28 June 2010
Available online 6 July 2010

Keywords:
Hypoxic pulmonary vasoconstriction
15-Hydroxyeicosatetraenoicacid
Protein kinase C isozymes
Signal pathway

ABSTRACT

15-Hydroxyeicosatetraenoic acid (15-HETE), a product of arachidonic acid (AA) catalyzed by 15-lipoxygenase (15-LOX), is an important mediator of hypoxic pulmonary vasoconstriction (HPV). We have previously reported that 15-HETE-induced pulmonary vasoconstriction occurs via protein kinase C (PKC) pathway, however, the role of PKC isoforms involved in 15-HETE-induced pulmonary vasoconstriction remains poorly understood. To examine the potential role of PKC-δ and PKC-ε isoforms that appear to be involved in 15-HETE-induced pulmonary artery (PA) contraction, a combination of immunofluorescence, western blotting, semi-quantitative PCR and functional contractile tension approaches on rat PA rings were utilized. We found that 15-HETE activates the translocation of PKC-δ and PKC-ε from the cytoplasm to the membranes of pulmonary arterial smooth muscle cells (PASMCs). However, the alteration was significantly reversed by nordihydroguairetic acid (NDGA), a 15-LOX inhibitor which blocked the formation of endogenous 15-HETE. Both endogenous and exogenous 15-HETE enhanced the expression of PKC-δ and PKC-ε in PASMCs exposure to hypoxia. The PKC inhibitor Gö6983 and rottlerin (PKC-δ selective), and the inhibitor selective for PKC-ε peptide significantly attenuated constriction effect of 15-HETE on isolated PA rings of rats maintained for 9 days in hypoxic environments ($FiO_2 = 0.12$) compared with siblings rats under normoxia. Thus, these findings indicate that PKC-δ and PKC-ε contributing to hypoxic pulmonary artery contraction elicited by 15-HETE.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Hypoxic pulmonary vasoconstriction (HPV) is an essential adaptive vasomotor response to alveolar hypoxia, which redistributes pulmonary blood flow from areas of low oxygen partial pressure to areas of high oxygen availability [1]. Alternatively, chronic, prolonged hypoxic exposure induces sustained pulmonary vasoconstriction, vascular remodeling and progressive pulmonary arterial hypertension, leading to right heart failure and death [2–4]. Despite intensive research, its exact mechanisms of hypoxic pulmonary vasoconstriction have remained elusive.

In previous studies, we have found that hypoxia up-regulates the activity of 15-LOX, which catalyzes AA into 15-HETE [5]. 15-HETE causes concentration-dependent constriction of PA ring through k^+ channels in vascular smooth muscle cells [6,7]. Furthermore, Guo et al. [8] reported that 15-HETE-induced pulmonary vasoconstriction and down-regulates the expression of K_V 1.5, K_V 2.1 and K_V 3.4 channels might through PKC other than PKA signaling pathway. It is well known that PKC consists of a family of serine/threonine kinases with at least 12 members; numerous PKC isozymes are expressed in vascular smooth muscle (α, β, ε, δ and ζ) [9,10]. No studies have yet identifiably addressed which PKC isozymes that appear to be involved in 15-HETE mediating pulmonary artery contraction.

In this study, we hypothesized that 15-HETE-induced hypoxic pulmonary vasoconstriction through members of the novel PKC isoforms. As PKC-δ and PKC-ε are expressed mainly in heart and the pulmonary vasculature, the first efforts of the study are focused on these isoforms. Immunofluorescence shows that 15-HETE and hypoxia activate the translocation of PKC-δ and PKC-ε from the cytoplasm to the membranes of PASMCs. Striking increases were observed in the expression of PKC-δ and PKC-ε at the protein and RNA levels exposure to hypoxia induced by 15-HETE. To examine the effects of PKC inhibitors on hypoxic pulmonary artery contraction, we used an animal model in which rats were exposed to low FiO_2 (0.12) for 9 days. We selected this model because continued exposure to hypoxia in this model results in increased right ventricular mass by 50–60 days of sustained hypoxia [11,12]. Our results show that inhibitors of PKC-δ and PKC-ε significantly attenuated

* Corresponding author. Tel.: +86 0451 53643849x5566.
E-mail addresses: enyouli@yahoo.cn, lixiaohong19731973@yahoo.com.cn (E. Li).
[1] These authors contributed equally to this work.

1098-8823/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.prostaglandins.2010.06.008

constriction effect of 15-HETE on isolated PA rings of rat under hypoxia. The data are consistent with PKC-δ and PKC-ε contributing to pulmonary artery contraction mediated by 15-HETE.

2. Materials and methods

2.1. Materials

15-HETE, cinnamyl 3,4-dihydroxy-[alpha]-cyanocinnamate (CDC), nordihydroguairetic acid (NDGA) were obtained from Cayman Chemical Company (Ann Arbor, MI). PKC-δ (sc-937), PKC-ε (sc-214) antibodies and peptide (sc-3095) were provided by Santa Cruz Biotechnology (CA, USA). Gö6983 was purchased from Biomol Company (Michign, USA) and rottlerin was purchased from Merck-Biosciences (Darmstadt, Germany). All other reagents were obtained from Sigma–Aldrich Co. (MO, USA).

2.2. Animals and lung tissues preparation

Adult male Wistar rats with a mean weight of 200 g were from the Experimental Animal Center of Harbin Medical University, which is fully accredited by the Institutional Animal Care and Use Committee (IACUC). Twelve-hour light exposure cycles, standard rat chow, and water ad libitum were provided to all rats. Adult male Wistar rats were randomized to 9 days of normal and hypoxic environments with fractional inspired oxygen (FiO_2) 0.21 and 0.12, respectively as previously described [5]. At the end of the 9 days exposure period, we anesthetized each rat with pentobarbital injection (50 mg/kg, i.p.), opened the thorax and removed the heart and lungs to ice-cold krebs solution (mmol/L: KCl 4.7, $MgSO_4$ 0.57, KH_2PO_4 1.16, $CaCL_2$ 2.5, $NaHCO_3$ 24, glucose 10 and NaCl 118, pH 7.4), followed by micro-dissection of PA rings.

2.3. Tension studies of PA rings

The arterial ring preparation was performed as our previous reports [5]. Briefly, microdissected distal PA were cut into rings ≈0.5–1.5 mm diameter and examined for isometric contractile responses as described [13]. The rings were attached to tension-measuring devices by tungsten wire hooks initially loaded with basal tension of 0.3 g. The experiments were divided into two groups depending on different PKC inhibitors. The PKC-δ inhibitors group: (1) (1) normoxia (2) normoxia + gö6983 (3) normoxia + rottlerin (2) (1) hypoxia (2) hypoxia + gö6983 (3) hypoxia + rottlerin. (3) (1) hypoxia (2) hypoxia + NDGA (3) hypoxia + NDGA + gö6983 (4) hypoxia + NDGA + rottlerin. The PKC-ε inhibitor group: (1) (1) normoxia (2) normoxia + peptide (3) hypoxia (4) hypoxia + peptide. (2) (1) hypoxia (2) hypoxia + NDGA (3) hypoxia + NDGA + peptide. PA rings isolated from normoxic and hypoxic rats were incubated with the 15-LOX inhibitor NDGA (30 μM), the PKC inhibitor gö6983 (50 nM), the PKC-δ inhibitor rottlerin (10 μM), the PKC-ε inhibitor peptide (1 μM) and NDGA plus gö6983, rottlerin or peptide for 30 min, respectively. The PA rings without any treatment were taken as control. Then the PA rings were treated with incremental amounts of 15-HETE from 10^{-7} to 10^{-5} mol/L. Tension data were relayed from the pressure transducers to a signal amplifier (600 series eight-channel amplifier, Gould Electronics). Data were acquired and analyzed with CODAS software (Data Q Instruments Inc.).

2.4. Cell culture and protocols

PASMCs were dispersed according to our previously published Protocol [8]. Cells were cultured in 20% fetal bovine serum (FBS) – Dulbecco's modified eagle's medium (DMEM) and in a 37 °C, 5% CO_2 humidified incubator. Cell viability determined by Trypan Blue exclusion was consistently greater than 98%. The purity of PASMCs in the primary cultures was determined by specific monoclonal antibodies raised against smooth muscle α-actin (Boehringer Mannheim, Germany). Passages 2–3 were used for further experimentations. Before each experiment, the cells were incubated in DMEM without serum for 24 h to stop cell growth. Quiescent (growth-arrested) PASMCs were divided into five groups for 24 h for western blot and PCR analysis: (1) normoxia (21% O_2/5% CO_2/balance N_2); (2) hypoxia (3% O_2, 5% CO_2, and balance N_2); (3) hypoxia with NDGA (30 μM) or CDC (5 μM); (4) hypoxia with NDGA (30 μM) or CDC (5 μM) plus 15-HETE (1 μM); and (5) hypoxia with 15-HETE (1 μM).

2.5. Immunofluorescence labeling and microscopy

PASMCs were cultured in 24 well culture clusters for 24 h under the following conditions: (1) normoxia (21% O_2/5% CO_2/balance N_2); (2) normoxia with 15-HETE (1 μM); (3) hypoxia (3% O_2, 5% CO_2/balance N_2); and (4) hypoxia with NDGA(30 μM). Then PASMCs were fixed with 4% paraformaldehyde solution at room temperature for 15 min. After washed out with PBS for two times, the cells were blocked in 3% bovine serum at 37 °C for 30 min, then incubated with anti-PKC-δ or anti-PKC-ε primary antibody (1:50) in PBS at 4 °C overnight. After rinsed twice with PBS, the cells were incubated with FITC-conjugated secondary antibody (1:100) diluted by PBS at 37 °C for 2 h in the dark. The FITC labeled cells were monitored the translocation of PKC-δ and PKC-ε and imaged with a fluorescent microscope (Nikon E800, Japan).

2.6. Western blot analysis

Cultured rat PASMCs were gently washed twice in cold PBS, scraped into 0.3 mL lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 μg/mL of phenylmethylsulfonyl fluoride, and 30 μL/mL of aprotinin), and incubated for 30 min on ice. Afterwards, the lysates were sonicated and centrifuged at 16,099 × g for 10 min, and the insoluble fraction was discarded. The protein concentrations in the supernatant were determined by the Bradford assay with bovine serum albumin as a standard [14]. Protein samples were subjected to 10% SDS-PAGE and then transferred to nitrocellulose membranes. After incubation for 1 h at 22–24 °C in a blocking buffer (Tris 20 mM, pH 7.6, NaCl 150 mM, and Tween 20 0.1%) containing 5% nonfat dry milk powder, the membranes were reacted with anti-PKC-δ or anti-PKC-ε antibody over night at 4 °C. The blots were then incubated with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents.

2.7. Gene expression analysis

Total RNA was extracted from cultured rat PASMCs using Trizol reagent and determined by ultraviolet spectrophotometry (absorbance at 260 nm/280 nm). RNA was reversely transcribed using SuperScript TM First-Strand cDNA synthesis system. Sequence of PKC-δ cDNA was obtained from the GenBank TM database (Accession # NM 133307), and also PKC-ε (Accession # NM 017171). Accordingly, PKC-δ: sense primer, 5′GAGGCACTCACCACAGAC3′ and anti-sense primer, 5′AGGTCCAGCCAGAACTCA3′, were designed for a 223-bp fragment. PKC-ε: sense primer, 5′CCCTTATCTAACCCAACTCTAT3′, and anti-sense primer, 5′ACCGCTGAATCTGGAACA3′, were designed for a 103 bp fragment. β-Actin control sense primer, 5′ACTATCGGCAATGAGCG3′, and anti-sense primer, 5′GAGCCAGGGCAGTAATCT3′, were used to obtain a 230 bp fragment. The PCR products were amplified in a DNA thermal cycler, followed by electrophoresis through a 1% agarose gel. The ampli-

Download English Version:

https://daneshyari.com/en/article/2019680

Download Persian Version:

https://daneshyari.com/article/2019680

Daneshyari.com