Mouse 17β-hydroxysteroid dehydrogenase type 2 mRNA is predominantly expressed in hepatocytes and in surface epithelial cells of the gastrointestinal and urinary tracts

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ABSTRACT

17β-Hydroxysteroid dehydrogenase (17HSD) type 2 efficiently catalyzes the conversion of the high activity 17β-hydroxy forms of sex steroids into less potent 17-ketosteroids. In the present study *in situ* hybridization was utilized to analyze the cellular localization of 17HSD type 2 expression in adult male and female mice. The data indicate that 17HSD type 2 mRNA is expressed in several epithelial cell layers, including both absorptive and secretory epithelia as well as protective epithelium. In both males and females, strong expression of 17HSD type 2 was particularly detected in epithelial cells of the gastrointestinal and urinary tracts. The mRNA was expressed in the stratified squamous epithelium of the esophagus, and surface epithelial cells of the stomach, small intestine and colon. The hepatocytes of the liver and the thick limbs of the loops of Henle in the kidneys, as well as the epithelium of the urinary bladder, also showed strong expression of 17HSD type 2 mRNA in both male and female mice. In the genital tracts, low 17HSD type 2 expression was detected in the seminiferous tubules, the uterine epithelial cells and the surface epithelium of the ovary. Expression of the mRNA was also detected in the sebaceous glands of the skin. The results indicate that in both male and female mice, 17HSD type 2 is expressed mainly in the various epithelial cell types of the gastrointestinal and urinary tracts, and therefore suggest a role for the enzyme in steroid inactivation in a range of tissues and cell types not considered as classical sex steroid target tissues.
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INTRODUCTION

17β-Hydroxysteroid dehydrogenases (17HSDs) catalyze the interconversion between neutral and phenolic 17β-hydroxy and 17-ketosteroids such as estrone (E1) and estradiol (E2), androstenedione (A-dione) and testosterone (T), 5α-dihydrotestosterone (DHT) and 5α-androstanedione (5α-A-dione). In general, the 17β-hydroxy forms of sex steroids have at least one order of magnitude higher affinity for estrogen and androgen receptors compared with the corresponding 17-ketosteroids. Hence, 17HSDs catalyze reactions between highly potent sex steroids and the far less potent 17-keto forms. The following evidence indicates that 17HSD enzymes play a significant role in several steps regulating the availability of the highly active ligands for receptor binding at the target cells. (i) Reductive 17HSD activity (17-keto to 17β-hydroxy) catalyzes one of the last steps in the biosynthesis of E2 and T in the ovaries and the testes respectively. (ii) Oxidative 17HSD-activity (17β-hydroxy to 17-keto) is one of the key metabolic reactions involved in the inactivation and excretion of sex steroids from the blood circulation. (iii) 17HSD enzymes present in steroid hormone target tissues regulate the relative concentrations of 17β-hydroxy and 17-keto forms

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of estrogens and androgens locally at the target tissue level.

At present five distinct 17HSD enzymes have been identified in rodents (Ghersevich *et al*. 1994*a*, Deyashiki *et al*. 1995, Normand *et al*. 1995, Akinola *et al*. 1996, Nokelainen *et al*. 1996, Sha *et al*. 1996, Mustonen *et al*. 1997), and four have been characterized in humans (Peltoketo *et al*. 1988, Wu *et al*. 1993, Geissler *et al*. 1994, Adamski *et al*. 1995). Each of the enzymes possesses unique enzymatic properties and has a distinct tissue distribution. Data obtained using rodent and human tissue specimens and cell lines indicate that reductive 17HSD type 1 and type 3 enzymes are principally involved in E2 and T biosynthesis in the ovaries and testes respectively (Geissler *et al*. 1994, Ghersevich *et al*. 1994*a*,*b*, Poutanen *et al*. 1995, Andersson *et al*. 1996, Miettinen *et al*. 1996). In contrast, type 2 and type 4 enzymes exclusively catalyze the opposite reaction, inactivating E2 to E1, and T to A-dione (Wu *et al*. 1993, Leenders *et al*. 1994, Adamski *et al*. 1995, Normand *et al*. 1995, Poutanen *et al*. 1995, Akinola *et al*. 1996, Andersson *et al*. 1996, Dieuaide-Noubhani *et al*. 1996, Miettinen *et al*. 1996, Mustonen *et al*. 1997). In addition, DHT is converted to 5α-A-dione by the type 2 enzyme (Wu *et al*. 1993, Elo *et al*. 1996).

In addition to the oxidation of E2 and T, the type 4 enzyme also catalyzes β-oxidation reactions of fatty acids and intermediates of bile acid biosynthesis (Dieuaide-Noubhani *et al*. 1996, Qin *et al*. 1997). The enzyme is constitutively expressed in all human and rodent tissues (Adamski *et al*. 1995, Mustonen *et al*. 1997), and our recent findings suggest that, when compared with 17HSD type 2, the type 4 enzyme is very inefficient at oxidizing E2 to E1 (Dieuaide-Noubhani *et al*. 1996, Qin *et al*. 1997). The data, hence, suggest that, of the 17HSD enzymes characterized to date, the type 2 enzyme is most efficient at converting 17β-hydroxysteroids into 17-keto forms. Up to now the cellular distribution of 17HSD type 2 has been only superficially characterized (Casey *et al*. 1994, Delos *et al*. 1995, Zhang *et al*. 1996). The present study, performed by utilizing *in situ* hybridization, shows that 17HSD type 2 is particularly expressed in numerous epithelial cell populations of the gastrointestinal and urogenital tracts of both male and female mice.

MATERIALS AND METHODS

Materials

Radiolabeled [α-^{35}S]dCTP (1300 Ci/mmol) was purchased from DupontNEN (Boston, MA, USA). Non-labeled nucleotides (rATP, rGTP and rUTP) were purchased from Promega (Madison, WI, USA). Restriction enzymes and DNA-modifying enzymes were from New England Biolabs (Beverly, MA, USA) and Boehringer (Mannheim, Germany). GTG agarose was from FCM BioProducts (Rockland, ME, USA) and the RNA ladder was from BRL (Gaithersburg, MD, USA). T7 and SP6 RNA polymerases were from Promega, and proteinase K and tRNA were from Boehringer. Other reagents not mentioned were purchased either from the Sigma Chemical Co. (St Louis, MO, USA) or Merck AG (Darmstadt, Germany) and were of the highest purity grade available.

Tissue specimens

In this study, formalin-fixed, paraffin-embedded mouse tissues were used. Tissues excised from adult BALB/c male and female mice were briefly washed with PBS, fixed overnight in 4% paraformaldehyde–PBS, dehydrated and embedded in paraffin (solidification point 51–53 °C, Merck). Thereafter, 7 μm sections were cut and collected on glass slides. The sections were dewaxed with xylene, and before hybridization, reactive aldehyde groups remaining after fixation were eliminated by 10-min treatment in 0·1 M glycine/0·2 M Tris HCl, pH 7·4.

In situ hybridization

A 737 bp fragment (nucleotides 584–1320) of mouse 17HSD type 2 cDNA (Mustonen *et al*. 1997) was cloned in pSP72 plasmid (Promega). Sense and antisense [α-^{35}S]CTP-labeled RNA probes were transcribed with SP6 and T7 RNA polymerases using linearized plasmids as templates. Before RNA transcription, the protruding 3′-overhang produced by SacI was destroyed by incubating the plasmid with Klenow DNA polymerase for 15 min at 22 °C. Specific activities of the synthesized RNA probes were approximately 5×10^7 c.p.m./μl.

The *in situ* hybridization protocol was based on that described by Chotteau-Lelievre *et al*. (1997), with minor modifications. Briefly, the sections were treated with proteinase K (1 μg/ml in 100 mM Tris HCl, 50 mM EDTA, pH 8·0) for 15 min at 37 °C. This was followed by post-fixation (30 min in 4% paraformaldehyde–PBS), acetylation (10-min treatment in 0·25% acetic anhydride in 0·1 M triethanolamine, pH 8·0) and dehydration in ethanol. After drying the sections, 70 μl sense or antisense RNA probes (20 000 c.p.m. labeled RNA/μl in 50% formamide, 0·3 M NaCl, 20 mM Tris HCl (pH 8·0), 5 mM EDTA, 100 mM DTT, 0·5 mg tRNA/ml, 1 × Denhardt's solution and 10% dextran

sulfate) were applied to the tissues. Coverslips were added and the slides were incubated at 60 °C overnight.

The sections were then washed with 4 × SSC and 10 mM DTT four times for 15 min each, followed by a stringent wash for 30 min at 60 °C in 50% formamide, 0·15 M NaCl, 30 mM Tris HCl, 5 mM EDTA, pH 8·0. Thereafter the sections were treated with RNase A solution (20 μg/ml) for 30 min at 37 °C, washed for 15 min in 2 × SSC at 60 °C and 15 min in 0·1 × SSC at 60 °C and then dehydrated with ethanol and air dried. The slides were then dipped in NTB2 emulsion (Eastman Kodak, Rochester, NY, USA; diluted 1:1 in 0·6 M ammonium acetate), and exposed in the dark at 4 °C for 15 days. The slides were then developed at 12 °C by treating with D-19 solution (Eastman Kodak) for 2·5 min, rinsed in distilled water, fixed for 5 min in Unifix (Eastman Kodak), and finally rinsed for 5 min in distilled water. Nuclei were further stained with Hoechst 33258 (Sigma), after which the slides were mounted with glycergel (DAKO A/S, Glostrup, Denmark). Hybridization with a sense probe was used as a control and no hybridization signals were detected in any of the tissues analyzed.

RESULTS

By using Northern analysis, two mouse 17HSD type 2 mRNA transcripts (0·9 and 1·2 kb) are detected with a constant ratio in various tissues. Sequencing results of several cDNA clones from a liver library indicate that the predominant form, the 0·9 kb mRNA, codes for the full length functional protein (Mustonen *et al*. 1997). Both of the mouse mRNAs are identically detected by a cDNA corresponding to the RNA-probe used in the present study (nucleotides 584–1320 of the cDNA) as well as with a full length cDNA probe (data not shown). All this information indicates that the present study reports the cellular localization of two functional mouse 17HSD type 2 mRNAs. Furthermore, our previous results on the tissue distribution of the mRNAs agree with the present data obtained with *in situ* hybridization. In line with our previous Northern analyses (Mustonen *et al*. 1997), type 2 mRNA was detected in several epithelial cell layers of the gastrointestinal and urogenital tracts of both male and female mice, including absorbtive and secretory epithelium as well as protective epithelium. No 17HSD type 2 expression was found in connective tissues or muscle cell layers in any of the tissue specimens analyzed. Neither was mRNA expression detected in any parts of the respiratory system, including epithelial cells of the trachea and bronchi, or in the respiratory epithelium of alveolar sacs. Furthermore, 17HSD type 2 mRNA was not detected in any of the cell types present in the pancreas, spleen, heart, brain or adrenals, in which signals obtained with antisense probe were indistinguishable from those found by hybridizing with the sense probe (data not shown).

Male and female gastrointestinal tract and liver

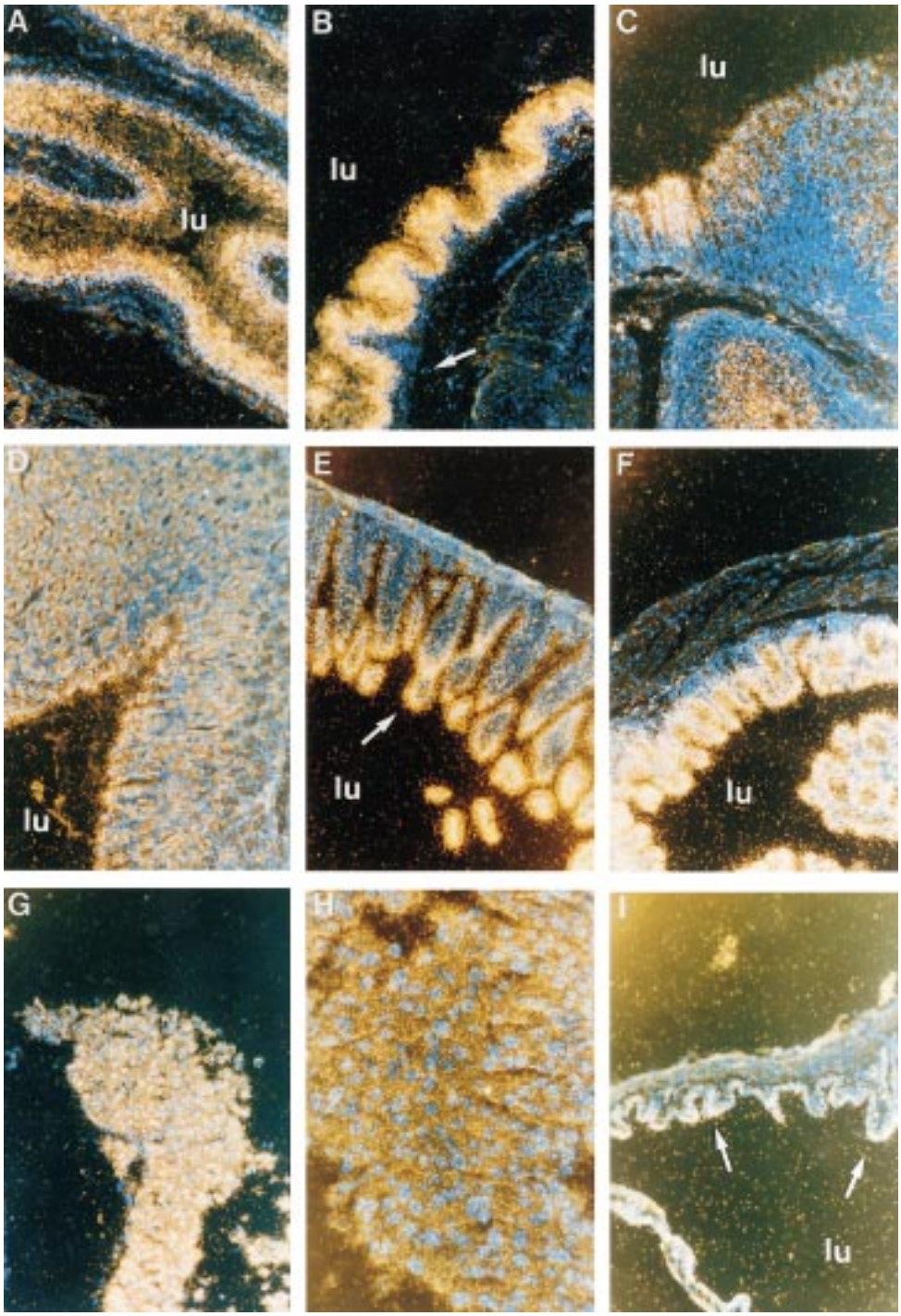
The localization and intensity of 17HSD type 2 mRNA expression were identical in the gastrointestinal tracts of both male and female mice. The mRNA was expressed in several epithelial cell types throughout the gastrointestinal tract. Strong expression of the mRNA was detected in the stratified squamous epithelium of the esophagus, both in the thoracic area (Fig. 1A) and at the esophago–gastric junction (Fig. 1B). Moderate mRNA expression was found in the surface epithelial cells of the stomach (Fig. 1C and D), while gastric glands did not show prominent expression. The mRNA for 17HSD type 2 was also strongly expressed in the surface epithelium of the small intestine (Fig. 1E) and colon (Fig. 1F), in which the highest expression was seen at the tips of mucosal villi. Interestingly, expression of the mRNA gradually increased towards the tips of the villi. No expression was found in the lamina propria or the muscularis mucosae, or the submucosal layers of the esophagus, stomach or intestine.

The hepatocytes, which develop from the endodermal epithelium during embryogenesis, showed equally strong expression of 17HSD type 2 mRNA in both male and female mice (Fig. 1G and H). In addition, the mucosal epithelium of the gall bladder showed slight expression of the mRNA (Fig. 1I).

Male and female urogenital tract

In both male and female kidneys, the mRNA was detected predominantly in deep layers of the cortex and outer medulla (cortico–medullary junction), most probably corresponding to the thick descending and/or ascending limbs of the loops of Henle (Fig. 2A). No expression was found in the renal corpuscles or in the collecting tubules of the medulla. The transitional epithelium of the urinary bladder showed a strong hybridization signal for 17HSD type 2 mRNA in both male and female mice (Fig. 2B). However, the connective tissue and the muscle cell layers did not show any expression for the enzyme.

Among the male reproductive organs, heterogeneous expression of 17HSD type 2 mRNA was



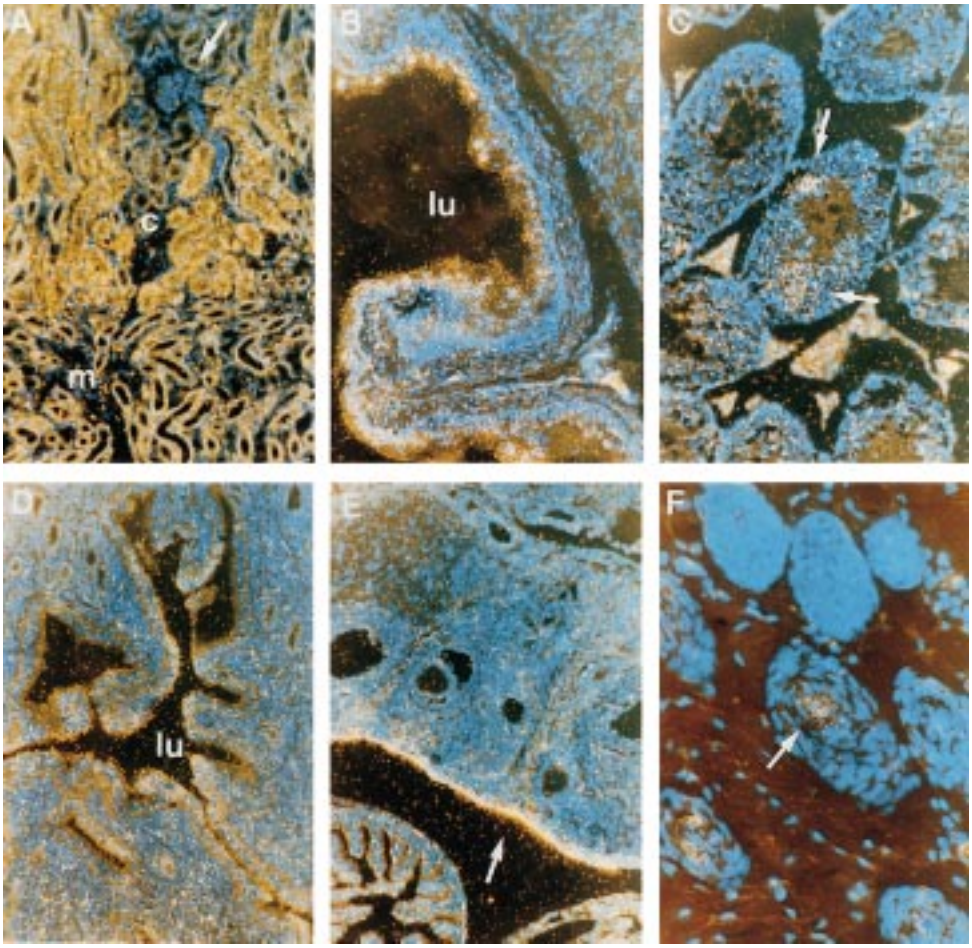


FIGURE 2. Darkfield microscope images of the urogenital tract and skin, showing hybridization signals for 17HSD type 2. (A) In kidney the mRNA was expressed moderately in the corticomedullary junction, i.e. in the thick descending and/or ascending limbs of the loops of Henle, but no expression was found in the collecting tubules of the medulla (m) or in the proximal tubules of the cortex (c) (arrow=glomerulus). (B) The epithelium of the urinary bladder showed a strong hybridization signal for 17HSD type 2 mRNA. (C) Heterogeneous expression of 17HSD type 2 mRNA was seen in the seminiferous tubules (arrows) of testes. (D) The mRNA for type 2 was only slightly expressed in uterine epithelial cells. (E) In the ovary, significant expression was found only in surface epithelial cells (arrow). (F) The enzyme was also expressed in the sebaceous glands of the skin (arrow). Magnification: A–E × 160; F × 400; nuclei were stained with Hoechst 33258 (blue color), lu=lumen.

FIGURE 1. Darkfield microscope images of the gastrointestinal tissues and liver of male mice, showing hybridization signals for 17HSD type 2. (A) A strong signal was detected in the stratified squamous epithelium of the thoracic esophagus. (B) mRNA signal in the esophagus was strongest near the esophago–gastric junction (arrow). (C and D) In surface epithelial cells of the stomach, the strongest expression was detected on the luminal surface, while no signal was detected in the muscle cell layers (lower right corner in Fig. C). (E) The mRNA for 17HSD type 2 was strongly expressed in the epithelial cells of mucosal villi of the small intestine. Expression of the mRNA increases gradually towards the tips of the villi (arrow). (F) Strong expression of the mRNA was also detected in epithelial cells of the colon. (G and H) A strong signal for 17HSD type 2 was seen in hepatocytes. (I) The mucosal epithelial cells of the gall bladder showed only weak expression of the mRNA (arrows). Magnification: A–G, I × 160; H × 400; nuclei were stained with Hoechst 33258 (blue color), lu=lumen.

detected in approximately 30% of the seminiferous tubules (Fig. 2C), suggesting that the enzyme is expressed either in developing sperm cells or in Sertoli cells. No detectable expression was found in the interstitial space (Leydig cells), or in any cell types of the epididymis, seminal vesicle or prostate. In females, the mRNA was slightly expressed in uterine epithelial cells (Fig. 2D). In the ovary, low expression was found in surface epithelial cells (Fig. 2E), and none in granulosa or theca cells at any stage of follicular development. Similarly, the mRNA was not detectable in corpora lutea.

Male and female skin

Expression of the mRNA was analyzed in female and male skin, and it was found exclusively only in sebaceous glands (Fig. 2F).

DISCUSSION

The substrate specificity of 17HSD type 2 is not fully characterized, but the data available indicate that the enzyme efficiently inactivates E2, T and DHT into their corresponding inactive 17-keto forms (Wu *et al*. 1993, Akinola *et al*. 1996, Elo *et al*. 1996, Miettinen *et al*. 1996, Mustonen *et al*. 1997), while the opposite reductive reaction is not catalyzed by the enzyme. In line with its enzymatic activity, the present results indicate that the enzyme is not expressed in ovarian follicles or testicular Leydig cells, but it is detectable in ovarian surface epithelial cells and in the seminiferous tubules of the testis. Based on these results, together with those of other recent studies (Wu *et al*. 1993, Geissler *et al*. 1994, Akinola *et al*. 1996, Miettinen *et al*. 1996, Mustonen *et al*. 1997), it is evident that the enzyme does not significantly contribute to E2 and T biosynthesis. However, using RT–PCR, low amounts of 17HSD type 2 mRNA have been detected in human corpora lutea (Zhang *et al*. 1996).

17HSD type 2 was initially cloned from a human prostate library by using an expression cloning technique with T as a substrate (Wu *et al*. 1993). Northern analyses have, furthermore, shown that, of the sex steroid target tissues, the enzyme is expressed at least in benign and malignant human prostate (Wu *et al*. 1993, Delos *et al*. 1995, Elo *et al*. 1996), and in normal human endometrium (Casey *et al*. 1994, Miettinen *et al*. 1996). Previous data suggest that in both human endometrium and prostate the enzyme appears to be present in the epithelial cells (Casey *et al*. 1994, Delos *et al*. 1995). In line with the results of these studies, the enzyme has also been detected in certain epithelial-like cancer cell lines originating from these tissues (Miettinen *et al*. 1996). It is likely therefore, that the enzyme down-regulates sex steroid action locally in the target tissues by inactivating circulating 17-hydroxysteroids into their inactive 17-keto forms (Casey *et al*. 1994, Elo *et al*. 1996, Miettinen *et al*. 1996). However, our recent Northern analyses have shown that the enzyme is only slightly expressed in rodent prostate and uterus (Akinola *et al*. 1996, Mustonen *et al*. 1997). This, together with the results of the present study, suggests a difference between humans and rodents in 17HSD type 2 expression in the genital tract.

Compared with the uterus and prostate, stronger expression of 17HSD type 2 mRNA is found in several human and rodent tissues which are not considered as classical steroid hormone target tissues, including intestine, liver, kidney and brain (Casey *et al*. 1994, Akinola *et al*. 1996, Miettinen *et al*. 1996, Mustonen *et al*. 1997). In the present study, strong expression of the mRNA was localized in the epithelium of the esophagus, stomach, small intestine, colon, urinary bladder and thick descending and/or ascending limbs of the loops of Henle in the kidney, as well as in hepatocytes. By using Northern analyses, 17HSD type 2 mRNA expression has been previously detected also in the brain as well as in adrenals (Carsol *et al*. 1996, Mustonen *et al*. 1997). However, in the present *in situ* hybridization study, the mRNA could not be detected in any of the sections obtained from different areas of the brain and adrenals. This suggests a low homogenous expression of the mRNA throughout these tissues. Our recent Northern analyses indicated that in the rat, 17HSD type 2 mRNA is similarly expressed in both male and female liver and small intestine, from late fetal life to 6-week-old animals (Akinola *et al*. 1997). This, together with the present findings, indicates that there are no differences in 17HSD type 2 expression in the various epithelial cell types in male and female mice. The data, therefore, suggest constitutive expression of the enzyme in the gastrointestinal tract, and that 17HSD type 2 expression is not related to the concentration or type of sex steroid in the blood circulation. However, estrogen receptors, but not androgen receptors, have been localized in many of the cell types of the gastrointestinal and urinary tracts expressing 17HSD type 2 (Riuzeweld de Winter *et al*. 1991, Pacchioni *et al*. 1993, Thomas *et al*. 1993), and additional studies are needed in order to characterize the role of 17HSD type 2 in the regulation of sex steroid action locally in the different cell types of the gastrointestinal tract.

The expression of 17HSD type 2 in several luminal epithelial cell types in both the gastrointestinal and urinary tracts suggests that the enzyme could have a role in inactivating sex steroids and steroid-like compounds found in the intestinal contents. It is well known that orally administered E2 and T are readily inactivated, and do not enter the blood circulation in significant amounts. Hence, it is likely that 17HSD type 2 is one of the enzymes involved in the rapid degradation and excretion of steroids in surface epithelial cells and hepatocytes in the intestine and liver respectively.

17HSD type 2 is one of the short chain dehydrogenases/reductases (SDR). In the family of SDR-enzymes, 17HSD type 2 has the highest identity (45%) with 11β-HSD (11HSD) type 2 (Baker 1995, Brown *et al*. 1996). The 11HSD type 2 enzyme possesses a predominant oxidative activity inactivating cortisol (11β-hydroxy) to cortisone (11-keto), and the activity of the enzyme has been shown to be crucial for aldosterone action in mineralocorticoid target tissues (Edwards *et al*. 1988, Funder *et al*. 1988, Mune *et al*. 1995). It has been suggested that the 11HSD and 17HSD type 2 enzymes have sufficiently conserved their tertiary structures such that 17HSD type 2 could also metabolize a corticosteroid (Baker 1995). Interestingly, the cellular distribution shown for 11HSD type 2 (Roland & Funder 1996, Smith *et al*. 1996) is very close to that resolved for 17HSD type 2 in the present study. However, in cultured cells, cortisol does not affect the E2 to E1 conversion catalyzed by 17HSD type 2 (data not shown), indicating that the putative 11HSD activity of the enzyme is not significant compared with its 17HSD activity. As E2 and T have not been shown to possess any significant affinity towards mineralocorticoid receptors (Arriza *et al*. 1987, Alnemri *et al*. 1991), the physiological role, if any, of 17HSD type 2 in mineralocorticoid target tissues remains to be characterized. In conclusion, the localization of 17HSD type 2 in several surface epithelial cells of the gastrointestinal and urinary tracts suggests a role for the enzyme in steroid inactivation in a range of tissues and cell types not considered as classical sex steroid target tissues.

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