Nitric Oxide 33 (2013) 18–41

Contents lists available at SciVerse ScienceDirect

Nitric Oxide

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

Contribution of iNOS/sGC/PKG pathway, COX-2, CYP4A1, and gp91^{phox} to the protective effect of 5,14-HEDGE, a 20-HETE mimetic, against vasodilation, hypotension, tachycardia, and inflammation in a rat model of septic shock ☆

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ARTICLE INFO

Article history:
Received 2 January 2013
Revised 23 April 2013
Available online 14 May 2013

Keywords:
Endotoxin
Hypotension
iNOS/sGC/PKG pathway
COX-2
CYP4A1
gp91^{phox}/NOX2

ABSTRACT

We have previously demonstrated that a stable synthetic analog of 20-hydroxyeicosatetraenoic acid (20-HETE), *N*-[20-hydroxyeicosa-5(*Z*),14(*Z*)-dienoyl]glycine (5,14-HEDGE), prevents vascular hyporeactivity, hypotension, tachycardia, and inflammation in rats treated with lipopolysaccharide (LPS) and mortality in endotoxemic mice. These changes were attributed to decreased production of inducible nitric oxide (NO) synthase (iNOS)-derived NO, cyclooxygenase (COX)-2-derived vasodilator prostanoids, and proinflammatory mediators associated with increased cytochrome P450 (CYP) 4A1-derived 20-HETE and CYP2C23-dependent antiinflammatory mediator formation. The aim of this study was to determine whether decreased expression and activity of iNOS, soluble guanylyl cyclase (sGC), protein kinase G (PKG), COX-2, gp91^{phox} (NOX2; a superoxide generating NOX enzyme), and peroxynitrite production associated with increased expression of COX-1 and CYP4A1 and 20-HETE formation in renal and cardiovascular tissues of rats contributes to the effect of 5,14-HEDGE to prevent vasodilation, hypotension, tachycardia, and inflammation in response to systemic administration of LPS. Mean arterial pressure fell by 28 mmHg and heart rate rose by 47 beats/min in LPS (10 mg/kg, i.p.)-treated rats. Administration of LPS also increased mRNA and protein expression of iNOS and COX-2 associated with a decrease in COX-1 and CYP4A1 mRNA and protein expression. Increased NOS activity, iNOS-heat shock protein 90 complex formation (an index for iNOS activity), protein expression of phosphorylated vasodilator stimulated phosphoprotein (an index for PKG activity), gp91^{phox}, p47^{phox} (NOXO2; organizer subunit of gp91^{phox}), and nitrotyrosine (an index for peroxynitrite production) as well as cGMP (an index for sGC activity), 6-keto-PGF$_{1\alpha}$ (a stable metabolite PGI$_2$) and PGE$_2$ levels (indexes for COX activity), and nitrotyrosine levels by LPS were also associated with decreased CYP hydroxylase activity as measured by 20-HETE formation from arachidonic acid in renal microsomes of LPS-treated rats. These effects of LPS, except iNOS mRNA and COX-1 protein expression, were prevented by 5,14-HEDGE (30 mg/kg, s.c.; 1 h after LPS). A competitive antagonist of vasoconstrictor effects of 20-HETE, 20-hydroxyeicosa-6(*Z*),

Abbreviations: 20-HEDE, 20-hydroxyeicosa-6(*Z*),15(*Z*)-dienoic acid; AA, arachidonic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; 5,14-HEDGE, *N*-[20-hydroxyeicosa-5(*Z*),14(*Z*)-dienoyl]glycine; 1,3-PBIT, phenylene-1,3-bis(ethane-2-isothiourea) dihydrobromide; cDNA, complementary deoxyribonucleic acid; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; CYP, cyctochrome P450; EET, epoxyeicosatrienoic acid; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; HR, heart rate; hsp, heat shock protein; IKK, IκB kinase; IκB, inhibitor of κB; IL, interleukin; iNOS, inducible nitric oxide synthase; i.p., intraperitoneally; LPS, lipopolysaccharide; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase 1; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor-κB; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PG, prostaglandin; PKG, protein kinase G; p-VASP, phosphorylated vasodilator stimulated phosphoprotein; RT-PCR, reverse transcription-polymerase chain reaction; s.c., subcutaneously; sEH, soluble epoxide hydrolase; sGC, soluble guanylyl cyclase; TNF, tumor necrosis factor; VASP, vasodilator stimulated phosphoprotein.

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1089-8603/$ - see front matter
http://dx.doi.org/10.1016/j.niox.2013.05.001

15(Z)-dienoic acid (30 mg/kg, s.c.; 1 h after LPS) reversed the effects of 5,14-HEDGE, except iNOS and COX-1 mRNA and protein expression as well as expression of CYP4A1 mRNA. These results suggest that increased CYP4A1 expression and 20-HETE formation associated with suppression of iNOS/sGC/PKG pathway, COX-2, and gp91^{phox} participate in the protective effect of 5,14-HEDGE against vasodilation, hypotension, tachycardia, and inflammation in the rat model of septic shock.

Introduction

The expression of inducible nitric oxide (NO) synthase (iNOS) is enhanced in many tissues in response to mediators released by the lipid A part of lipopolysaccharide (LPS), also known as endotoxin, which is the most potent microbial mediator in the pathogenesis of septic shock [1]. This leads to increased generation of NO, which contributes to a fall in blood pressure, vascular hyporeactivity, multiple organ failure, and high mortality rate that are associated with septic shock [1]. In many models, endotoxin-induced vascular hyporeactivity to vasoconstrictors is associated with an enhanced formation of NO within the blood vessels, involving activation of not only iNOS, but also endothelial NOS (eNOS) [2]. NO is a potent activator of soluble guanylyl cyclase (sGC) and exerts many of its effects by activating sGC, which produces cyclic guanosine monophosphate (cGMP) [3]. NO plays an important role in cGMP-mediated smooth muscle relaxation by activating protein kinase G (PKG) leading to phosphorylation of vasodilator stimulated phosphoprotein (VASP) [4,5]. NO also reacts with superoxide generated by mainly gp91^{phox} (also known as NOX2) in the presence of p47^{phox} (also known as NOXO2; organizer subunit of gp91^{phox}) to form peroxynitrite, a powerful oxidant and nitrating molecule, and subsequent reaction of peroxynitrite with proteins results in nitrotyrosine formation [6,7]. *In vivo*, peroxynitrite generation represents a NO-dependent pathogenic mechanism in conditions such as circulatory shock and chronic inflammatory diseases. In addition to NO, increased production of prostanoids by cyclooxygenase (COX)-2 has also been shown to contribute to systemic hypotension and related organ damage and decreased survival in animals and humans with sepsis [1]. Systemic blockade of iNOS or COX-2 opposes the fall in blood pressure in sepsis and septic shock [1]. This is not only due to withdrawal of the vasodilator effects of NO and prostanoids, but also associated with enhanced production of vasoconstrictor mediators including catecholamines, endothelin-1, and 20-hydroxyeicosatetraenoic acid (20-HETE) as well as activation of the renin-angiotensin system and increased sensitivity of baroreceptor reflex mechanisms [1].

20-HETE is an ω-hydroxylation product of arachidonic acid (AA) that is produced by cytochrome P450 (CYP) enzymes, mainly by the CYP4A and CYP4F isoforms in the kidney, heart, liver, brain, lung, and vasculature [1,8–10]. In the vasculature, 20-HETE causes vasoconstriction in several vascular beds, including renal, cerebral, aortic, mesenteric, and coronary arteries [11–15]. Activation of protein kinases, such as mitogen-activated protein kinase (MAPK),

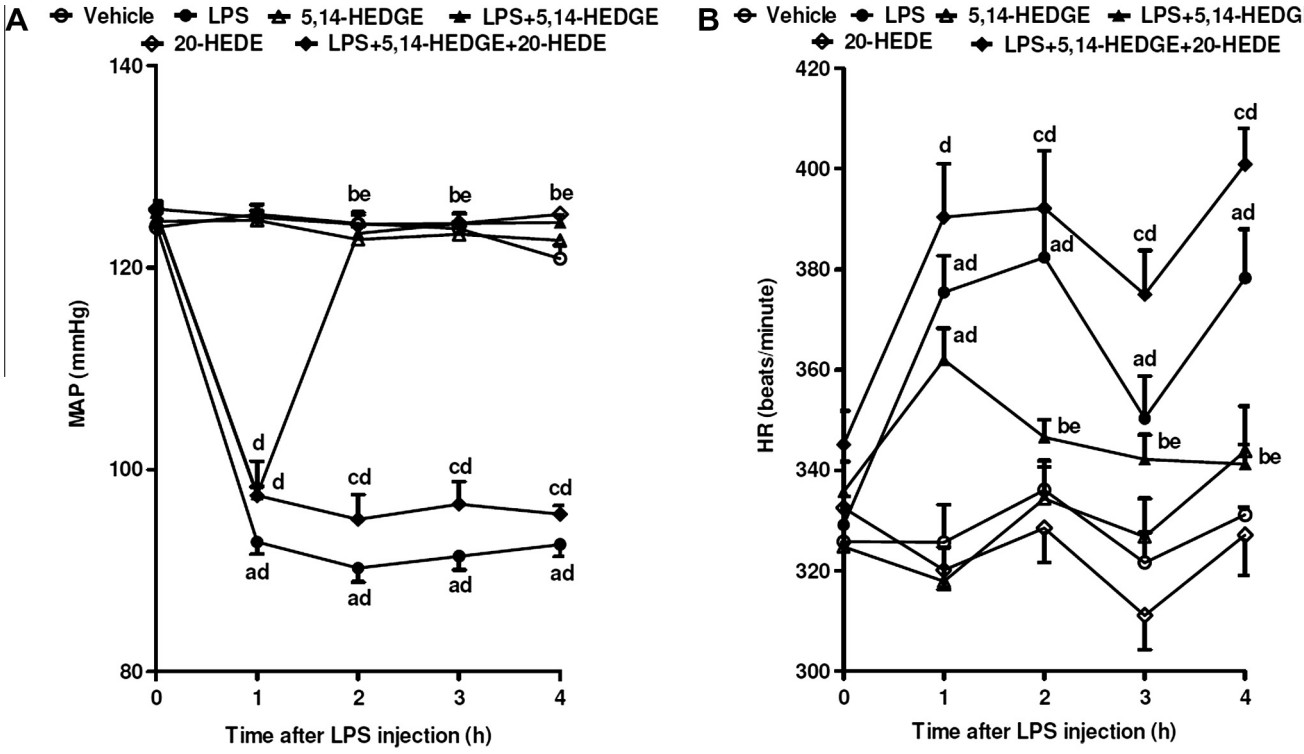

Fig. 1. Time course of the effects of 5,14-HEDGE and 20-HEDE on (A) MAP and (B) HR following administration of saline (vehicle) (4 ml/kg, i.p.) or LPS (10 mg/kg, i.p.) to conscious rats. 5,14-HEDGE (30 mg/kg, s.c.) and/or 20-HEDE (30 mg/kg, s.c.) were given 1 h after administration of saline or LPS. Data are expressed as means ± S.E.M. of 10 animals. [a]Significant difference from the corresponding value seen in rats treated with saline (vehicle) ($p < 0.05$). [b]Significant difference from the corresponding value seen in the rats treated with LPS ($p < 0.05$). [c]Significant difference from the corresponding value seen in the rats treated with LPS and 5,14-HEDGE ($p < 0.05$). [d]Significant difference from the time 0 h value within a group ($p < 0.05$). [e]Significant difference from the time 1 h value within a group ($p < 0.05$).