

Localization of 17 β -hydroxysteroid dehydrogenase type 1 mRNA in mouse tissues

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Abstract

The enzyme 17 β -hydroxysteroid dehydrogenase (17 β -HSD) type 1 catalyzes the conversion of estrone (E₁) into 17 β estradiol (E₂). To gain information about the cellular localization of 17 β -HSD mRNA type 1 expression, we performed *in situ* hybridization using a ³⁵S-labeled cRNA probe in several tissues of adult mice of both sexes. In the ovary, high expression was found in granulosa cells of growing follicles. No specific labeling could be observed in corpora lutea or interstitial cells. In the pituitary gland of animals of both sexes, 17 β -HSD type 1 mRNA was expressed in the intermediate lobe melanotrophs while no specific signal could be detected in the anterior or posterior lobes of the pituitary. In the prostate, 17 β -HSD type 1 mRNA was exclusively found in the epithelial cells. In both male and female mouse dorsal skin, a specific hybridization signal was seen in the sebaceous glands while the epidermis, stroma, hair follicles and sweat glands were unlabeled. In the testis, a hybridization signal was detected in germ cells of the seminiferous tubules, Leydig cells being unlabeled. The present data indicate that E₂ can be formed through the action of 17 β -HSD type 1 in specific cells of the gonads and peripheral tissues. In the testes and peripheral tissues, the action of E₂ is probably limited to the cells involved in its formation in an intracrine fashion.

Journal of Molecular Endocrinology (2004) **33**, 459–465

Introduction

The 17 β -hydroxysteroid dehydrogenase (17 β -HSD) enzymes play essential roles in steroid hormone formation by catalyzing the final steps in androgen and estrogen biosynthesis (Labrie *et al.* 2000, 2003, Luu-The 2001, Mindnich *et al.* 2004). The molecular structure of human 17 β -HSD type 1 cDNA and of its corresponding gene which encodes a predicted protein of 327 amino acids was the first to be elucidated (Peltoketo *et al.* 1988, Luu-The *et al.* 1989). This enzyme, a member of the short-chain alcohol dehydrogenase superfamily, is a cytosolic protein that exists in a homodimeric form, that predominantly catalyzes the interconversion of estrone (E₁) to estradiol (E₂) using NADP(H) as a cofactor (Dumont *et al.* 1992, Lin *et al.* 1992). An analysis of the tissue distribution revealed the expression of 17 β -HSD type 1 mRNA in steroidogenic as well as various other peripheral tissues, including the placenta, ovary, breast,

endometrium, prostate, skin and adipose tissue (Peltoketo *et al.* 1988, Dumont *et al.* 1992).

A mouse 17 β -HSD type 1 cDNA has also been cloned and found to encode a protein of 344 amino acid residues (Nokelainen *et al.* 1996, Mustonen *et al.* 1997). The mouse enzyme shows 63% overall identity with that of the human. The mouse 17 β -HSD type 1 mRNA transcript was found in ovarian granulosa cells, but not in term placenta (Nokelainen *et al.* 1996). Transient expression studies of mouse recombinant 17 β -HSD type 1 have shown that the enzyme efficiently converts E₁ to E₂ and also androstenedione to testosterone (Nokelainen *et al.* 1996, Mustonen *et al.* 1997).

The localization of 17 β -HSD type 1 mRNA at the cellular level has not been reported so far. We therefore used *in situ* hybridization (ISH) to localize the 17 β mRNA type 1 in several tissues in adult mice of both sexes to gain information about the exact sites of expression of the enzyme.

Materials and Methods

Animals

Four adult male (26–30 g) and female (24–27 g) C57BL6 mice were housed under constant temperature (21 ± 1 °C) and light (lights on from 0600 to 2000 h) regimen. The animals received Purina Chow (Ralston-Purina, St-Louis, MO, USA) and tap water *ad libitum*. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals. The animals were all perfused between 0900 and 1000 h for histological procedures as described below. The females were on diestrous day 1.

Histological procedures

All the animals were deeply anesthetized with an i.m. injection of ketamine hydrochloride (80 mg/kg body weight (bw); Bayer Inc. Toronto, Canada) and zylazine (10 mg/kg bw). They were perfused transcardially with 50 ml 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The different tissues, i.e., liver, kidney, lung, skin, adrenal, pituitary, testis, prostate, ovary, uterus, vagina, mammary gland and brain were excised and postfixed in the same fixative for 24 h at 4 °C. The tissues were placed in 15% sucrose in 0.1 M phosphate buffer before being quickly frozen in isopentane cooled in liquid nitrogen.

ISH

Frozen sections (10 μ m thick) were serially cut at -20 °C and mounted on gelatin- and poly-L-lysine-coated slides. The vector used for the production of the cRNA probe was constructed by the insertion of a cDNA fragment (504 bp) of mouse 17 β -HSD type 1 (GeneBank accession number NM_010475) into a pBSKSII+ vector (Statagene, La Jolla, CA, USA). The cDNA fragment located at position 47–550 downstream from the ATG start codon was obtained by amplification using PCR. ISH with the antisense and sense ³⁵S-labeled cRNA probes was performed as previously described (Givalois *et al.* 1997). Following hybridization procedures, the sections were then dehydrated and exposed onto

Kodak Biomax MR films for 3–8 days before being coated with liquid photographic emulsion (Kodak-NTB2; diluted 1:1 with water). Slides were exposed for 3–45 days, developed in Dektol developer (Kodak, Rochester, NY, USA) for 2 min, and fixed in rapid fixer (Kodak) for 4 min. Thereafter, the sections were rinsed and coverslipped with Permount (Fisher Scientific, Montreal, Canada).

Results

After 3 to 8 days of exposure of the films, specific radiolabeling was observed in the ovary, testis, prostate, pituitary gland and skin. No specific hybridization signal could be detected in the mammary gland, uterus, vagina, adrenal gland, kidney, liver, brain or lung. Identification of the cell types expressing 17 β -HSD type 1 mRNA in the different tissues was achieved following examination of photographic emulsion-coated sections. In the ovary, high labeling was only observed over the granulosa cells in growing follicles at all stages of development (Fig. 1). On the other hand, no specific labeling could be detected in primordial and primary follicles nor in corpora lutea and interstitial cells. In the testis, a specific hybridization signal was seen over seminiferous tubules, Leydig cells being devoid of any specific labeling (Fig. 2). Since we used frozen sections, a procedure which does not totally preserve the integrity of the tissues, it was not possible to identify all the cell types expressing type I 17 β -HSD mRNA in the tubules. However, it clearly appears that labeling was present over the basal region of the tubules that contain spermatogonia, spermatocytes and round spermatids.

In the pituitary gland of animals of both sexes, the hybridization signal was detected in the cells of the intermediate lobe, the anterior and posterior lobes exhibiting no specific labeling (Fig. 3). In the intermediate lobe, all the cells exhibited labeling. Since the vast majority of intermediate lobe cells are melanotrophs (Tong & Pelletier 1992), it can be concluded that melanotrophs are expressing the enzyme. On the other hand, it cannot be totally excluded that labeling occasionally observed in the interstices between melanotrophs could be associated with folliculo-stellate cells which are few in number (Tong & Pelletier 1992). In the prostate, specific labeling was only detected over the epithelial cells bordering the acinar lumen (Fig. 4).

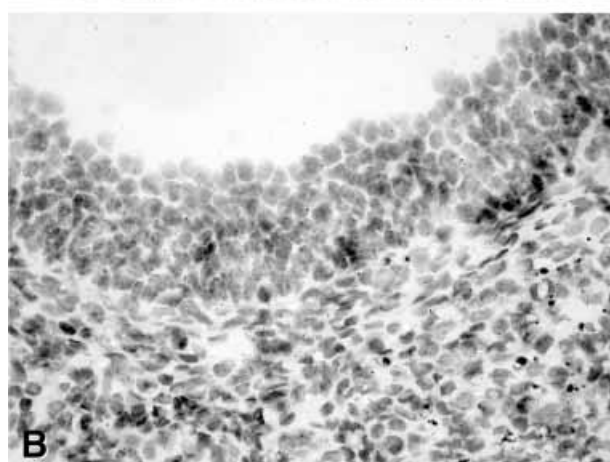
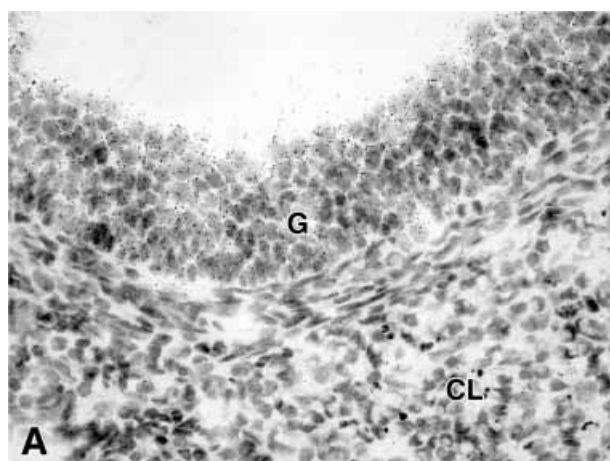


Figure 1 (A) Section through an ovary. After hybridization with the antisense probe, strong labeling is detected over granulosa cells (G) of a large follicle. An adjacent corpus luteum (CL) is unlabeled. (B) Consecutive section hybridized with the sense probe, only weak diffuse background can be seen. Exposure time: 3 days. Magnification: $\times 600$.

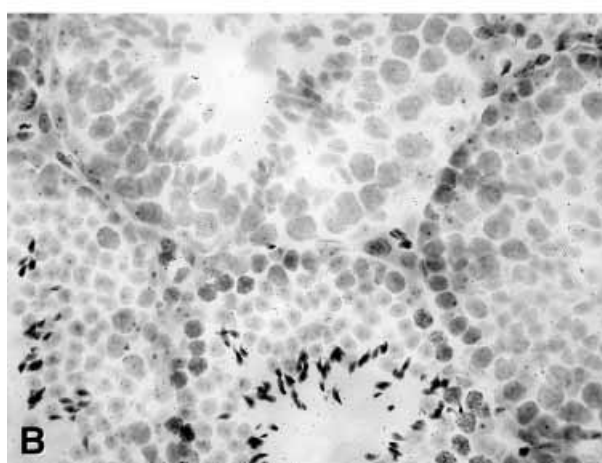
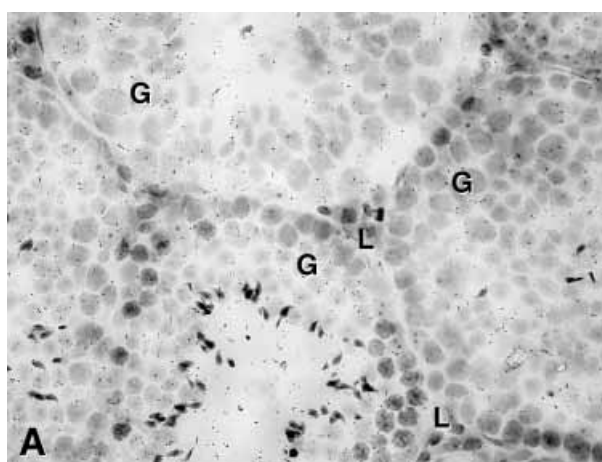


Figure 2 (A) Micrograph illustrating the hybridization signal obtained in the testis. Specific labeling is detected over germ cells (G). The Leydig cells (L) are unlabeled. (B) In control consecutive section hybridized with the sense probe, only diffuse background is present. Exposure time: 21 days. Magnification: $\times 600$.

On the other hand, in both male and female mouse dorsal skin, a specific hybridization signal was seen in the sebaceous glands, while the epidermis, stroma, hair follicles and sweat glands were unlabeled (Fig. 5). In all the tissues examined, hybridization with the radiolabeled sense probe generated only a light uniform labeling (Figs. 1B, 2B, 3B, 4B, 5B).

Discussion

The enzyme 17 β -HSD type 1 plays an important role in the formation of E₂ in the human ovary and

placenta (Peltoketo *et al.* 1988, Luu-The *et al.* 1989, Dumont *et al.* 1992, Lin *et al.* 1992, Mustonen *et al.* 1997, Luu-The 2001). The recent cloning of the cDNA encoding mouse 17 β -HSD type 1 has allowed studies on the histological localization of the corresponding mRNA in a series of male and female mouse tissues. In the ovary, 17 β -HSD type 1 was found to be highly expressed in granulosa cells of growing follicles without any detectable hybridization signal in the corpora lutea and interstitial cells. These results agree with previous reports indicating the presence of 17 β -HSD type 1 mRNA transcripts in the human, rat and mouse granulosa cells (Nokelainen *et al.* 1996, Akinola *et al.*

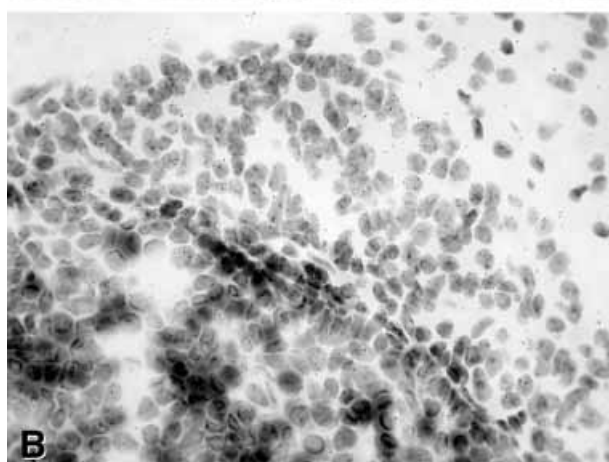
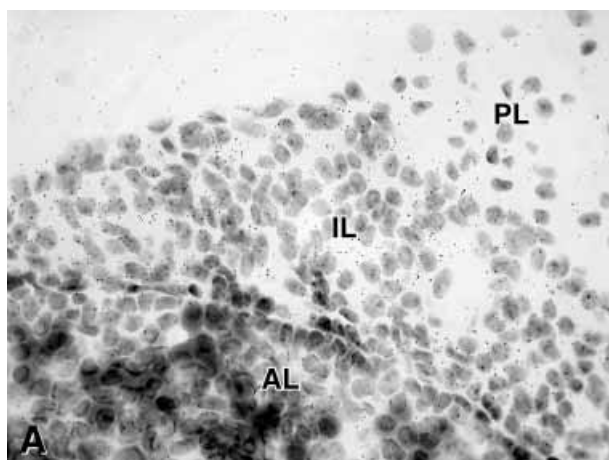


Figure 3 (A) Section through the pituitary gland of a male mouse. Accumulation of silver grains are seen over cells of the intermediate lobe (IL). The anterior (AL) and posterior (PL) lobes do not exhibit any specific labeling. (B) Control sections hybridized with the sense probe. Only diffuse background can be observed. Exposure time: 28 days. Magnification: $\times 600$.

1997, Mustonen *et al.* 1997, Luu-The 2001). Using microdissection procedures, Zhang *et al.* (1996) have reported the expression of 17 β -HSD type 1 mRNA in human corpora lutea. This discrepancy between results obtained in rodent and human ovaries might be due to species differences or contamination of corpora lutea specimens with granulosa cells during the dissection procedure. By immunocytochemistry, it has been shown that in the human ovary, the 17 β -HSD type 1 protein was only expressed in granulosa cells (Sawetawan *et al.* 1994). These results clearly suggest that 17 β -HSD type 1 is involved in the formation of ovarian E₂ which is eventually released into the general circulation. Female mice at

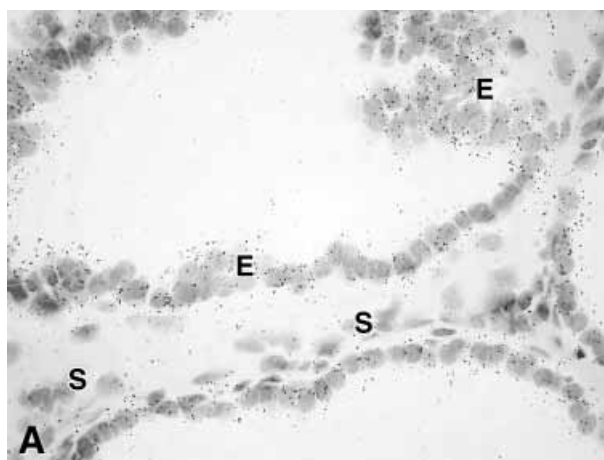


Figure 4 (A) Section through the prostate. Silver grains are found over the epithelial cells (E) bordering the acinar lumen. The stroma cells (S) are unlabeled. (B) Adjacent section hybridized with the sense probe. Few dispersed silver grains can be seen. Exposure time: 28 days. Magnification: $\times 600$.

diestrous day 1 were used since at this stage of the estrous cycle, the estrogen levels are low and quite stable. It cannot be excluded that the variation in circulating levels of estrogens occurring during the estrous cycle might modulate 17 β -HSD type 1 mRNA expression. We have recently observed that ovariectomy and estrogen administration to ovariectomized mice did not modify 17 β -HSD type 1 mRNA levels in the skin (G Pelletier, V Luu-The, S Li, L Ren and F Labrie unpublished data), suggesting that circulating estrogens do not influence 17 β -HSD type 1 expression at least in the skin.

In the testis, 17 β -HSD type 1 mRNA was expressed in germ cells, especially spermatogonia,

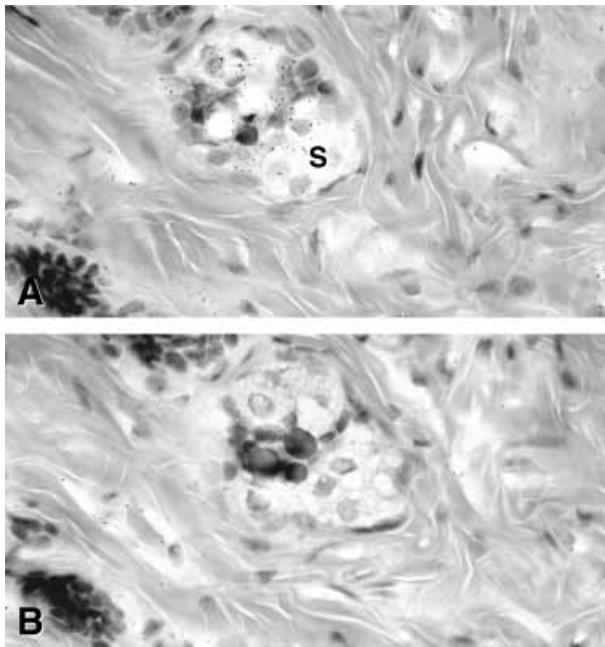


Figure 5 (A) Section through the dorsal skin of a female mouse. Specific labeling is detected over a sebaceous gland (S). (B) In control adjacent section hybridized with the sense probe, only few dispersed silver grains are present. Exposure time: 35 days. Magnification: $\times 600$.

spermatocytes and round spermatids, but not in Leydig cells, thus suggesting that the enzyme may contribute to the local production of E_2 in the germinal epithelium. A low level of 17 β -HSD type 1 has been detected by RT-PCR in the human testis (Nokelainen *et al.* 1996), but the cell type(s) expressing the enzyme have not been identified so far. The present findings, however, agree with previous studies indicating that estrogens are synthesized by germ cells (de Jong *et al.* 1974, Nitta *et al.* 1993, Kivan *et al.* 1995), although the role of estrogens in germ cell functions and/or development is not fully understood. On the other hand, estrogen receptors α have been localized in rat germ cells (Pelletier *et al.* 2000), thus suggesting that E_2 can exert intracrine and/or paracrine activity in the seminiferous tubules.

In the brain, including the posterior lobe of the pituitary gland, which is an extension of the medio-basal hypothalamus, no specific hybridization signal could be found. In fact, only the intermediate lobe which contains a homogenous population of cells (melanocytes) with very few folliculo-stellate cells (Tong & Pelletier 1992) exhibited expression of 17 β -HSD type 1 mRNA.

Since the labeling was rather uniform, it can be concluded that the vast majority of the melanocytes, if not all of them, express the enzyme and are thus involved in E_2 formation. In the rat intermediate lobe, Shugrue *et al.* (1998) reported that ER β mRNA but not ER α was expressed. On the other hand, Mitchner *et al.* (1998) found that both ER α and ER β mRNAs were expressed in the rat intermediate lobe, with a higher expression of ER α mRNA. By immunocytochemistry, neither ER α nor ER β protein could be detected in the rat intermediate lobe of the pituitary (Pelletier *et al.* 2000). It remains to be determined whether or not locally produced E_2 can exert an intracrine activity in mouse intermediate lobe melanotrophs. The role of estrogens on melanotroph functions is still unclear. In fact, under *in vivo* conditions, estrogen treatment could induce an increase in the release of α -melanocyte stimulating hormone (Ellerkman & Nagy 1992), but there is no evidence that estrogen can directly modulate melanotrophs.

In the prostate, 17 β -HSD type 1 mRNA was exclusively detected in the epithelial cells bordering the acinar lumen (luminal cells). In the mouse prostatic acini, the majority of epithelial cells are luminal cells, while the basal cells are rather scarce. In fact, the ratio of luminal to basal cells is approximately 10:1 (El-Alfy *et al.* 2000). It can thus be logically assumed that most of the specific labeling is originating from the luminal cells. The presence of 17 β -HSD type 1 has been reported in the human prostate (Luu-The *et al.* 1990, Dumont *et al.* 1992), but identification of prostate cell types expressing the enzyme have not been reported. Since ER β has also been reported in luminal cells in the prostate of several species, including the mouse (Couse & Korach 1999, Pelletier 2000, Pelletier *et al.* 2000), it can be suggested that E_2 synthesized in luminal cells through the action of 17 β -HSD type 1 might exert an intracrine activity. The physiological role of estrogens in the prostate is still unclear. In ER α or ER β knockout mice, no abnormality of the development of the prostate has been found (Couse *et al.* 2000, Dupont *et al.* 2000). On the other hand, we have reported that E_2 administered to castrated rats could induce moderate hypertrophy of prostate epithelial cells as well as an increase in androgen receptor expression in epithelial and stromal cells (Pelletier 2002).

In the dorsal skin of mice of both sexes, 17 β mRNA type 1 was only detected in sebocytes.

Sebocytes have been shown to express both ER α and ER β with a much higher expression of ER β (Pelletier 2000, Thornton *et al.* 2003). It thus appears that locally produced estrogens can likely be involved in the regulation of sebaceous gland activity. Recent studies using sebaceous glands in culture have shown that physiological levels of estradiol significantly decreased lipogenesis without affecting the rate of cell division (Guy *et al.* 1996). The mechanisms involved in the regulation of the local production of estrogens in the skin remain to be elucidated.

By RT-PCR analyses, Nokelainen *et al.* (1996) have detected very low levels of 17 β -HSD type 1 mRNA in the uterus and adrenal glands of female mice. In the present study, we have been unable to detect any hybridization signal in the uterus or adrenal glands. This discrepancy between the two approaches can simply be explained by a lower sensitivity of the ISH technique.

In summary, we report for the first time the identification of cell types expressing 17 β -HSD type 1 mRNA in mouse gonads and peripheral tissues. In the testis and peripheral tissues, the action of E₂ formed through 17 β -HSD type 1 is probably limited to the cells involved in the formation of E₂ in an intracrine fashion (Labrie 1991). It is noteworthy that tissues expressing 17 β -HSD type 1 are also expressing aromatase (Sharpe 1998, Simpson *et al.* 2000), thus suggesting that E₂ is produced by aromatization of 4-androstenedione (4-dione) into E₁ followed by the conversion of E₁ into E₂ by 17 β -HSD type 1, rather than by aromatization of testosterone into E₂.

Acknowledgements

ATLAS program supported by Genome Canada and Genome Quebec.

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Received 2 April 2004

Accepted 11 June 2004

Made available online as an

Accepted Preprint 23 June 2004