Biochimica et Biophysica Acta 1801 (2010) 1090–1097

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Effects of CYP7B1-mediated catalysis on estrogen receptor activation

Hanna Pettersson, Johan Lundqvist, Maria Norlin *

Division of Biochemistry, Department of Pharmaceutical Biosciences, University of Uppsala, Box 578, S-751 23 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 12 February 2010
Received in revised form 12 May 2010
Accepted 19 May 2010
Available online 26 May 2010

Keywords:
Estrogen
CYP7B1
Hydroxylation
ER-mediated response
Steroid metabolism

ABSTRACT

Most of the many biological effects of estrogens are mediated via the estrogen receptors ERα and β. The current study examines the role of CYP7B1-mediated catalysis for activation of ER. Several reports suggest that CYP7B1 may be important for hormonal action but previously published studies are contradictory concerning the manner in which CYP7B1 affects ERβ-mediated response. In the current study, we examined effects of several CYP7B1-related steroids on ER activation, using an estrogen response element (ERE) reporter system. Our studies showed significant stimulation of ER by 5-androstene-3β,17β-diol (Aene-diol) and 5α-androstane-3β,17β-diol (3β-Adiol). In contrast, the CYP7B1-formed metabolites from these steroids did not activate the receptor, indicating that CYP7B1-mediated metabolism abolishes the ER-stimulating effect of these compounds. The mRNA level of HEM45, a gene known to be stimulated by estrogens, was strongly up-regulated by Aene-diol but not by its CYP7B1-formed metabolite, further supporting this concept. We did not observe stimulation by dehydroepiandrosterone (DHEA) or 7α-hydroxy-DHEA, previously suggested to affect ERβ-mediated response. As part of these studies we examined metabolism of Aene-diol in pig liver which is high in CYP7B1 content. These experiments indicate that CYP7B1-mediated metabolism of Aene-diol is of a similar rate as the metabolism of the well-known CYP7B1 substrates DHEA and 3β-Adiol. CYP7B1-mediated metabolism of 3β-Adiol has been proposed to influence ERβ-mediated growth suppression. Our results indicate that Aene-diol also might be important for ER-related pathways. Our data indicate that low concentrations of Aene-diol can trigger ER-mediated response equally well for both ERα and β and that CYP7B1-mediated conversion of Aene-diol into a 7α-hydroxymetabolite will result in loss of action.

1. Introduction

Estrogens exert a number of biological effects throughout the body, most of which are mediated via the estrogen receptors ERα and β. Important functions of estrogens include effects in the female as well as the male reproductive system and in several non-reproductive tissues [1–3]. There are also adverse effects of estrogens, for instance related to hormone-dependent carcinogenesis [4,5]. One of the suggested roles of estrogens concerns cellular growth [6]. The role of estrogens in growth is however not well understood. It has been suggested that estrogenic effects on growth may be different for different types of estrogens or may be mediated via differential effects by the two ER receptors [7,8]. For instance, it has been proposed that ERα might stimulate proliferation whereas ERβ acts as an antiproliferative receptor [9,10].

Sex hormone precursors, including dehydroepiandrosterone (DHEA), are excreted in large amounts from the adrenals into the circulation [11]. However, metabolic events in peripheral tissues are essential for local formation of estrogens as well as other sex hormones. Cellular estrogen levels are dependent on the availability of hormone precursors in the circulation but also on local formation in many tissues, which is subject to cell-specific regulatory mechanisms [12,13]. Tissue-specific formation of steroids may be very important for the local requirement(s) of sex hormones.

One physiologically potent estrogen is 17β-estradiol (E2). In addition, there are several other endogenous estrogens with effects in various organs. For instance, an estrogenic steroid formed in prostate as well as in some other tissues, 5α-androstane-3β,17β-diol (3β-Adiol), is a ligand of both ERα and β with highest affinity for ERβ [14–16]. 3β-Adiol is present in high levels in human prostate and is reported to be a major intraprostatic estrogen [17]. 3β-Adiol is a substrate for CYP7B1, a steroid hydroxylase which is widely expressed in humans and other species. CYP7B1 metabolizes a number of steroids, including the sex hormone precursors DHEA and pregnenolone as well as ER ligands and certain cholesterol derivatives [13,18–22]. The chemical structures of some CYP7B1 substrates of interest in this study are shown in Fig. 1.

CYP7B1-mediated catalysis has been suggested to play a role for ER signalling but the manner in which this enzyme affects estrogenic action is not clear. In some previous studies, it was proposed that activation of ERβ by 3β-Adiol may be important for suppression of

Abbreviations: 3β-Adiol, 5α-androstane-3β,17β-diol; 3β-Atriol, 5α-androstane-3β,7α,17β-triol; Aene-diol, 5-androstene-3β,17β-diol; Aene-triol, 5-androstene-3β,7α,17β-triol; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; ER, estrogen receptor; ERE, estrogen response element; E2, 17β-estradiol; HEK, human embryonic kidney

* Corresponding author. Division of Biochemistry, Department of Pharmaceutical Biosciences, University of Uppsala, Box 578, S-751 23 Uppsala, Sweden. Tel.: +46 18 471 4331; fax: +46 18 558778.
E-mail address: Maria.Norlin@farmbio.uu.se (M. Norlin).

1388-1981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2010.05.011

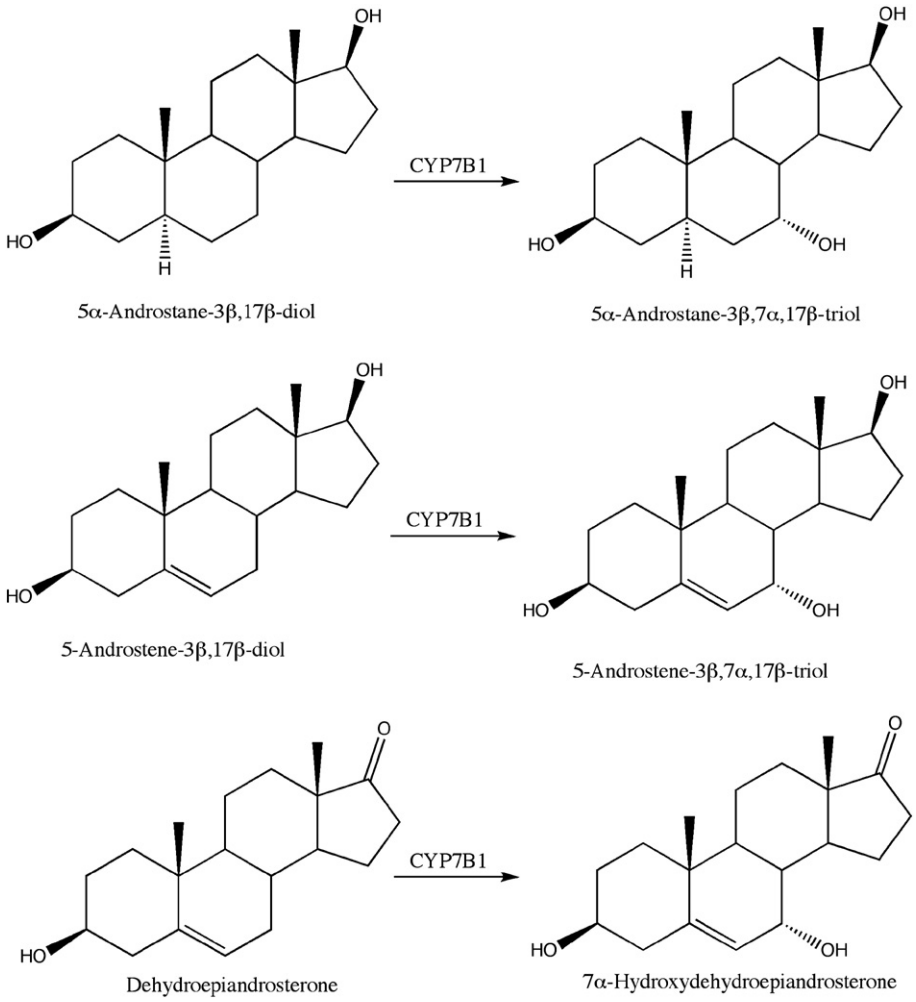

Fig. 1. Chemical structures of some CYP7B1 substrates and CYP7B1-formed products.

prostate growth [17]. Since 3β-Adiol is metabolized by CYP7B1, it was suggested that this enzyme might decrease ERβ action via control of 3β-Adiol levels. In contrast, other investigators reported that a steroid metabolite formed by CYP7B1, 7α-hydroxy-DHEA, activates ERβ and that CYP7B1 catalysis therefore should increase ERβ action [23]. Furthermore, a cholesterol derivative metabolized by CYP7B1, 27-hydroxycholesterol, was recently found to exert pro-estrogenic actions in some cells but suppress ER activation in others [24,25].

In addition to a potential role for ER signalling, 7α-hydroxy-DHEA as well as other CYP7B1-formed steroid metabolites has been proposed to exert several actions in various tissues, including for instance effects on the immune system and in the CNS [26–28]. It is in many cases unclear whether CYP7B1-mediated catalysis represents inactivation mechanisms or biosynthetic pathways to form active hormones.

Since estrogen receptors affect a wide range of physiological systems, it is important to understand events that regulate their function. In the current study, we investigated the roles of CYP7B1-mediated catalytic reactions for estrogen receptor-mediated response.

2. Materials and methods

2.1. Materials

Human embryonic kidney (HEK293) cells (CRL-1573) and breast cancer MCF-7 cells (HTB-22) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Materials for cell culturing were obtained from Invitrogen™. Dehydroepiandrosterone, 5α-androstane-3β,17β-diol, 17β-estradiol and pregnenolone were from Sigma Chemical Co. 7α-Hydroxy-DHEA, 5-androstene-3β,17β-diol, 5-androstene-3β,7α,17β-triol and 7α-hydroxy-pregnenolone were from Steraloids Inc. (Wilton, NH). Radiolabeled DHEA was purchased from Perkin Elmer. 27-Hydroxycholesterol, prepared from kryptogenin [29], was a kind gift from Dr. L. Tökes, Syntex, Palo Alto, CA, USA. The human ERβ and ERα expression vectors and the ERE (estrogen response element) luciferase reporter vector were generous gifts from Dr. P. Chambon, Institute de génétique et de biologie moléculaire et cellulaire, Strasbourg, France and Dr. K. Arcaro, University of Massachusetts, MA, USA, respectively. All remaining chemicals were of analytical grade and purchased from commercial sources.

2.2. Microsomal preparation

Pig liver tissue was weighed, minced and homogenized in sucrose buffer containing 0.25 M sucrose, 10 mM Tris–Cl, pH 7.4, and 1 mM EDTA to a 20% suspension. Microsomes were prepared as previously described [13] and were suspended in 50 mM Tris–acetate buffer, pH 7.4, containing 20% glycerol and 0.1 mM EDTA and stored at −80 °C until incubation. Protein contents of the microsomes were assayed by the method of Lowry *et al.* [30].

2.3. Preparation of ^{3}H-labeled 5-androstene-3β,17β-diol (Aene-diol)

^{3}H-Labeled Aene-diol was prepared from ^{3}H-labeled DHEA by bioconversion with a commercially available hydroxysteroid