Sex steroid hormone metabolism takes place in human ocular cells

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Abstract

Steroids are potentially important mediators in the pathophysiology of ocular diseases. In this study, we report on the gene expression in the human eye of a group of enzymes, the 17β-hydroxysteroid dehydrogenases (17HSDs), involved in the biosynthesis and inactivation of sex steroid hormones. In the eye, the ciliary epithelium, a neuroendocrine secretory epithelium, co-expresses the highest levels of 17HSD2 and 5 mRNAs, and in lesser level 17HSD7 mRNA. The regulation of gene expression of these enzymes was investigated in vitro in cell lines, ODM-C4 and chronic open glaucoma (GCE), used as cell models of the human ciliary epithelium. The estrogen, 17β-estradiol (10^{-7} M) and androgen agonist, R1881 (10^{-8} M) elicited in ODM-C4 and GCE cells over a 24 h time course a robust up-regulation of 17HSD7 mRNA expression. 17HSD2 was up-regulated by estradiol in ODM-C4 cells, but not in GCE cells. Under steady-state conditions, ODM-C4 cells exhibited a predominant 17HSD2 oxidative enzymatic activity. In contrast, 17HSD2 activity was low or absent in GCE cells. Our collective data suggest that cultured human ciliary epithelial cells are able to metabolize estrogen, androgen and progesterone, and that 17HSD2 and 7 in these cells are sex steroid hormone-responsive genes and 17HSD7 is responsible to keep on intra/paracrine estrogenic milieu.
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1. Introduction

Steroids have been long viewed as important factors in the development of hormone dependent cancers such as breast cancer [1]. In eye diseases, steroids have also been considered playing a key role particularly in the development of certain forms of glaucoma. In one form known as "steroid-induced glaucoma", topical or systemic application of corticosteroids in the eye leads to an acerbated elevation of the intraocular pressure and to secondary glaucoma [2]. On the other hand, linkage analysis have identified a number of genes harboring mutations associated with different clinical forms of glaucoma [3]. Among these figured cytochrome P450 (*CYP 1B1*) encoding an enzyme involved in the metabolism of estradiol [4,5], and the *MYOC* (Myocilin/TIGR) gene which exhibits glucocorticoid responsiveness [6,7]. In normal eye donors these genes are abundantly expressed in the tissues associated with the regulation of intraocular pressure such as the trabecular meshwork and the ciliary body. A common feature of many of the clinical forms of glaucoma is an abnormal elevation in the intraocular pressure, which is considered a high risk factor in the development and progression of the disease [8].

Recent studies have shown that the human ciliary epithelium displays characteristic neuroendocrine functions, including the synthesis, processing and release of neuropeptides and hormones restricted to endocrine tissues and cells [9–11]. Since some of the peptides and hormones expressed in the ciliary epithelium have been shown separately to exhibit hypotensive and/or hypertensive effects in the vascular system, we have hypothesized that same peptides and hormones in the ciliary epithelium regulate the intraocular pressure by endocrine mechanism [12]. Furthermore, the ciliary epithelium serves as a protective function to the avascular tissues of the eye, including the cornea, the lens, and the trabecular meshwork, by the abundant expression of genes encoding antioxidant and detoxification enzymes [13,14]. These protecting enzymatic systems are involved to minimize the potential of damage from light-induced free radicals and from xenobiotics by removing them from the aqueous humor.

To further investigate on the endocrine functions of the human ciliary epithelium, we report here on the identification of a group of genes encoding different members of a family of enzymes known as 17β-hydroxysteroid dehydrogenases

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(17HSDs). The 17HSDs mediate the interconversions between 17-keto- and 17β-hydroxysteroids, thereby playing an important role in the biosynthesis and metabolism of sex steroid hormones [15].

At least nine types of human 17HSDs have been identified so far, sharing very low homology among them (types 1–5, 7, 8 and 10 and 11) [15,16]. Types 1, 3, 5 and 7 catalyze reductive reactions whereas types 2, 4 and 8 catalyze oxidative reactions. The 17HSD1 and 7 catalyze the reduction reactions of estrone (E1) to estradiol (E2), a biologically more active estrogen [17]. In addition, 17HSD7 catalyzes the reaction from dihydrotestosterone (DHT) into 5α-androstane-3β-17β-diol (3β-Adiol) [18]. 17HSD2 catalyzes the oxidative reactions between E2 and E1, testosterone (T) and androstenedione (A-dione), and DHT and 5α-androstanedione (5α-A-dione) [19,20], and 20α-hydroxyprogesterone (20-OH-P) into progesterone (P). 17HSD2 metabolizes also xenobiotics [19]. 17HSD5 catalyzes reactions of DHT into 5α-androstane-3α-17β-diol (3α-Adiol) and P into 20-OH-P [21].

In this work we document the pattern of expression of 17HSD2, 5 and 7 mRNA in human ocular tissues and their distinct responsiveness to estrogen and to androgen. We present evidence of the 17HSD-specific oxidative and reductive enzymatic activities in cell lines established from the ciliary epithelium of eye donors. We propose that the 17HSDs in the ocular ciliary epithelium may subserve the endocrine functions of this tissue in vivo by metabolizing sex steroid substrates.

2. Materials and methods

2.1. Tissues and chemicals

Human eyes were obtained from cadavers within 24 h after enucleation through the National Disease Research Interchange (Philadelphia, PA). Under a dissecting microscope, the cornea, lens, ciliary processes, iris and retina were microdissected and stored in liquid nitrogen for further analysis. Investigation of the human subjects was approved by the Human Subjects Committee of Yale University and, as far as it applies, followed the tenets of the Declaration of Helsinki.

Two human cell lines established from the human non-pigmented ciliary epithelium were included in this study. One cell line (ODM-C4) was established from a normal male eye donor [22], whereas the second cell line was from a female eye donor with chronic open glaucoma (GCE) as previously described [23]. These cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and humidified in 5% CO_2/air at 37 °C. Treatment of ODM-C4 and GCE cells with 17β-estradiol (Sigma) or the androgen agonist methyltrienolone (R1881) (New England Nuclear) was carried out in a serum-free medium QBSF 56 (Sigma) for 1 h. After treatment, cells were washed three times with PBS and incubated up to 24 h in QBSF 56 serum-free, and drug-free medium.

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) assays

Oligonucleotide sets of primers used in PCR were synthesized in the DNA Synthesis Facility at Yale University. Primers were selected based on published cDNA nucleotide sequences for specific gene and selected with the aid of a Primer-select program of DNASTAR (DNASTAR Inc., Madison, WI). The oligonucleotide primers used in this work are indicated in Table 1. To eliminate possible genomic DNA contamination in the RNA preparation, samples were pre-treated with RNase-free DNase (Boehringer Mannheim, Indianapolis, IN) before RT-PCR reactions were performed using a RT-PCR kit (Stratagene, La Jolla, CA), according to the manufacturer's protocol. Each PCR cycle consisted of a denaturation step at 94 °C for 1 min, 1 min of annealing at the optimal temperature and 1 min of polymerization at 72 °C. This cycle was repeated 35 times for each of the set of primers used in this work. The final polymerization step was extended an additional 5 min. Each predicted DNA product amplified by PCR was gel purified and sequenced in an automated DNA sequencer ABI PRISM 310 Genetic Analyzer, using the rhodamine terminator cycle sequencing ready reaction from a DNA sequencing kit (PE Applied Biosystems, Foster City, CA). Nucleotide sequences were aligned and verified to share 100% homology with the corresponding published human cDNA sequences in GenBank.

2.3. RNA extraction and Northern blot

Total RNA was isolated from cultured cells using TRIzol reagent (GIBCO BRL, Gaithersburg, MD), according to manufacturer's protocol. Northern hybridization was carried out to determine the patterns of expression of 17HSD2, 5 and 7 mRNAs in cultured cells and in ocular tissues from human eye donors (cadavers), ranging in age between 56 and 78 years old. Total RNA was separated by electrophoresis on 1% agarose gels containing formaldehyde and transferred by capillary action onto Nytran filters (Schleicher and Schuell, Keene, NH), as previously described [9]. DNA probes for 17HSD2, 5, and 7 for hybridization were prepared by RT-PCR, using specific oligonucleotide primers, as indicated in Table 1. Specific DNA probes were labeled with ^{32}P-dCTP (Amersham Corp.) by random priming, and hybridization was carried out in ULTRAhyb Hybridization buffer (Ambion, Austin, TX) at 42 °C overnight. Subsequent washing of the filters was carried out at high stringency for 15 min in 0.1× SSCPE with 0.1% SDS at 60 °C. Filters were autoradiographed at −70 °C with intensifying screen. Quantification of hybridization signal for individual mRNAs from autoradiographies was carried out with the gel scan program of a IS-1000 digital imaging system (Alpha

Table 1
Oligonucleotide primer pairs

Gene	Forward/reverse	Product length (bp)	Annealing temperature (°C)	GenBank accession no.
17β-Hydroxysteroid dehydrogenase 2 (17HSD2)	5′-AAACAATGCATGGCCGTGAACTT-3′/ 5′-CTCTGGGCATGGGCTTGTCTTG-3′	571	58	NM_002153
17β-Hydroxysteroid dehydrogenase 5 (17HSD5)	5′-ACTTCATGCCTGTATTGGGATTTG-3′/ 5′-CTGCCTGCGGTTGAAGTTTGA-3′	476	56	NM_003739
17β-Hydroxysteroid dehydrogenase 7 (17HSD7)	5′-CTGCTGGCGGAAGATGATGAG-3′/ 5′-CTTGCACTGCGAGATGATGTCC-3′	461	57	BC007068
Estrogen receptor α (ERα)	5′-AATTCAGATAATCGACGCCAG-3′/ 5′-GTGTTTCAACATTCTCCCTCCTC-3′	345	57	XM_004331
Estrogen receptor β (ERβ)	5′-CACCTGGGCACCTTTCTCCTTTAG-3′/ 5′-GCTCGTCGGCACTTCTCTGTCTC-3′	468	58	NM_001437
Androgen receptor (AR)	5′-CGAAATGGGCCCCTGGATGGATAG-3′/ 5′-AGTCGGGCTGGTTGTTGTCGTGTC-3′	524	59	NM_000044
β-Actin	5′-TGCGCAGAAAACAAGATGAGATT-3′/ 5′-TGGGGGACAAAAAGGGGGAAGG-3′	438	60	X00351

Innotech Corp.) and normalized with β-actin as previously described [24].

2.4. Measurement of 17HSD2, 5 and 7 activities in cultured ciliary epithelial cell lines

Cells were plated on six-well plates 2×10^6 cells per well, or grown to confluence on 175 cm² T-flasks (25×10^6 cells). At the beginning of the experiment, the culture medium was replaced with either 2 ml per well or 25 ml per 175 cm² T-flasks of medium containing 10% FCS charcoal-dextran treated. The metabolism of the steroids was followed by adding ³H-labeled substrates (1.8 nM [2,4,6,7-³H]estrone (94–95 Ci/mmol), 1.8 nM [2,4,6,7-³H]estradiol (88 Ci/mmol), 1.7 nM [1,4,6,7-³H]testosterone (95 Ci/mmol), 1.5 nM [1,4,6,7-³H]androst-4-ene-3,17-dione (99 Ci/mmol), 1.4 nM 5α-dihydro[1,2,4,5,6,7-³H]testosterone (125 Ci/mmol) or 1.8 nM [1,2,6,7-³H]progesterone (86 Ci/mmol) (Amersham Pharmacia Biotech, Little Chalfont, UK) and corresponding unlabeled steroids E1, E2, T, A-dione, DHT and P (Steraloids Inc., Newport, RI) to final concentration of 1 nM. The cells were incubated for 1, 2, 4, 6, 10 and 20 or 24 h at 37 °C in 5% CO_2 atmosphere. The reactions were stopped by immediately freezing the medium samples, 1 ml aliquots. The steroids were extracted into an organic phase (diethyl ether–ethyl acetate, 9:1) from medium samples. Enzyme activities were analyzed by determining the conversion percentage of substrates to specific products using a HPLC system (Waters, Milford, MA) as previously described [25,26]. The substrates and products were separated in a Symmetry C18 reverse-phase chromatography column (3.9 mm × 150 mm) using an acetonitrile/water (48:52, v/v) solution as a mobile phase, and radioactivity was measured by an on-line β-counter (150TR, FLO-ONE Radiomatic, Packard, Meriden, CT) connected to the HPLC system. Ecoscint A (National Diagnostics, Atlanta, GA) was used as a scintillation solution.

3. Results

3.1. Members of the 17β-hydroxysteroid dehydrogenase gene family are expressed in the human eye

Among a large number of cDNA clones isolated by suppression subtractive hybridization from the human ciliary epithelium (manuscript in preparation), we identified one clone that exhibited 100% homology with the human type 7 17β-hydroxysteroid dehydrogenase (17HSD7). Further analysis by RT-PCR and nucleotide sequencing revealed that in addition to 17HSD7, it also co-expressed types 2 and 5 mRNAs. The expression of these genes was also demonstrated in the two human ciliary epithelial cell lines (ODM-C4 and GCE) used in this work (Fig. 1; panels A, C and E; lanes 2 and 3). To verify the pattern of mRNA expression of 17HDSs in the human eye, we prepared Northern blots from ocular tissues from eye donors (cadavers) ranging in age between 56 and 78 years old. Blots were sequentially hybridized with type-specific 17HSDs DNA probes prepared by RT-PCR. The lack of homology (less than 25%) between these 17HSD DNA probes and the high stringency conditions used on Northern blot hybridizations provided the bases to distinguish the 17HSD type-specific transcript species expressed in ocular tissues. Fig. 1 (panels B, D and F) shows a representative profile of the relative abundance of 17HSD2, 5 and 7 mRNAs in ocular tissues from a pair of eyes of a 78-year-old eye donor (cadaver). The 571 bp 17HSD2-specific DNA probe (Fig. 1, panel A) hybridized to a main transcript of approximately 1.5 kb most abundantly in the ciliary epithelium (Fig. 1, panel B, lane 3) and to a lesser level in iris (lane 4), cornea (lane 1), lens (lane 2), and retina (lane 5). The 476 bp 17HSD5-specific DNA probe (Fig. 1, panel C) hybridized to a transcript slightly smaller in size than 17HSD2 mRNA in the ciliary epithelium (Fig. 1, panel D, lane 4), and in lower level in iris (lane 4) and retina (lane 5). Finally, the
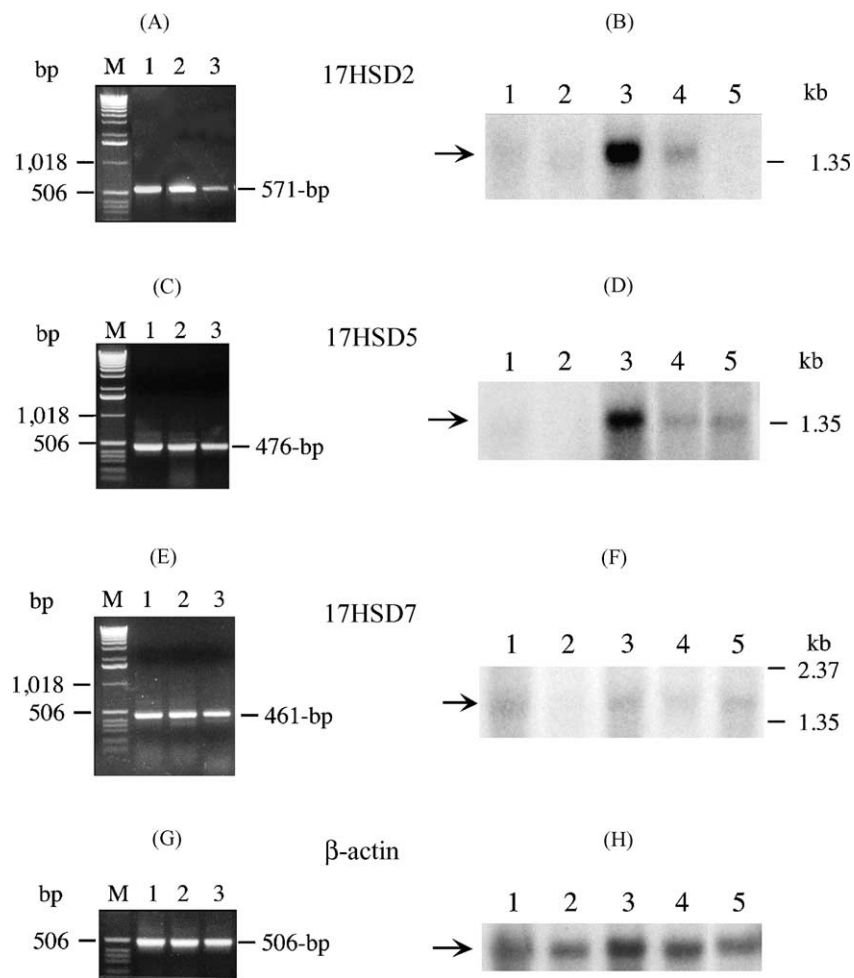
Fig. 1. Expression of 17HSD2, 5 and 7 mRNAs in ocular tissues of a postmortem 78-year-old donor, and in human ciliary epithelial cell lines. For RT-PCR amplifications (panels A, C, E, and G), the cDNAs were synthesized *in vitro* from RNA prepared from the ciliary epithelium (lanes 1), and the cell lines ODM-C4 (lanes 2) and GCE (lanes 3) and annealed with 17HSD type specific and β-actin oligonucleotide primers respectively (see Table 1). The predicted DNA size products amplified by PCR (571-bp, for 17HSD2; 476-bp for 17HSD5; 461-bp for 17HSD7 and 506-bp for β-actin) were resolved on agarose gels, gel purified and their nucleotide sequence determined. For Northern blot hybridization (panels B, D, F and H), total RNA from the cornea (lane 1); the lens (lane 2); the ciliary epithelium (lane 3); the iris (lane 4), and the retina (lane 5), of the above donor eyes was prepared, separated by electrophoresis (20 μg/lane), transferred onto a filter and hybridized sequentially with 17HSD type-specific and β-actin radiolabeled DNA probes generated by RT-PCR. In panels B, D, F and H the arrow at left indicates the main transcript specie hybridized, and at right an RNA standard ladder is indicated. M = 1 kb DNA ladder.

461 bp 17HSD7-specific DNA probe (Fig. 1, panel E) hybridized to a transcript specie of 1.7 kb long at low level in cornea (Fig. 1, panel F, lane 1), ciliary body (lane 3) and retina (lane 5). These results revealed that in the human eye the ciliary epithelium expresses 17HSD2, 5 and 7 mRNA, being types 2 and 5 most abundantly, and in a lesser level 17HSD7 mRNA.

3.2. Distinct responsiveness of 17HSD genes to estrogen and androgen in cultured ciliary epithelial cells

Earlier studies have indicated that members of the 17HSD family of genes may be targets of estradiol action in vivo [27]. Due to the difficulty to perform this study on intact human eye tissue, we determined the 17HSDs responsiveness to estrogen or androgen on two available cell lines

(ODM-C4 and GCE) representative of the human ciliary epithelium as indicated in Section 2. We confirmed earlier evidence that the human ciliary body express estrogen and androgen receptors, and determined by RT-PCR that the two human ciliary epithelial cell lines used in this work also expressed estrogen and androgen receptors, indicating that these cell lines conserve this phenotype from the intact tissue. Oligonucleotide-specific primers to the estrogen receptor α (ERα), estrogen receptor β (ERβ) and androgen receptor (AR) (Table 1), were annealed to cDNA templates synthesized in vitro from RNA prepared from human ciliary tissue, and cultured ODM-C4 and GCE cells. The predicted DNA products amplified from the ciliary epithelium, ODM-C4 and GCE for ERα, ERβ and AR were resolved on agarose gels, gel purified and their nucleotide sequences verified to share 100% homology with

the corresponding published human cDNA sequences (data not shown).

3.2.1. 17HSD7 and 2 expression are highly responsive to 17β-estradiol

ODM-C4 and GCE cells were cultured and exposed up to 60 min in serum-free medium to 17β-estradiol (10^{-7} M) or to the androgen agonist R1881 (10^{-8} M), and then harvested at 0 h (control), 6, 14 and 24 h post-treatment in serum-free and drug-free medium. Total RNA was prepared from the cells at each of the post-treatment times and Northern blots hybridized sequentially, with 17HSD7, 2, 5 and β-actin DNA-specific probes as indicated in Fig. 1. ODM-C4 cells responded to 17β-estradiol by up-regulating both 17HSD7 and 2 mRNA, but no significantly 17HSD5 mRNA expression in a time-dependent manner (Fig. 2, panel A). After normalizing the hybridization signal of 17HSD7, 2 and 5 bands

to β-actin, we estimated an increase in 17HSD7 mRNA expression of 2-fold at 6 h, 7-fold at 14 h and 9.5-fold at 24 h post-treatment. On the other hand, 17HSD2 mRNA increased 3-fold at 6 h, 5-fold at 14 h and 7.5-fold at 24 h after treatment. No significant changes in 17HSD5 mRNA were detected up to 24 h post-treatment with estradiol (Fig. 2, panel A). In contrast to ODM-C4 cell line, cultured GCE cells responded moderately to 17β-estradiol exposure. Cells responded by up-regulating 17HSD7 mRNA expression approximately 2-fold at 14 h and up to 2.5-fold after 24 h post-exposure (Fig. 2, panel A). No detectable changes in expression were observed on 17HSD2 and 5 mRNA after treatment.

3.2.2. 17HSD7 expression is highly responsive to androgen

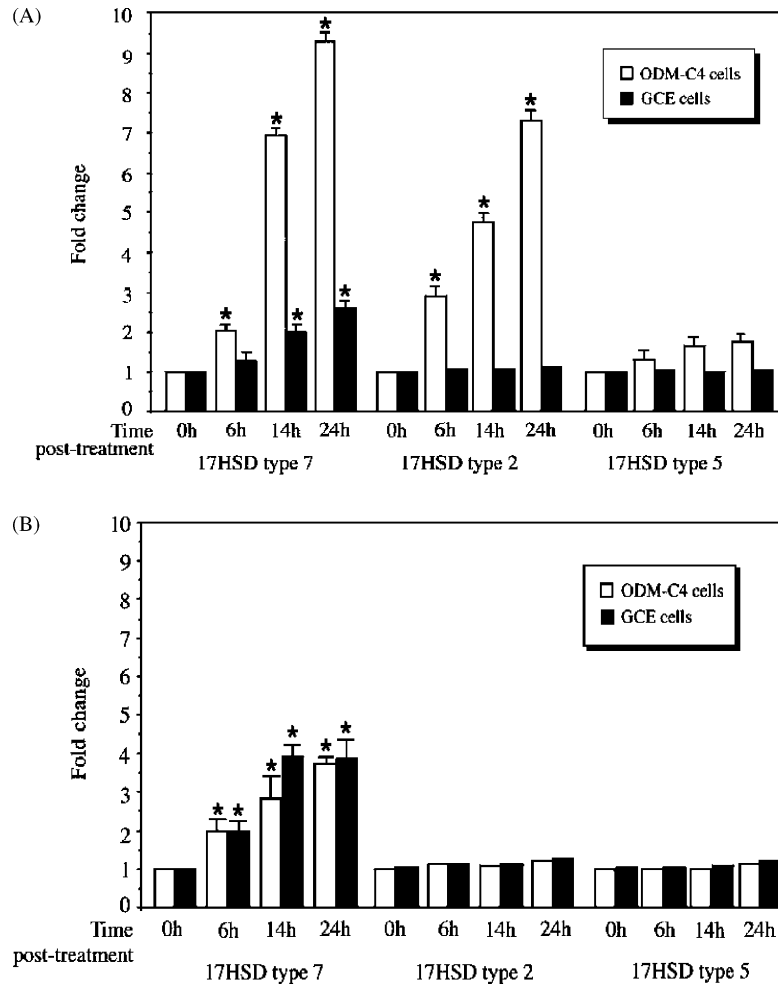ODM-C4 cells responded to the androgen agonist R1881 (10^{-8} M) by up-regulating 17HSD7 mRNA expression in a

Fig. 2. Time course responses of 17HSD7, 2 and 5 mRNA expression to 17β-estradiol (panel A) and to androgen R1881 (panel B) in cultured human ciliary epithelial ODM-C4 and GCE cells. Both cell lines were grown in 25 cm^2 size flasks, in culture medium containing serum to semiconfluency, and then exposed to 17β-estradiol (10^{-7} M) or R1881 (10^{-8} M) for 1 h in serum-free medium. Total RNA was prepared from the cells at 0, 6, 14 and 24 h post-treatment, and hybridized in a Northern blot sequentially to 17HSD7, 2, 5 and β-actin DNA probes. Hybridization signals for the main 17HSD7, 2 and 5 transcripts were scanned and the values normalized to β-actin, and expressed as fold change. Asterisks indicate statistical significance calculated using Student's *t*-test ($P < 0.05$). Results shown are means ± S.E.M. (three independent experiments).

time-dependent manner (Fig. 2, panel B). In contrast, R1881 had no effect on 17HSD2 or 5 mRNA expressions. After normalizing the hybridization signal of the 17HSD7 band to β-actin, as indicated above for the estradiol response, we determined an increase in 17HSD7 mRNA in ODM-C4 cells of two-fold at 6 h, three-fold at 14 h and four-fold at 24 h post-treatment. Under the same conditions GCE cells responded very similarly to ODM-C4 cells by up-regulating 17HSD7 mRNA expression. As shown for the ODM-C4 cells, the expression of 17HSD2 and 5 in GCE cells were unresponsive to androgen treatment (Fig. 2, panel B).

3.3. 17HSD2, 5 and 7-specific enzymatic activities in cultured ciliary epithelial cells

We examined whether the expression of 17HSD2, 5 and 7 mRNAs in ODM-C4 and GCE cell lines correlated with the 17HSD-specific activities they mediate. We determined the

oxidative (17HDS2) and reductive (17HSD5 and 7) activities over a period of 20–24 h. Under steady-state, ODM-C4 cells were able to catalyze the inactivation of 17β-estradiol (E2) by its oxidation to estrone (E1) (Fig. 3, panel A); the conversion of testosterone (T) into androstenedione (A-dione) (Fig. 3, panel B), and the conversion of dihydrotestosterone (DHT) into 5α-androstanedione (5α-A-dione) (Fig. 3, panel C). In contrast, GCE cells were able to catalyze these oxidative interconversions at very low rate.

Interestingly, the reductive interconversions of E1 into E2 and DHT into 3β-Adiol, respectively, which are catalyzed by 17HSD7 were clearly detected in GCE cells (Fig. 4, panels A and B). It has been documented that 3β-Adiol had some estrogenic activity [28]. Furthermore, the interconversions of P into 20-OH-P and DHT into 3α-Adiol, respectively, which are mediated by 17HSD5 [21], were seen also in GCE cells (Fig. 4, panels C and D), but 17HSD5 reaction of interconversion of A-dione to T was not detected.
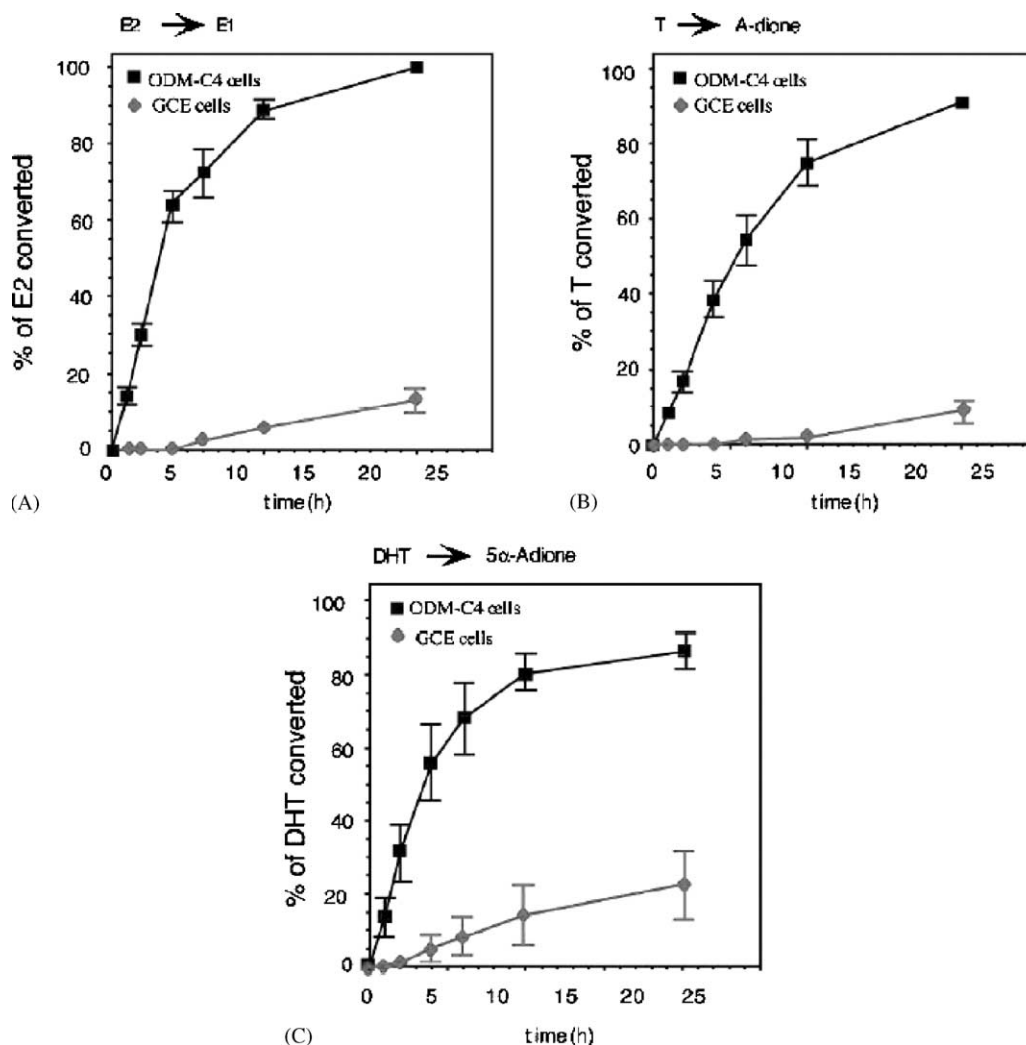

Fig. 3. Characterization of oxidative 17HSD2-specific activities in cultured ODM-C4 and GCE cells. Panel A, E2 → E1; panel B, T → A-dione; and panel C, DHT → 5α-A-dione. Activities were determined at 1, 2, 4, 6, 10 and 20 h, and shown as percentage of substrates converted to product. Each value is the average of three independent determinations. Error bars indicate standard deviations. E1, estrone; E2, 17β-estradiol; T, testosterone; A-dione, androstenedione; 5α-A-dione, 5α-androstanedione; DHT, dihydrotestosterone.
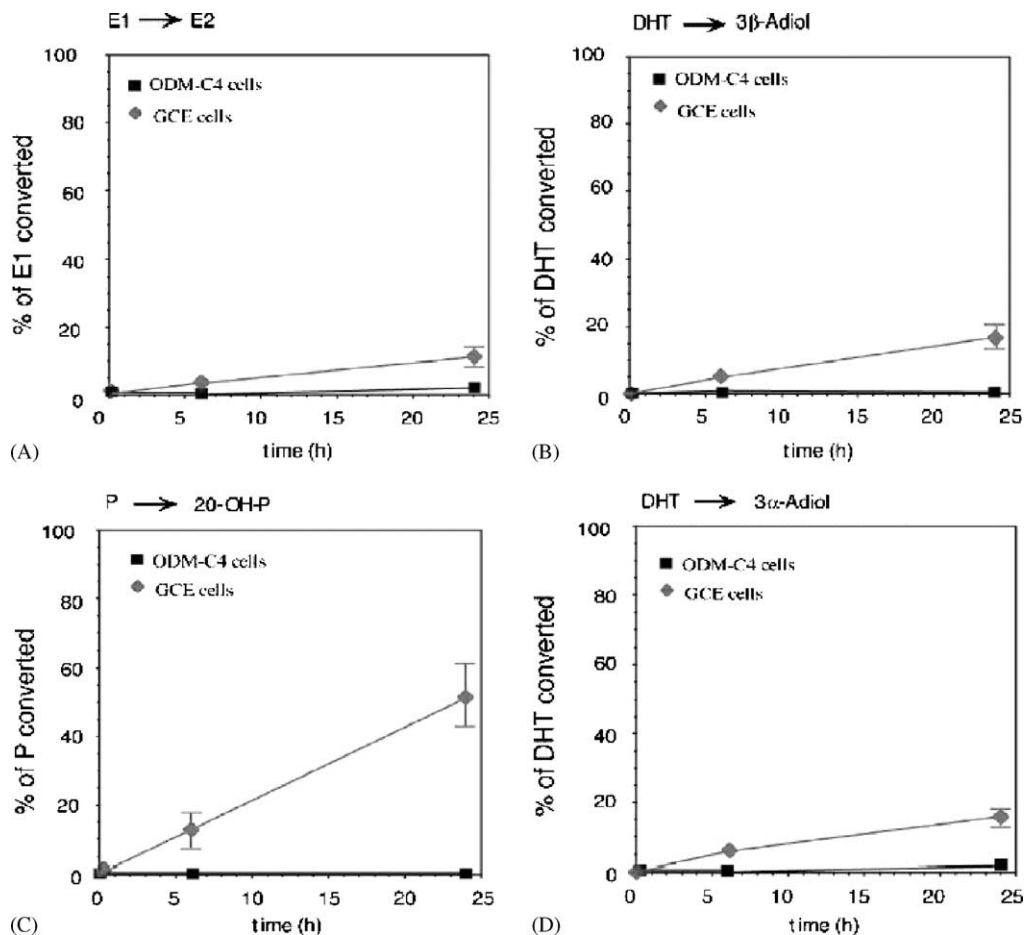
Fig. 4. Characterization of reductive17HSD7 and 5 activities in cultured ODM-C4 and GCE cells. Panels A and B: 17HSD7 activities. Panel A, E1 → E2 and panel B, DHT → 3β-Adiol. Panels C and D, 17HSD5 activities. Panel C, P → 20-OH-P and panel D, DHT → 3α-Adiol. Activities were determined at 6 and 24 h, and are shown as percentage of substrates converted to product. Each value is the average of six independent determinations. Error bars indicate standard deviations. E1, estrone; E2, 17β-estradiol; DHT, dihydrotestosterone; 3β-Adiol, 5α-androstane-3β-17β-diol; 3α-Adiol, 5α-androstane-3α-17β-diol; P, progesterone; 20-OH-P, 20α-hydroxyprosgesterone.

Surprisingly, ODM-C4 did not exhibit detectable reductive 17HSD5 or 7 enzymatic activities.

4. Discussion

Emerging evidence indicates that the human ocular ciliary epithelium is a neuroendocrine tissue [14]. This tissue regulates the intraocular pressure in conjunction with the trabecular meshwork. It modulates the rate of aqueous humor secretion, a fluid that nourishes the avascular tissues of the anterior segment of the eye. The neuroendocrine nature of the ciliary epithelium has been established by the expression of regulatory peptides, hormones and endocrine-processing enzymes usually restricted to neuroendocrine cells and tissues [9,24]. Interestingly, some of the hormones processed and secreted by the ciliary epithelium included the natriuretic peptides (ANP and BNP) which have been shown experimentally to display potent effect in lowering intraocular pressure [29]. These hormones upon their release by the ciliary epithelial cells are believed to target their cognate receptors on the same peptide-producing cells by an autocrine mechanism, or on the trabecular meshwork cells by a paracrine mechanism [11]. A number of reports have suggested the role of sex steroid hormones in the regulation of intraocular pressure [30,31], and documented the expression of androgen, estrogen and progesterone receptors in ocular tissues including the ciliary epithelium [32]. So far, the most effective ways of reducing the risk of glaucomatous visual field are to lower the intraocular pressure by reducing the rate of secretion of aqueous humor at the site of formation (i.e. ciliary epithelium) [33] and/or by increasing the drainage of aqueous humor through the trabecular [34] and uveoscleral outflow pathways [35]. Therefore, these sites are potentially important targets for sex steroid action in intraocular pressure.

The 17HSD family of enzymes regulates the last key step in the synthesis and inactivation of sex steroid hormones. These enzymes have been reported to exhibit tissue-specific expression, display substrate specificity, and the enzymatic

activities they catalyze are also intracrine or paracrine [36]. The finding that certain members of the 17HSD family genes are highly restricted to the human ciliary epithelium adds a new potentially important function to the endocrine nature of this tissue. The capability of the ciliary epithelial cells, in vitro, to metabolize androgen, estrogen and progesterone, suggests that the ciliary epithelium may play an important role in the intracrine formation and metabolism of sex steroid hormones. These results could provide insight information on the possible role of estrogen overexposure in the pathogenesis of ocular diseases (i.e. glaucoma). The present results also indicate that androgen and estrogen can modulate the 17HSD expression in a type-specific manner in the ciliary epithelial cells and therefore influence their activities in a positive feedback manner.

Although multiple genetic and environmental components are involved in the development of glaucoma [3], still controversial whether sex differences play a role in glaucoma, or whether incidence of increasing intraocular pressure in post-menopausal women relates to an increasing risk of developing glaucoma [31]. Interestingly, the *HSD17B7* gene maps on human chromosome 1q23 and is very close to the *GLC1A* gene, localized on 1q23–24, which is associated to different subset forms of glaucoma [6]. Whether the 17HSDs enzymes might influence any of the glaucoma genes so far identified (i.e. *GLC1A; CYP 1B1*) in the etiology of the disease is at present unknown.

The two 17HSDs found abundantly expressed in the ciliary epithelium of eye donors were 17HSD2 and 5 (Fig. 1). Due to its ability to convert 17β-estradiol into estrone, or T into A-dione or DHT into 5α-A-dione or to metabolize xenobiotics [19] via oxidative reactions it has been suggested that 17HSD2 provides a protective function to the tissues where it is expressed [37–39]. In addition, 17HSD5 mediate reactions between androgens, progestins and xenobiotics. Finally, 17HSD7 expressed in other ocular tissues in addition to the ciliary epithelium, mediates the interconversion of E1 to E2 and DHT to 3β-Adiol, and therefore is estrogenic.

The expression of 11β-hydroxysteroid dehydrogenase isoenzymes which are involved in the metabolic interconversions of hormonally active cortisol and inactive cortisone have been also documented in the human ciliary epithelium and cornea, respectively [40]. These studies are supported by the classical methods of steroid metabolism in rabbit eye tissues [41]. Earlier studies also have documented the identification of sex steroid hormones (testosterone, estrone and 17β-estradiol) in aqueous humor and in the vitreous of rabbit eyes [42]. Overall these studies support the view that the eye is a target for sex steroid hormones and glucocorticoids action.

Due to the obvious difficulties inherent to the procurement of fresh eye tissue from human donors, the type-specific 17HSD activities and their gene regulation was carried out on two ciliary epithelial cell lines established so far from a normal, and a glaucoma eye donor (cadavers). Although this could represent a limitation, it allowed investigation of the 17HSD responsiveness to sex steroids and to determine the type-specific 17HSD activity in homogenous cell populations. A lesson from the cell lines was that the expression of 17HSD2 and 7 in the normal-derived cell line ODM-C4 was estrogen-responsive. The lack of estrogen responsiveness by the 17HSD2 in the glaucoma-derived cell line GCE could be interpreted by differences between the two cell lines, including: (1) the age of the eye donors; (2) gender and race of eye donors; and/or (3) the presence or lack of the disease in one of the eye donors.

However, the distinct estrogen responsiveness of 17HSD2 was in contrast to the similar and 17HSD7-specific responsiveness to estrogen and androgen by the two cell lines. Thus, while the 17HSD2 responsiveness to estrogen in GCE cells was severely reduced or absent when compared to ODM-C4 cells, the 17HSD7 response to estrogen and androgen was preserved in both cell lines. This suggested that age and gender differences of eye donors may not be the reasons for the lack of 17HSD2 responsiveness by GCE cells to estrogen treatment.

There is another possible interpretation to explain the differences in 17HSD2 responsiveness to estrogen in the two cell lines. This could however be based on the nature that one cell line derived from a healthy donor, and the other from a donor with glaucoma. The predominant oxidative 17HSD2 activities found in the normal-derived cell line, were significantly reduced or absent in the glaucoma-derived cell line. This could explain why the 17HSD7 enzymatic activity detected in the glaucoma-derived cell line could lead to an overexposure to estrogen, which is increased by the fact that 17HSD5 activity to convert A-dione to T was missing (data not shown). In addition, the 17HSD7-specific activity to convert DHT into estrogenic 3β-Adiol in GCE cells will increase the estrogenic effect. 17HSD7 is inactivating progesterone [43], which opposes estrogenic effect [44]. Thus, when estrogen up-regulates 17HSD7 expression the possible positive feedback mechanism will sustain this effect. Simultaneously, 17HSD5 will decrease the androgenic effect by converting DHT into 3α-Adiol. Progesterone is also metabolized into less active 20-OH-P by 17HSD5 and when 17HSD2, which is able to convert 20-OH-P to P is low or missing, the estrogenic effect is increased. Androgens are up-regulating 17HSD7 in both cell lines and 17HSD5 is not influenced by sex steroid hormones. These results suggest that 17HSD2 can be an important regulator of hormonal balance in ciliary body as it is in many other cells [17,37–39].

Although the exact physiological role of 17HSDs in peripheral tissues is not known, there is growing evidence that androgens and estrogens exert actions on vascular functions, and metabolism that are not directly related to reproductive processes [45]. The present study provides a possible role of the 17HSDs in the ocular ciliary epithelium. The 17HSDs may contribute to the endocrine functions of the ciliary epithelium in the metabolism of steroid hormones.

Acknowledgements

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