Biochemical and Biophysical Research Communications 402 (2010) 265–271

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Oleate and eicosapentaenoic acid attenuate palmitate-induced inflammation and apoptosis in renal proximal tubular cell

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ARTICLE INFO

Article history:
Received 29 September 2010
Available online 16 October 2010

Keywords:
Renal lipotoxicity
PKCθ
Inflammation
Apoptosis
Free fatty acid
Dgat2

ABSTRACT

Free fatty acid (FFA)-bound albumin, which is filtrated through the glomeruli and reabsorbed into proximal tubular cells, is one of the crucial mediators of tubular damage in proteinuric kidney disease. In this study, we examined the role of each kind of FFA on renal tubular damage *in vitro* and tried to identify its molecular mechanism. In cultured proximal tubular cells, a saturated fatty acid, palmiate, increased the expression of monocyte chemoattractant protein-1 (MCP-1), but this effect was abrogated by co-incubation of monounsaturated fatty acid, oleate, or ω-3 polyunsaturated fatty acid, eicosapentaenoic acid (EPA). Palmitate led to intracellular accumulation of diacylglycerol (DAG) and subsequent activation of protein kinase C protein family. Among the several PKC inhibitors, rottlerin, a PKCθ inhibitor, prevented palmitate-induced MCP-1 expression via inactivation of NFB pathway. Overexpression of dominant-negative PKCθ also inhibited palmitate-induced activation of MCP-1 promoter. Furthermore, palmitate enhanced PKCθ-dependent mitochondrial apoptosis, which was also prevented by co-incubation with oleate or EPA through restoration of pro-survival Akt pathway. Moreover, oleate and EPA inhibited palmitate-induced PKCθ activation through the conversion of intracellular DAG to triglyceride with the restoration of diacylglycerol acyltransferase 2 expression. These results suggest that oleate and EPA have protective effects against the palmitate-induced renal tubular cell damage by inhibiting PKCθ activation.

1. Introduction

In proteinuric kidney disease, lipotoxicity has been proposed as a pathogenic mechanism of progressive renal tubulointerstitial damage [1–3], which correlates with renal prognosis [4]. Serum free fatty acids (FFAs) bound to albumin are filtered through the glomeruli, reabsorbed by the proximal tubular cells and followed by their metabolism. Several experimental studies using FFA-bound albumin-overload animal models have shown that excess FFA-load in proteinuria induces severe tubulointerstitial injury consisting of inflammatory cell infiltration and renal tubular cell apoptosis [1–3].

Serum FFAs are composed of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, and this composition is modified by metabolic state such as metabolic syndrome and obese [5], which may affect renal prognosis. However, at present, the contribution of the composite change in serum FFA to tubulointerstitial damage remains unclear. Therefore, understanding the effect and mechanism of each type of FFA on tubular cell damage should provide a new therapeutic strategy to improve renal prognosis of obese or diabetic patients with overt proteinuria.

Several molecular pathways are involved in FFAs-mediated tissue or cell dysfunction [6–8]. Among these, recent studies have shown that accumulation of FFAs-mediated diacylglycerol (DAG) contributes to the activation of protein kinase C isoforms (PKCs) and subsequent tissue impairments [9,10]. PKCs are divided into classified into conventional, novel, and atypical subfamilies, and several of these have been identified in the kidney. However, until recently, there are no studies on the contribution of each kind of PKC to the FFA-mediated tubular damage.

In the present study, we evaluated the effects and molecular mechanism of saturated FFA (palmitate), monounsaturated FFA (oleate), and ω-3 polyunsaturated FFA (eicosapentaenoic acid;

Abbreviations: MCP-1, monocyte chemoattractant protein-1; EPA, eicosapentaenoic acid; DAG, diacylglycerol; FFA, free fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFAs, polyunsaturated fatty acids; TNFα, tumor necrosis factor-α; LPS, lipopolysaccharide; PKC, protein kinase C; mPTEC, mouse proximal tubular epithelial cell; LDs, lipid droplets; Dgat, diacylglycerol acyltransferase; TLRs, Toll-like receptors; hPTEC, human proximal tubular epithelial cell.

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doi:10.1016/j.bbrc.2010.10.012

EPA) on the expression of monocyte chemoattractant protein-1 (MCP-1) and apoptosis in cultured proximal tubular cells, with a special focus on FFA-mediated PKCs activation.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA; fatty acid free, fraction V) was obtained from Nacalai Tesque (Kyoto, Japan). BSA did not contain a high level of endotoxin (<3.0 ng/ml), as confirmed by the Endospacy method (FALCO, Kyoto, Japan). Anti-PARP, anti-cleaved caspase 3, anti-phospho-PKCθ(Thr^{538}), anti-PKC, anti-phospho-PKCμ(Ser^{916}), anti-phospho-PKCδ(Thr^{505}), anti-PKCδ, anti-phospho-PKCβII(Ser^{660}), anti-phospho-PKCα/βII(Thr$^{638/641}$), anti-phospho-PKCζ/λ(Thr$^{410/403}$), anti-phospho-p44/p42 MAPK(Thr^{202}/Tyr^{204}), anti-phospho-p38 MAPK(Thr^{180}/Tyr^{182}), anti-phospho-IκBα(Ser176/^{180}), anti-phospho-JNK(Thr^{183}/Tyr^{185}), anti-p44/p42 MAPK, anti-p38 MAPK, anti-IκBα, anti-Bad, anti-phospho-Bad(Ser^{136}), anti-histone, anti-phospho-Akt(Ser^{473}) and anti-JNK antibodies were purchased from Cell Signalling Technology (Beverly, MA). Anti-Akt, anti-PKCα, anti-PKCζ/λ and anti-PKCβII antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Rottlerin, GÖ6850, GÖ6976, and GÖ6983 were purchased from Calbiochem (San Diego, CA). Plasmid vectors encoding the mouse PKCβII, PKCμ, PKCδ, and PKCθ were kindly provided by Dr. A. Reifel Miller (Lilly Research Laboratories, Indianapolis, IN) [11]. Wild-type PKCθ and mutated PKCθ(K/R) and (A/E) were kindly provided by Dr. Gottfried Baier (Innsbruck Medical University, Innsbruck, Austria) [12].

2.2. Cell culture

Mouse proximal tubular epithelial cells (mPTEC) were cultured as described [13]. Primary human proximal tubular epithelial cells (hPTEC) were purchased from Primary Cell Co., Ltd. (Hokkaido, Japan). Lipid-containing media were prepared by conjugation of FFA with FFA-free bovine serum albumin. Briefly, sodium palmitate (Sigma, St. Louis, MO), oleic acid (Sigma), eicosapentaenoic acid

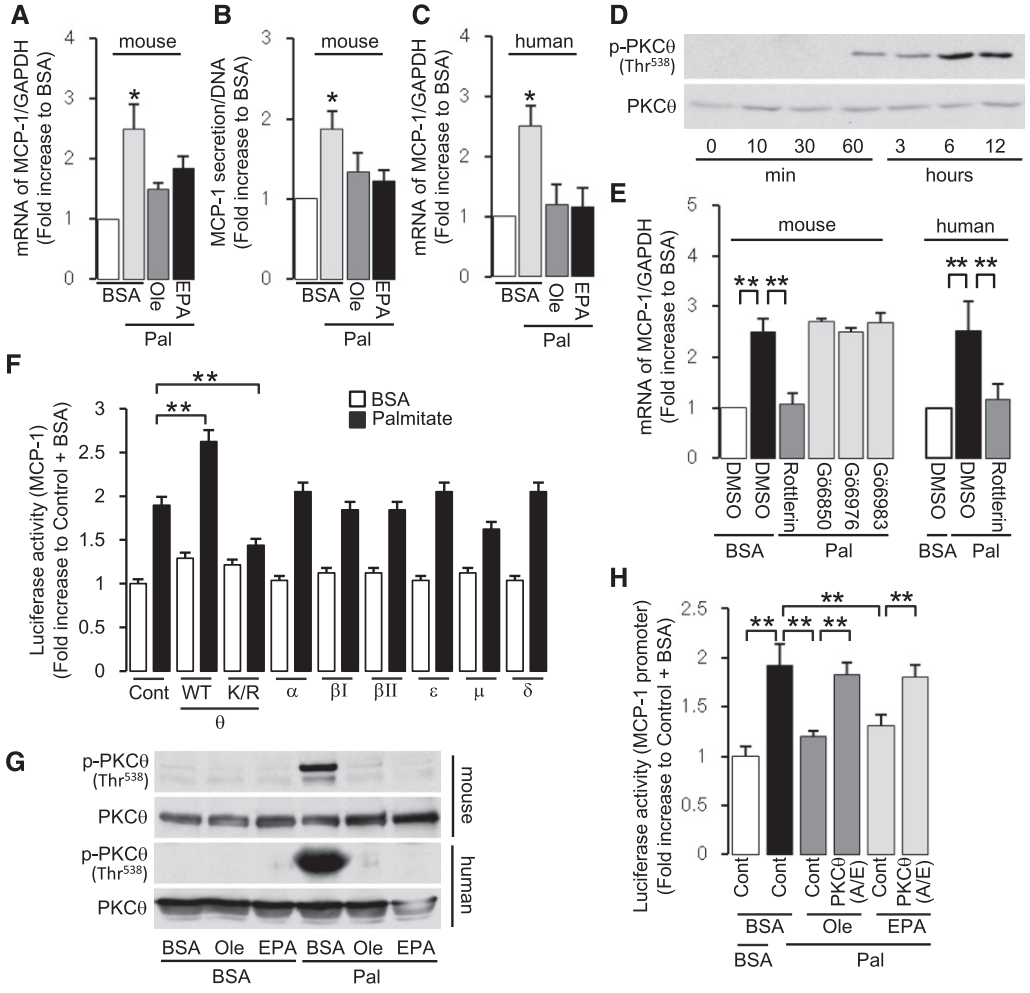

Fig. 1. Oleate and EPA abrogate palmitate-induced MCP-1 expression through the inhibition of PKCθ in renal tubular cell. (A) mRNA expression of MCP-1 in mPTEC incubated with 150 μM of palmitate and supplemented with either 150 μM oleate or 50 μM EPA. (B) MCP-1 secretion in the culture medium, determined by ELISA, incubated for 18 h in the presence of different FFAs. (C) mRNA expression in hPTEC incubated with palmitate (150 μM) and supplemented with either 150 μM oleate or 50 μM EPA. (D) Immunoblot showing phosphorylation of PKCθ in mPTEC exposed to palmitate (150 μM). (E) mRNA expression of MCP-1 in mPTEC and hPTEC exposed to palmitate (150 μM) for 12 h in the presence of each PKCs inhibitor. Rottlerin; PKCθ and δ inhibitor, Gö6850; PKCα, βI, βII, γ, δ and ε inhibitor, Gö6976; PKCα, βI and μ inhibitor, Gö6983: PKCα, βI, βII, γ, δ and ζ/λ inhibitor. (F) MCP-1 promoter activity in each PKCs overexpression vector-transfected mPTEC under treatment with palmitate (150 μM) for 12 h. Data were expressed as fold increase to BSA + Control vector. (G) Immunoblot showing phosphorylation of PKCθ in mPTEC and hPTEC exposed to palmitate (150 μM) for 6 h in the presence of either oleate (150 μM) or EPA (50 μM). (H) MCP-1 promoter activity in mutated PKCθ(A/E)-transfected mPTEC under treatment with palmitate (150 μM) for 12 h, supplemented with oleate or EPA. All data are expressed as mean ± SD of three independent experiments. $^{*}P < 0.01$ vs. other groups. $^{**}P < 0.05$ vs. indicated groups. WT; wild type of PKCθ. K/R; dominant-negative mutated PKCθ. A/E; constitutive-active mutated PKCθ.