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Progesterone rapidly down-regulates the biosynthesis of 5-lipoxygenase products in human primary monocytes

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H89 (PubChem CID: 449241)
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cPLA$_2\alpha$ inhibitor RSC-3388, N-{(2S,4R)-4-(biphenyl-2-ylmethyl-isobutyl-amino)-1-[2-(2,4-difluorobenzoyl)-benzoyl]-pyrrolidin-2-ylmethyl}-3-[4-(2,4-dioxothiazolidin-5-ylidenemethyl)-phenyl]acrylamide (PubChem CID: 9833099)
Ca^{2+}-ionophore A23187 (PubChem CID: 11957499)
1-Oleoyl-2-acetyl-*sn*-glycerol (OAG) (PubChem CID: 6437085)
8-Br-cAMP (PubChem CID: 32014)
Prostaglandin E_2 (PubChem CID: 5280360)

ABSTRACT

5-Lipoxygenase (5-LO), the key enzyme in the biosynthesis of pro-inflammatory leukotrienes (LTs) from arachidonic acid, is regulated by androgens in human neutrophils and monocytes accounting for sex differences in LT formation. Here we show that progesterone suppresses the synthesis of 5-LO metabolites in human primary monocytes. 5-LO product formation in monocytes stimulated with Ca^{2+}-ionophore A23187 or with lipopolysaccharide/formyl peptide was suppressed by progesterone at concentrations of 10–100 nM in cells from females and at 1 μM in cells from males. Progesterone down-regulated 5-LO product formation in a rapid and reversible manner, but did not significantly inhibit 5-LO activity in cell-free assays using monocyte homogenates. Also, arachidonic acid release and its metabolism to other eicosanoids in monocytes were not significantly reduced by progesterone. The inhibitory effect of progesterone on LTs was still observed when mitogen-activated protein kinases were pharmacologically blocked, stimulatory 1-oleoyl-2-acetyl-*sn*-glycerol was exogenously supplied, or extracellular Ca^{2+} was removed by chelation. Instead, suppression of PKA by means of two different pharmacological approaches (i.e. H89 and a cell-permeable PKA inhibitor peptide) prevented inhibition of 5-LO product generation by progesterone, to a similar extent as observed for the PKA activators prostaglandin E_2 and 8-Br-cAMP, suggesting the involvement of PKA. In summary, progesterone affects the capacity of human primary monocytes to generate 5-LO products and, in addition to androgens, may account for sex-specific effects on pro-inflammatory LTs.

Abbreviations: AA, arachidonic acid; cPLA$_2$, cytosolic phospholipase A$_2$; CDC, cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate; COX, cyclooxygenase; ERK, extracellular signal-regulated kinases; FLAP, 5-lipoxygenase-activating protein; fMLP, N-formyl-methionyl-leucyl-phenylalanine; 12-HHT, 12-hydroxyheptadecatrienoic acid; 5-H(P)ETE, 5-hydro(pero)xyeicosatetraenoic acid; LO, lipoxygenase; LT, leukotriene; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; PBMC, peripheral blood mononuclear cells; PKA, protein kinase A; PR, progesterone receptor.

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Introduction

5-Lipoxygenase (5-LO) is a key enzyme in the biosynthesis of arachidonic acid (AA)-derived lipid mediators with biological roles as modulators of inflammation. In intact cells, AA is released from membrane phospholipids by cytosolic phospholipase (cPL)A_2 and then metabolized by 5-LO in a two-step reaction involving formation of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and further conversion to leukotriene LTA_4 [1]. LTA_4 is an unstable epoxide, which then serves as precursor for the generation of LTB_4, which induces leukocyte chemotaxis and activation, or for the synthesis of cysteinyl-containing LTC_4, LTD_4, and LTE_4 (cys-LTs), which increase vascular permeability and induce smooth muscle contraction [2]. 5-LO is mainly expressed in mature leukocytes, such as granulocytes and monocytes, and cellular formation of 5-LO products appears to be a highly regulated event, involving different factors and additional proteins [1,3]. Thus, Ca^{2+}, various glycerides and phosphorylations are important for 5-LO activity, and also regulate the amount of AA available and accessible to 5-LO, by affecting $cPLA_2$ activity and 5-LO translocation. In fact, 5-LO associates with coactosin-like protein [4] and translocates upon activation from a soluble cellular compartment (either the cytosol or the nucleus, depending on the cell type) to the nuclear membrane, where it interacts with membrane phospholipids and with 5-LO-activating protein (FLAP) for metabolism of endogenous AA [5,6], while exogenously supplied AA can also be converted at different cellular compartments [7]. Phosphorylation of 5-LO may particularly occur at Ser271 by p38 mitogen-activated protein kinase (MAPK)-regulated MAPK-activated protein kinase (MK)-2/3 [8], at Ser663 by extracellular signal-regulated kinases (ERKs) [9], and has provided a molecular basis for the 5-LO stimulatory effect of cell stress via p38MAPK [10]. On the other hand, protein kinase A can phosphorylate 5-LO at Ser523 which may suppress 5-LO activity and may account for the inhibitory effect of PKA-activating agents, such as prostaglandin (PG)E_2 and 8-Br-cAMP in neutrophils [11,12].

As inflammatory mediators, LTs have established roles in bronchial asthma and allergic rhinitis, but may also be involved in cardiovascular disease and cancer [13]. Accordingly, 5-LO represents a drug target for respiratory and cardiovascular diseases [14] and 5-LO regulation at both expression and activity level has long been investigated in presence of pathological factors and inflammation-related agents, including bacterial products (e.g., lipopolysaccharide, LPS; and formyl peptide, fMLP), complement, growth factors, cytokines and chemokines [1]. Because of well-recognized sex differences in the incidence of LT-related diseases, such as a female prevalence for severe asthma [15], we and others have recently focused on the influence of sex and sex hormones as physiological modulators of 5-LO. We observed that female neutrophils and monocytes produced higher amounts of LTs than males, which depended on androgen-mediated differences in 5-LO subcellular localization in neutrophils and in 5-LO/co-factor interactions in monocytes [16,17]. Recently, we demonstrated sex differences in LT synthesis and related inflammatory reactions in an in vivo model of inflammation, the mouse zymosan-induced peritonitis [18]. Moreover, it was reported that dual deletion of 5-LO and 12/15-LO in mice resulted in sex-specific reduction of atheroma formation [19], and only female mice were protected in the platelet-activating factor (PAF)-induced shock by deletion of BLT_1 receptor [20]. In comparative experiments with androgens, we observed a partial down-regulation of LTs by progesterone in monocytes [17] but not in neutrophils [16]. Also, a high capacity for LT biosynthesis in peripheral blood was observed during pregnancy and the stimulatory effect of plasma from pregnant women was lost when monocytes were resuspended in plasma of women in the third trimester [21], suggesting regulation of 5-LO by progesterone in monocytes. Since monocytes are pivotal cells in inflammation and may affect LT-related diseases [22], we here aimed to pharmacologically characterize the influence and signaling of progesterone on 5-LO product synthesis in human monocytes.

Material and methods

Materials

HPLC solvents were from Merck (Darmstadt, Germany). The myristoylated PKA inhibitor peptide (fragment 14–22; Myr-Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ala-Ile-NH_2) and cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate (CDC) were from Enzo Life Sciences International Inc. (Lörrach, Germany). The $cPLA_{2\alpha}$ inhibitor N-{(2S,4R)-4-(biphenyl-2-ylmethyl-isobutyl-amino)-1-[2-(2,4-difluorobenzoyl)-benzoyl]-pyrrolidin-2-ylmethyl}-3-[4-(2,4-dioxothiazolidin-5-ylidenemethyl)-phenyl]acrylamide was from Calbiochem (Bad Soden, Germany). Ca^{2+}-ionophore A23187, fMLP, ionomycin, LPS, 1-oleoyl-2-acetyl-*sn*-glycerol (OAG), H89 and all other chemicals were purchased from Sigma (Deisenhofen, Germany), unless stated otherwise.

Isolation of monocytes

Human monocytes were isolated from leukocyte concentrates as previously reported [17]. Thus, venous blood was collected from fasted (12 h) adult male and female healthy volunteers, with consent (Blood Center, University Hospital, Tuebingen, Germany). The subjects had no apparent inflammatory conditions and had not taken oral contraceptives (or other sex hormones) or anti-inflammatory drugs for at least ten days prior to blood collection. Blood was subjected to centrifugation at $4000 \times g$/20 min/20 °C for preparation of leukocyte concentrates. Peripheral blood mononuclear cells (PBMC) were promptly isolated by dextran sedimentation and centrifugation on Nycoprep cushions (PAA Laboratories, Linz, Austria). Cells were collected, washed three times with cold PBS and then monocytes were separated by adherence for 1 h at 37 °C to culture flasks (Greiner, Nuertingen, Germany; cell density was 2×10^7 cells/ml of RPMI 1640 medium containing 2 mM L-glutamine and 100 U/ml penicillin and 100 μg/ml streptomycin), which gave a purity of >85%, defined by forward- and side-light scatter properties and detection of the CD14 surface molecule by flow cytometry (BD FACS Calibur). Monocytes were finally resuspended in ice-cold PBS plus 1 mg/ml glucose (PG buffer) or in PG buffer supplemented with 1 mM $CaCl_2$ (PGC buffer).

Determination of 5-LO products, 15-LO1 products, and 12-HHT

For determination of cellular 5-LO product formation, human monocytes (1×10^6) in 1 ml PGC buffer were pre-incubated with the indicated compounds at 37 °C, and 5-LO product formation was started by addition of the respective stimuli. The reaction was stopped with 1 ml methanol and 30 μl 1 N HCl, 200 ng prostaglandin B_1 and 500 μl PBS were added. Formed 5-LO metabolites, the 15-LO1 products 12(S)-hydro(pero)xy-6-trans-8,11,14-cis-eicosatetraenoic acid (12-H(P)ETE) and 15(S)-hydro(pero)xy-5,8,11-cis-,13-trans-eicosatetraenoic acid (15-H(P)ETE) and the cyclooxygenase (COX) product 12-hydroxyheptadecatrienoic acid (12-HHT) were extracted and analyzed by HPLC [23]. 5-LO products include LTB_4 and its all-trans isomers, and 5-H(P)ETE.

For determination of 5-LO product formation in homogenates, 1 mM EDTA was added to monocytes resuspended in PBS. Samples were cooled on ice (5 min), sonicated (3×10 s) at 4 °C, and 1 mM ATP was added. As indicated, homogenates were prepared from intact monocytes treated with vehicle (0.05% EtOH) or progesterone (1 μM) for 10 min at 37 °C, or incubation with vehicle or