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1 **Expression patterns of 17 β hydroxysteroid dehydrogenase 14 in human tissues**

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20 **Running Head:** Systemic distribution and activity of 17 β HSD14

Abstract

17 β HSD enzymes catalyse the stereospecific oxidation/reduction at carbon 17 β of androgens and oestrogens, and are important players in intracrine sex hormone synthesis. The biological relevance of 17 β HSD14, first named retSDR3, is largely unknown. We generated and validated an antibody targeting the 17 β HSD14 antigen and used this for immunohistochemical evaluation of expression patterns in 33 healthy human tissues. Furthermore, sex steroid conversional activity in *HSD17B14* over-expressing HEK293 and MCF10A cells was investigated by assessing interconversion products of oestrone, oestradiol, androstenedione, testosterone and dehydroepiandrosterone. Immunohistochemical staining patterns of 17 β HSD14 with the enzyme being primarily expressed in glandular epithelial tissue reveal an enzyme with possible implications in the secretion or conversion of externally derived compounds. A role for 17 β HSD14 in sex steroid metabolism is supported by the finding that 17HSD14 oxidises both oestradiol and testosterone into less bioactive steroid metabolites oestrone and androstenedione respectively.

Key words: 17 β HSD, immunohistochemistry, radio-HPLC

1. INTRODUCTION

The gene product of *DHRS10*, initially named retSDR3, and later 17 β hydroxysteroid dehydrogenase (17 β HSD) 14, was cloned from a retinal epithelium cDNA-library in the year 2000 [1]. Based on sequence analysis, the protein was determined to be a short-chain dehydrogenase/reductase (SDR), and suggested to be involved in retinol metabolism; however such functional properties could not be shown. In 2006, Lukacik and colleagues further investigated the *DHRS10* gene product [2]. Based on structural and functional studies which revealed features of the protein equivalent to those of 17 β HSDs, the *DHRS10* gene product was denoted 17 β HSD14 [2].

Using the oxidised or reduced forms of NAD(P)(H) as hydrogen donors or acceptors, 17 β HSD enzymes catalyse the stereospecific oxidation/reduction at carbon 17 β of androgens and oestrogens [3]. Upon receptor binding, the 17 β -hydroxy conformation of androgens and estrogens triggers a greater biological response than the corresponding keto-conformation of the steroids [4], and the 17 β HSD enzymes are thus important mediators in pre-receptor regulation of sex hormone action. Of the 15 types described to date, 14 are found in human, and structurally, all but 17 β HSD type 5 which is an aldo-keto reductase, belong to the SDR-family [5,6]. The 17 β HSD enzymes are a diverse protein family, varying in tissue distribution as well as in substrate preferences. While the major substrates of 17 β HSD enzymes are sex steroids [2,7-11], a few are believed to be dedicated primarily to other substrates, such as fatty acids [12,13], cholesterol [14] bile acids [15] or retinoids [16]. Several of the 17 β HSDs can work essentially as both reducing and oxidising agents depending on cofactor concentrations and pH, yet *in vivo* they tend to be unidirectional, and according to experimental data the 17 β HSD enzymes are grouped as either oxidative or reductive enzymes based on preferred cofactor utilization under biological conditions. From this classification, human 17 β HSD types 2, 4, 6, 8, 10, 11 and 14 are considered *in-vivo* oxidative enzymes catalyzing the NAD(P)⁺-dependent inactivation of oestrogens/androgens whereas types 1, 3, 5, 7, 12 and 15 catalyze the NAD(P)H-dependent reduction, and hence activation, of oestrogens and androgens [5,6].

By catalyzing the final steps in steroid metabolism, the 17 β HSD enzymes, alongside enzymes such as 3 β -hydroxysteroid dehydrogenases, aromatase and sulfotransferases/sulfatases, are thought to be

important contributors in intracrinology. This element in endocrinology deals with peripheral sex steroid synthesis from adrenally derived precursors, e.g. dehydroepiandrosterone (DHEA), into more potent metabolites such as estradiol (E2) and testosterone (T) [17]. In intracrinology, the biological effect of a sex steroid is the result of local synthesis rather than an outcome mediated by circulating active sex steroids derived from endocrine organs, i. e. the gonads. The local distribution and expression levels of oxidative versus reductive steroidogenic enzymes will eventually affect the fine-tuning of hormonal profiles in a given target tissue or cell type. In addition to significant roles in normal physiology, many of the enzymes involved in intracrine sex-steroid synthesis have been implicated in steroid-associated pathological conditions such as breast and prostate cancer [18,19].

The current study further elucidates the biological role of 17 β HSD14. An antibody targeting 17 β HSD14 was prepared and used for determining expression patterns in 33 human tissues. Moreover, the enzymatic potential in converting E2, oestrone (E1), androstenedione (A) and T, was assessed in media from cells transiently over-expressing *HSD17B14*.

2. MATERIAL AND METHODS

2.1 Preparation of polyclonal anti-17 β HSD14 antibody

The peptide sequence (NH₂)-CKASRSTPVDAPDIP(-CONH₂) corresponding to amino acids 255 to 269 of human 17 β HSD14 with an additional terminal cystein residue for affinity purification purposes, was synthesised and sequenced by AgriSera (Vännäs, Sweden). A breed of New Zealand white rabbits/French lop received initial subcutaneous injections of 200 μ g peptide dissolved in a buffer emulsified in Freund's Complete Adjuvant. Three booster injections were administered at 3 months interval; the first including 200 μ g peptide and the two following 100 μ g. Freund's Incomplete Adjuvant was used with the booster injections. The animals were finally bled one week after the last immunisation and were thereafter sacrificed by injection of an intravenous pentobarbital (Apoteket Stockholm, Sweden). The anti-17 β HSD14 antibody was affinity-purified on a column containing a peptide-coated gel matrix (Ultralink; Thermo Fischer Scientific, Waltham, MA). The preparation of

the 17 β HSD14 antibody was performed with permission given by the Swedish animal welfare authority (dnr A112-06). Peptide-neutralisation assay as well as immunoblot analysis of *HSD17B14*-transfected HEK293- and MCF10A cells confirmed antibody specificity.

2.2 Immunohistochemical evaluation of 17 β HSD14 expression in various human tissues

Paraffin-embedded tissue micro array (TMA)-slides composed of totally 33 human tissues from healthy individuals including adrenal gland (n=3), myeloid bone (n=8), breast (n=60), brain (n=14), colon (n=60), eye (n=3), esophagus (n=8), heart (n=3), kidney (n=8), larynx (n=3), liver (n=8), lung (n=8), lymph node (n=5), mesothelium (n=3), nerve (n=3), ovary (n=8), pancreas n= 8), parathyroid gland (n=3), pituitary (n=3), prostate (n=60), rectum (n=5), salivary gland (n=3), skeletal muscle (n=3), skin (n=8), duodenum (n=5), spleen (n=8), stomach (n=8), testis (n=8), thymus (n=8), thyroid gland (n=3), tonsil (n=3), uterine cervix (n=8), uterus (n=3), all in 1 mm diameter cores of 5 μ m thickness were purchased from Tissue Array Network (Rockville, MD). TMA slides were deparaffinised in xylene and then hydrated in descending concentrations of ethanol. The sections were thereafter treated in a decloaking buffer (Biocare Medical, Concord, CA) in a decloaking chamber to retrieve antigenicity. The temperature was allowed to reach 120°C at which the heat was turned off. To reduce non-specific staining, sections were immersed in phosphate-buffered saline (PBS) supplemented with 0.1 % bovine serum albumin (BSA) and then incubated with a commercial protein blocking solution (Spring Bioscience, Pleasanton, CA) for 10 minutes. The tissue sections were incubated with the in-house polyclonal rabbit anti-human 17 β HSD14 antibody diluted to 1:1200 in antibody diluent (DakoCytomation, Glostrup, Denmark) at 4°C over night. After a washing step in PBS/BSA, sections were incubated with EnVision horseradish peroxidase-conjugated anti-rabbit polymer (DakoCytomation) for 25 minutes. The immune reaction was visualised by incubating the samples in a solution containing 3,3-diaminobenzidine chromogen supplemented with hydrogen peroxide for eight minutes. Sections were briefly counterstained with hematoxylin, dehydrated in ascending concentrations of ethanol and finally mounted. A test for antibody specificity was performed by peptide neutralising assay. Pre-incubation of antibody and 17 β HSD14-peptide was done

for two hours in RT with peptide (10^{-6} M) and 17 β HSD14 antibody (1:600) at a molar ratio of 100:1 in tris-buffered saline with tween (TBST) without blocking reagent. The pre-incubation solution was mixed with an equal volume of TBST supplemented with blocking solution and added to the TMA tissue for overnight incubation. The TMAs were investigated microscopically using a Leica LB30T microscope (Leica Microsystems, Wetzlar, Germany) by two independent investigators blinded to clinical data (T.S and A.J), and the findings were confirmed by a pathologist (HO). Representative slides were photographed using an Olympus SC20 digital camera (Olympus Europe GmbH, Hamburg, Germany).

2.3 Transient transfection of cultured cells with HSD17B14

HEK293 cells (American Type Culture Collection, ATCC, Manassas, VA; ATCC number CRL-1573) were seeded at 16 000 cells/cm² in 24-well plates in 0.5 ml/well OPTIMEM phenol-red free Opti-MEM medium (Invitrogen, Carlsbad CA) supplemented with 4% charcoal-treated serum (CTS) (Invitrogen). MCF10A cells (ATCC number CRL-10781), were seeded at at 16 000 cells/cm² in 24-well plates in 0.5 ml/well in ATCC complete growth medium supplemented with 20 ng/ml epidermal growth factor, 100 ng/ml cholera toxin, 0.01 mg/ml insulin and 500 ng/ml hydrocortisone. All cells were cultured at 37°C in 5% CO₂. Twenty-four hours after seeding, the cells were transfected with a commercial *HSD17B14*-plasmid (Origene, Rockville, MD, USA), using Xtremegene transfection reagent (Roche Diagnostics, Indianapolis, IN). Mock-transfected cells were incubated with the same amount of a vector missing the *HSD17B14* insert. (Roche Diagnostics, Indianapolis, IN). Transfection efficiency was evaluated by measuring 17 β HSD14 expression in transfected cells with western blot.

2.4 Western blot

30 μ g of protein from lysates of non-transfected, mock-transfected cells was subjected to electrophoretic separation on 5-15 % SDS-PAGE gels (BioRad, Hercules, CA) at 200 V for 30 minutes. The proteins were then blotted onto a PVDF-membranes for 60 minutes at 100 V.

Electrophoresis and western blotting was performed using the Criterion precast gel System (BioRad). The membranes with blotted proteins were blocked in TBS-tween (TBST) supplemented with 5 % non-fat skimmed milk (BioRad) for 60 minutes on a shaker after which it was incubated with an in-house polyclonal rabbit anti-17 β HSD14 antibody, diluted in blocking solution to 1:1000 at 4°C overnight. The membranes were then washed three times with TBST solution and incubated at ambient temperature for 60 minutes with a secondary HRP-conjugated anti-rabbit IgG (DakoCytomation), diluted to 1:2000 in blocking solution. The blot was washed three times in TBST and bound antibodies were detected using ECL plus detection reagents (Amersham, Arlington Heights, IL). Results were visualised using LAS1000 CCD-camera detection system (FujiFilm, Tokyo, Japan). A monoclonal anti human β -aktin antibody (Cell Signaling, Beverly, MA) was used to control for equal loading.

2.5 Assessment of enzymatic conversion of tritiated substrates in cultured cells over-expressing HSD17B14

Twenty four hours after transfection, cell culture media was aspirated and replaced by Opti-MEM I with 4 % CTS supplemented with 10 nM of tritiated sex steroids; androst-4-ene-3, [1,2,6,7-³H(N)]-, 17-Dione (98.1 Ci/mmol; range 70-110 Ci), oestradiol, [2,4,6,7-³H(N)]- (70 Ci/mmol; range 70-115 Ci), oestrone, [2,4,6,7-³H(N)]- (89.3 Ci/mmol; range 50-100 Ci), testosterone, [1,2,6,7-³H(N)]- (70 Ci/mmol; range 70-105 Ci) and dehydroepiandrosterone, [1,2,6,7-³H(N)]- (50 Ci/mmol; range 60-100 Ci) (Perkin Elmer, Waltham, MA). Cell culture media was collected at 0, 4, 8, 24, 48 and 72 hours, and stored at -70°C until analysis. Experiments were run in duplicates and the whole procedure was repeated at least three times. Media samples with tritiated sex steroid metabolites were incubated without cells in order to monitor spontaneous steroid degradation within the given time interval.

2.6 Sample preparation and HPLC-analysis of steroid interconversion

The procedure for sample preparation, separation and detection of sex steroid interconversion product has been published elsewhere [20] and was used with minor modifications. Briefly, cell culture media

1 samples containing tritiated steroid interconversion products were precipitated using cold zinc
2 sulphate solution (10%) and 1 M sodium hydroxide solution. After centrifugation, supernatants were
3 transferred to injection vials for analysis. Samples were injected onto a Beckman Coulter Ultrasphere
4 ODS column, 250 x 4.6 mm, 5 μ m (Bromma, Sweden), maintained at 40°C. The retention time for
5 androstenediol was experimentally determined using UV detection of unlabelled substrate. During the
6 run the autosampler was kept at 15°C. Between two and 14 minutes scintillation fluid (Ultima-Flo,
7 Perkin Elmer) was mixed with the column eluent in a 2:1 ratio enabling radiochemical detection of the
8 tritium labeled steroids.

10 3. RESULTS

11 3.1 *Antibody specificity*

12 Immunoblot analysis of lysates from HEK293 and MCF10A cells transiently transfected with
13 *HSD17B14* revealed a single band at expected size 28 kDa. Weak or no bands were present in mock-
14 transfected or non-transfected cells (Fig. 1). When neutralized by a 17 β HSD14-peptide,
15 immunopositivity in liver was not evident (Fig. 3).

17 3.2 *17 β HSD14 distribution in human tissues*

18 In order to evaluate the localisation of 17 β HSD14 on a sub-tissue level we generated an antibody
19 towards the antigen and used this for immunohistochemical analysis of the protein in 33 human tissues
20 (Table 1). In specimens representing female reproductive organs (Fig. 2 A-D), uterus showed weak to
21 moderate cytoplasmic immunoreactivity in the secretory glandular epithelium of the endometrium,
22 whereas peritubular area, comprised of mainly smooth muscle, was unstained. Ovarian specimens
23 showed immunoreactivity in granulosa cells of dominant follicles as well as in atretic corpus luteum.
24 Breast specimens showed moderate to strong staining of 17 β HSD14 in the cytoplasm of ductal
25 epithelial cells. Periductal stromal areas were unstained. In male reproductive organs (Fig. 2 E and F),

prostate samples showed negative to weak immunoreactivity. In testis, immunoreactivity was seen in most of the seminiferous epithelium as well as in peritubular areas harbouring Leydig cells. 17 β HSD14 was highly expressed in kidney, with samples showing strong immunoreactivity in glandular cells of both proximal and distal tubules while cells of Bowman's capsule and endothelial cells of glomeruli remained unstained (Fig. 3 A). In retina, 17 β HSD14 was present in cytoplasmic projections of the plexiform layers whereas nuclear layers were negative, with an exception for the inner nuclear layer, where staining was prominent in some nuclei (Fig. 3 B). 17 β HSD14 expression was also evident in the gastrointestinal (GI) area (Fig. 3 C-F); in stomach samples, staining was relatively strong in upper mucosal areas whereas underlying stroma was unstained. Most 17 β HSD14 appeared to be located cytoplasmically although some nuclear staining was evident. In duodenal specimens, staining was weak to moderate throughout the mucosal layer. In colon and rectal samples, the presence of 17 β HSD14 was especially marked at the absorptive edges of the mucosa facing the GI-lumen. Both enterocytes and goblet cells of the mucosal crypts stained, and although staining was mostly cytoplasmic, as in the stomach, some nuclear staining was evident. In the deeper layers of the mucosa, staining diminished gradually and was virtually absent below the inner muscular layer. When tissues of both sexes were available, no apparent differences in staining intensities or distribution patterns were seen when taking gender of tissue donor into account.

3.3 Steroid interconversion

In order to assess steroid conversion by 17 β HSD14, HEK293 cells were transfected with an expression vector containing the *HSD17B14* insert. Transfected cells were thereafter exposed to radiolabelled steroids, and generated tritiated products were separated by HPLC and detected by β -scintillation. The rate of steroid interconversion of oestrone/oestradiol and androstenedione/testosterone was obtained by integrating generated peak areas and computing ratios of E1/E2 and A/T. *HSD17B14* transfected cells oxidised oestradiol and testosterone to oestrone and androstenedione respectively with net efficiencies of approximately 15% for the oestradiol to oestrone reaction and 8% for the testosterone to androstenedione conversion compared with mock-transfected

cells. No reduction of E1, A or DHEA exceeding that of mock-transfected cells was seen for *HSD17B14*-transfected cells (Fig. 4 A-E).

4. DISCUSSION

To date, 15 isoenzymes of the 17 β HSD family have been described [5,6]. The extent to which individual enzymes of this family have been studied varies. The relevance of the 17 β HSD14 protein in normal physiology has not been addressed, although we have previously shown tumoural expression of both *HSD17B14* mRNA and the 17 β HSD14 protein to be of importance in breast cancer, both for predicting prognosis [21] and in predicting tamoxifen treatment response [22]. In both cases, 17 β HSD14 appears to be a factor predicting good clinical outcome in breast cancer; however, functional mechanisms underlying this remain elusive. The current study was initiated in order to learn more of the normal physiology of the 17 β HSD14 protein. An antibody targeting the 17 β HSD14 antigen was raised and used for immunohistochemical analysis of healthy human tissues. Furthermore, enzymatic properties of the protein in intact cells were assessed. Overall, distribution patterns and activity measurements of 17 β HSD14 support the theory of the enzyme being an actor in intracrine sex steroid interconversion.

17 β HSD14 was first cloned from a retinal cDNA library in 2000 by Haeseleer and Palczewski [1]. Based on amino acid sequence, the enzyme was predicted to utilize NADPH as a cofactor, which would render the enzyme reductive. However, using recombinant protein expressed in insect cells, the authors could not detect transfer of ³H from [³H]NADPH to any of the steroid substrates tested. Lukacik et al re-evaluated the same gene product in 2006 and also solved the crystal structure of 17 β HSD14. The purified recombinant enzyme was screened against a compound library and was found to convert NAD⁺ to NADH in the presence of oestradiol, testosterone and 5-androstenediol. Oestradiol oxidation to oestrone was shown in a cell based system overexpressing *HSD17B14* [2]. In the current study, we utilized a similar cell based system and found significantly higher oxidation of both oestradiol and testosterone into oestrone and androstenedione respectively for transient *HSD17B14* overexpression compared with mock-transfected cells. The relevance of 17 β HSD14 as a steroid-converting enzyme was strengthened by the immunohistochemical findings, which showed

1 presence of 17 β HSD14 in several steroidogenic tissues including uterus, testis and breast. Among
2 female and male reproductive tissues, 17 β HSD14 was most strongly expressed in uterus and testis
3 respectively, staining endometrial glands as well as male germ cells and Leydig cells, where
4 17 β HSD14 may participate in the fine-tuning of the levels of bioactive sex hormones which is crucial
5 for the homeostasis in these tissues. 17 β HSD14 was not expressed in prostate tissue. Among a large
6 number of normal breast samples analysed, 17 β HSD14 was highly expressed in the majority. It has
7 been suggested that the predominant enzymatic direction in normal breast is oxidative, favouring E2 to
8 E1 conversion, whereas tumour progression is associated with a shift towards a reductive
9 environment, and these changes have been shown to concur with changes in local protein expression
10 of steroidogenic enzymes from a dominance of oxidative enzymes such as 17 β HSD2, towards
11 increased expression of reducing enzymes, e.g. 17 β HSD1 [23-25].

12 17 β HSD14 was expressed throughout the gastrointestinal tract with most prominent staining seen in
13 stomach as well as in colonic and rectal samples. In colon and rectal samples, immunoreactivity of
14 enterocytes and goblet cells of mucosal crypts was prominent in the outer absorptive border facing the
15 lumen, whereas positivity towards the deeper situated glands gradually diminished. The same pattern,
16 although not as marked, was seen in duodenal samples. Based on expression patterns in mucosal
17 layers of the colon and rectum, which resemble those published regarding other oxidizing 17 β HSD
18 enzymes, e.g. type 2 and 4 in colon [26,27], it is suggestive that 17 β HSD14 has a role in protecting
19 tissues from exposure to excess reduced steroidal agents such as ingested or bacterially derived
20 oestradiol. As proposed by English et al, the primary enzymatic activity in colonic tissue is oxidative,
21 favouring the inactivation of potent sex steroids [27].

22 The kidney harbours several steroidogenic enzymes [28-30]. The exact significance of steroid
23 metabolism in renal cells has not been extensively studied, although observed gender differences in
24 e.g. blood pressure response to salt stimuli and the tendency to form urinary stones suggests
25 involvement of sex steroids in the regulation of tubular reabsorption [28]. In our study, 17 β HSD14
26 positivity in kidney was intense and specific, present in epithelial cells of proximal and distal tubules
27 whereas renal corpuscles were negative. Specific immunopositivity for 17 β HSD14 and other 17 β HSD

enzymes such as 17 β HSD5 in sites for selective ion and water transport in the kidney could possibly suggest a role for 17 β HSD enzymes in normal reabsorption physiology [28,31].

The *HSD17B14* gene product was first cloned from a retinal cDNA-library and was also found to be highly expressed therein [1]. We found 17 β HSD14 immunoreactivity in cytoplasmic projections of the plexiform layers of the retina, whereas nuclear layers were negative, with an exception for the inner nuclear layer, where staining was prominent in specific nuclei, most likely belonging to amacrine cells. Interestingly, the inner nuclear layer of the retinal epithelium has been shown to harbour the highest density of steroidogenic enzymes in rat retina [32]. The presence of 17 β HSD14 in retina could be related to a role in steroid metabolism, but it could also be related to a possible role in retinoid metabolism. 17 β HSD enzymes share homology with retinol dehydrogenase enzymes, and some 17 β HSD enzymes have been shown to metabolise retinoids [16,33,34]. As recently shown by Haller *et al.*, the exchange of a single amino acid in the 17 β HSD1 enzyme makes the enzyme efficiently reducing all-*trans*-retinal to its alcohol [35].

Although conversion data support a role for 17 β HSD14 in sex steroid metabolism, the conversion rates of these reactions are relatively low, with approximately 8-15 % of the added hydroxysteroids being oxidized after 72 hours. In comparison, and as a methodological control, using the same transfection system, we transiently over-expressed *HSD17B2* and found 100% of added oestradiol to be oxidised to oestrone within less than 24 hours. The low efficiency of 17 β HSD14 in oxidising hydroxysteroids could possibly suggest that the enzyme in addition operates on other, yet unknown substrates. A role for 17 β HSD14 in inflammatory responses is proposed by a recent interesting publication by Saijo *et al.*, in which 17 β HSD14 is shown to act immunomodulatory by reducing DHEA into androstenediol, which in turn is shown to be a significant inducer of anti-inflammatory responses mediated by ER β in the central nervous system [36]. When adding DHEA as a substrate to transiently *HSD17B14* over-expressing MCF10A cells, we detected no reduction exceeding that of mock-transfected cells within 72 hours using our HPLC-based system. The reason for this apparent discrepancy remains elusive, and the suggestion that 17 β HSD14 would potentially act as a reductive enzyme as shown by Saijo *et al.*, is intriguing as neither we, nor Lukacik *et al.*, could detect reductive

activity when adding ketosteroids such as E1 or A as substrates to purified enzyme [2] or cells over-expressing *HSD17B14*. It has been shown that oxidation or reduction reactions catalysed by 17 β HSD enzymes are essentially reversible reactions, however, in intact cells these enzymes cause equilibrium, favouring either the 17 β -hydroxy conformation or the 17-keto conformation of the sex steroid, and the enzymes are thus classified as either reductive or oxidative [37]. Sherbet et al., have shown that whereas the E2/E1 ratio in cell culture media of cells expressing *HSD17B1* could be altered by glucose deprivation which causes changes in the NADPH/NADP⁺ ratio and thus alters the preference from E1 to E2 as a substrate, the preference of *HSD17B2* for E2 oxidation was not affected by changes in the NAD⁺/NADH gradient [3]. The influence on the enzymatic properties of 17 β HSD14 of factors that could affect cofactor availability, such as prolonged incubation times with subsequent changes in cell density and pH, have not been investigated, hence there remains a possibility that differences in cell culture conditions could be the cause of the dissimilar results. The discrepancy could also stem from the fact that a different cell line is used or even the means of analysis used which differs between our studies. Whereas Saijo et al used non-labelled steroids which after extraction from cell culture media were derivatised and then separated/analysed with gas chromatography, we used radiolabelled steroids which were separated via reversed phase HPLC and detected by an on-line scintillographer. Further studies on the topic of 17 β HSD14 substrate preference are warranted.

In conclusion, the 17 β HSD14 enzyme is expressed in several classical steroidogenic tissues such as breast, ovary and testis which supports the theory of 17 β HSD14 being an actor in intracrine sex steroid interconversion. This theory is further strengthened by the finding that 17 β HSD14 oxidises both oestradiol and testosterone in intact cells. Immunohistochemical staining patterns of 17 β HSD14 with the enzyme being primarily expressed in glandular epithelial cells, may implicate a role for the enzyme in modulating the levels of secreted sex steroids, either endogenous or externally derived.

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LEGENDS

Figure 1

Immunoblot analysis of lysates from HEK293 (A) and MCF10A (B) cells over-expressing HSD17B14. A single band at 28 kDa corresponding to 17βHSD14 is seen in lane 2 of both blots. The band is not present in lanes 3 and 4 (row 2) representing mock-transfected and untransfected cells respectively. β-actin (upper row), at 45 kDa is used to control for equal loading.

Figure 2

17βHSD14 immunolocalisation in reproductive tissue; A, uterus, B, ovary, C, uterus, D, breast, E, testis and F, prostate. Scale bars represent 50 (C) or 100 (A, B, D, E, F) μm.

Figure 3

Immunolocalisation of 17βHSD14 in A, kidney, B, retina (arrows depicting inner nuclear layer), C stomach, D colon, E rectum and F duodenum. Peptide neutralisation assay; in G, liver section with 17βHSD14 immunoreactivity and H, liver immunostained with an anti-17βHSD14 antibody neutralised with a 17βHSD14-peptide. Scale bars represent 50 (A, B, G, H) or 100 (C, D, E, F) μm.

Figure 4

Interconversion of tritiated steroids in HEK293 (A to D) and MCF10A (E) transiently transfected with HSD17B14 expressed as the fraction of added compound compared to the total amount of added and generated steroid metabolite. Interconversion profiles for HSD17B14-transfected cells compared with that of a mock-transfected cells treated with (A) oestrone, (B) oestradiol, (C) androstenedione, (D) testosterone and (E) dehydroepiandrosterone (DHEA). Representative experiments are shown. Experiments have been performed in duplicates and repeated at least three times.

Table 1

Summary of immunohistochemical staining patterns and grading of 17βHSD14 in human tissue

Table 1

<i>Tissue</i>	<i>Female n (median age)</i>	<i>Male n (median age)</i>	<i>Grading</i>	<i>Immunolocalisation</i>
Adrenal gland	2 (17.5)	1 (0,5)	Strong	<i>Cortex layer</i>
Bone (myeloid)	5 (50)	3 (56)	moderate	
Breast	60 (43.5)		Moderate/strong	<i>Ductal epithelial cells</i>
Brain	14 (24)		Moderate	<i>Gray and white matter</i>
Colon	19 (54)	41 (52)	Moderate/strong	<i>Glandular surface epithelium</i>
Eye	3 (52)		Strong	<i>Retinal plexiform layers, inner nuclear layer amacrine cells</i>
Esophagus	1 (42)	7 (30)	Strong	<i>Mucosal cells</i>
Heart	3 (21)		Strong	
Kidney	5 (21)	3 (22)	Strong	<i>Cortical tubular epithelium</i>
Larynx		3 (45)	Moderate	<i>Outer mucosal layer</i>
Liver	6 (21)	2 (35)	Strong	<i>Glandular parenchyma</i>
Lung	2 (21)	6 (39.5)	Weak	<i>Epithelial cells in bronchial areas</i>
Lymph node	2 (38.5)	3 (61)	Negative	
Mesothelium	2 (21)	1 (43)	Weak	
Nerve	2 (18)	1 (43)	Weak	
Ovary	8 (21)		Weak	<i>Dominant follicles</i>
Pancreas	6 (21)	2(20.5)	Moderate/strong	<i>Acinar cells</i>
Parathyroid gland		3 (33)	Moderate/strong	
Pituitary	2 (16.5)	1 (56)	Moderate/strong	
Prostate		60 (66)	Weak	<i>Tubuloalveolar epithelium</i>
Rectum	1 (35)	4 (50)	Strong	<i>Gland cell surface epithelium</i>
Salivary Gland	1 (38)	2 (36)	Strong	
Skeletal muscle		3 (40)	Strong	
Skin	1 (50)	7 (35)	Moderate	<i>Stratum spinosum and glandular structures</i>
Small Intestine	2 (49)	3 (40)	Moderate	<i>Mucosal cells</i>
Spleen	4 (21)	4 (32.5)	Moderate	
Stomach	3 (14)	5 (35)	Moderate/strong	<i>Glandular</i>
Testis		8 (40)	Strong	<i>Spermatogonia, spermatocytes, Leydig cells</i>
Thymus gland	2 (18)	6 (2)	Moderate	<i>Centers of Hassals' corpuscles</i>
Thyroid	1 (21)	2 (37.5)	Weak	
Tonsil	2 (21)	1 (48)	Negative	
Uterus	3 (21)		Moderate/strong	<i>Epithelial cells of endometrial glands</i>