Biochimica et Biophysica Acta 1821 (2012) 973–979

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Effects of CYP7B1-related steroids on androgen receptor activation in different cell lines

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ARTICLE INFO

Article history:
Received 27 June 2011
Received in revised form 29 February 2012
Accepted 5 March 2012
Available online 28 March 2012

Keywords:
Androgen
CYP7B1
Hydroxylation
AR-mediated response
Steroid metabolism

ABSTRACT

The widely expressed steroid hydroxylase CYP7B1 is involved in metabolism of a number of steroids reported to influence estrogen and androgen signaling. Several studies by us and other investigators have linked this enzyme to effects on estrogen receptor activation. In a previous report we examined the effect of CYP7B1-mediated hormone metabolism for estrogen-mediated response in kidney-derived HEK293 cells. In the current study we used an androgen response element (ARE) reporter system to examine androgen-dependent response of some CYP7B1 substrates and CYP7B1-formed metabolites in several cell lines derived from different tissues. The results indicate significantly lower androgen receptor activation by CYP7B1-formed steroid metabolites than by the corresponding steroid substrates, suggesting that CYP7B1-mediated catalysis may decrease some androgenic responses. Thus, CYP7B1-dependent metabolism may be of importance not only for estrogenic signaling but also for androgenic. This finding, that CYP7B1 activity may be a regulator of androgenic signaling by converting AR ligands into less active metabolites, is also supported by real-time RT-PCR experiment where a CYP7B1 substrate, but not the corresponding product, was able to stimulate known androgen-sensitive genes. Furthermore, our data indicate that the effects of some steroids on hormone response element reporter systems are cell line-specific. For instance, despite transfection of the same reporter systems, 5-androstene-3β,17β-diol strongly activates an androgen-dependent response element in prostate cancer cells whereas it elicits only ER-dependent responses in kidney HEK293 cells. Potential roles of cell-specific metabolism or comodulator expression for the observed differences are discussed.

1. Introduction

Androgenic signaling is important in a broad range of physiological processes, including normal prostate development as well as prostate carcinogenesis. A majority of all prostate cancers are androgen dependent when diagnosed (*i.e.* they rely on androgens to proliferate) [1]. Therefore, decreased androgen production and/or inhibited androgenic signaling have become key strategies in prostate cancer treatment [2].

Androgenic effects are mediated by the androgen receptor (AR). Ligand-activated AR binds to specific DNA sequences named androgen response elements (ARE) in gene promoters and recruits a variety of comodulators. This complex of ligand-activated AR and comodulators can then alter the transcriptional activity of the promoter of target genes.

The major sex hormone precursor dehydroepiandrosterone (DHEA) is excreted in large amounts from the adrenals. In peripheral tissues, DHEA can be converted into different steroids *via* enzyme-catalyzed reactions (Fig. 1). AR can be activated by a number of these steroids, with dihydrotestosterone (DHT) and testosterone as the most potent ligands.

CYP7B1 is a steroid hydroxylase that metabolizes a number of steroids, including DHEA, 5-androstene-3β,17β-diol (Aene-diol), 5α-androstane-3β,17β-diol (3β-Adiol) and 5α-androstane-3α,17β-diol, to the corresponding 7α-hydroxy steroids [3,4]. Conflicting data have been published regarding the effect of DHEA and related steroids on the androgen receptor. Mo et al. [5] have reported that DHEA, but not 7α-hydroxy-DHEA, exerts androgenic effects while Chen et al. [6] suggest that DHEA rather acts as an antagonist to the androgen receptor.

We have previously reported that the CYP7B1 substrates Aene-diol and 3β-Adiol can activate both estrogen receptors α and β

Abbreviations: 3β-Adiol, 5α-androstane-3β,17β-diol; Aene-diol, 5-androstene-3β,17β-diol; Aene-triol, 5-androstene-3β,7α,17β-triol; AR, androgen receptor; ARE, androgen response element; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; ELISA, enzyme-linked immunosorbent assay; FKBP5, FK506 binding protein 5; 3β-HSD/KSI, 3β-hydroxysteroid dehydrogenase/ketosteroid isomerase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 4-MA, 17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one; NCoR-1, nuclear receptor co-repressor 1; PSA, prostate-specific antigen; SMRT, nuclear receptor co-repressor 2; SRC-1, human steroid receptor coactivator; SRA, homo sapiens steroid receptor RNA activator; TLC, thin layer chromatography

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doi:10.1016/j.bbalip.2012.03.007

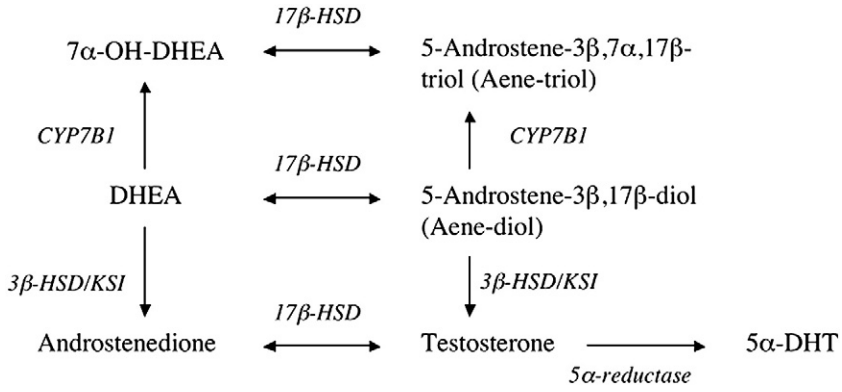

Fig. 1. Enzyme-catalyzed reactions of importance for the studied steroids.

while the corresponding 7α-hydroxylated products cannot [7]. This indicates that the expression level and activity of CYP7B1 could alter estrogenic signaling.

It remains unclear whether Aene-diol exerts androgenic effects. It has been reported by Miyamoto et al. [8] and Mizokami et al. [9] that Aene-diol acts as an androgen in prostate cells while Evaul et al. [10] have suggested that Aene-diol has to be converted to testosterone to gain androgenic effect.

The aim of the present study has been to examine if CYP7B1-catalyzed conversion of steroids could alter the androgenic signaling. This has been conducted by studying the androgen-dependent response to some CYP7B1 substrates and products in an androgen response element (ARE) reporter system.

2. Materials and methods

2.1. Materials

5-Androstene-3β,17β-diol, 5-androstene-3β,7α,17β-triol and 7α-hydroxy-DHEA were purchased from Steraloids Inc. (Wilton, NH). Unlabeled DHEA, androstenedione and DHT were purchased from Sigma. Radiolabeled DHEA was obtained from Perkin Elmer Life Sciences. The human androgen receptor expression vector and the androgen response element luciferase reporter vector were generous gifts from Dr. A. Brinkmann and Dr. J. Trapman, Erasmus Medical Centre, Rotterdam, The Netherlands. Luciferase assay reagent was purchased from Promega. Lipofectamine transfection reagent was purchased from Invitrogen. 17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA) was generously provided by Dr. D.W. Russell, University of Texas Southwestern Medical Center, Dallas, USA. The ELISA kit for assay of testosterone (DE1559) was purchased from Demeditec Diagnostics GmbH, Germany. All other chemicals were of analytical grade and purchased from commercial sources.

2.2. Cell culture

Human embryonic kidney HEK-293 cells (ATCC CRL-1573) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco) and 1% antibiotic/antimycotic (Gibco). Human adrenocortical carcinoma NCI-H295R cells (ATCC CRL-2128) were cultured in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Ham (Sigma) supplemented with 1% ITS Plus premix (BD Biosciences), 2.5% NuSerum (VWR), 1% L-glutamine (Gibco) and 1% antibiotic/antimycotic (Gibco). Human prostate adenocarcinoma LNCaP cells (ATCC CRL-1740) and human prostate carcinoma DU145 cells (ATCC HTB-81) were cultured in RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco) and 1% antibiotic/antimycotic (Gibco). All cells were cultured as monolayers in a humidified environment at 37 °C with 5% CO_2.

2.3. Analysis of AR activation by luciferase reporter assay

Cells were transiently transfected with an AR-responsive luciferase reporter vector together with an expression vector containing cDNA for human androgen receptor (AR) and a pCMVβ-galactosidase plasmid (in order to standardize for transfection efficiency), using Lipofectamine (NCI-H295R cells, DU145 cells and LNCaP cells) in accordance with the manufacturer's recommendations or calcium co-precipitation (HEK-293) as previously described [7]. The AR-responsive vector contains an androgen response element (ARE) coupled to luciferase. Transfected cells were treated for 24–40 h with different steroids dissolved in ethanol and the levels of androgen-dependent luciferase activity were compared with the luciferase levels in cells treated with the same volume of ethanol. In some experiments, ketoconazole (inhibitor of CYP enzymes) was added to the steroid mixture. Luciferase and β-galactosidase activities were assayed as previously described [11,12]. Luciferase activity is expressed as relative light units (RLU) divided by β-galactosidase activity. Data are expressed as fold change compared to control ± standard deviation. In some experiments, the cells were co-treated with 10 μM of trilostane (Sanofi) or 4-MA, dissolved in DMSO, to inhibit 3β-hydroxysteroid dehydrogenase (3β-HSD/KSI)-catalyzed conversion of the added steroids DHEA and Aene-diol.

2.4. RT-PCR

RT-PCR was used to examine the expression of mRNA for human androgen receptor (AR), 3β-hydroxysteroid dehydrogenase (3β-HSD/KSI) types 1 and 2, 5α-reductase type 2, NCoR-1, SMRT, SRC-1 and SRA in NCI-H295R cells, LNCaP cells, HEK-293 cells and DU-145 cells. RNA was isolated using RNeasy Mini Kit (Qiagen) and reverse transcribed to cDNA by Reverse Transcription System (Promega) with oligo dT primer. Amplification was performed using the AmpliTaq Gold system (Applied Biosystems) in accordance with the manufacturer's recommendations. Primers and program were as described by Montgomery et al. [13] for AR, by Hammer et al. [14]for 3β-HSD/KSI type 1, by Asif et al. [15] for 3β-HSD/KSI type 2, by Suzuki et al. [16] for 5α-reductase type 2, and by Scott et al. [17] for nuclear receptor co-repressor 1 (NCoR-1), nuclear receptor co-repressor 2 (SMRT), human steroid receptor coactivator (SRC-1) and homo sapiens steroid receptor RNA activator (SRA). Control experiments with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were performed to confirm RNA integrity.