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

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Abstract

The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 catalyzes the conversion of estrone (E1) into 17β estradiol (E2). To gain information about the cellular localization of 17β-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, 17β-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, 17β-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 17β-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.


Introduction

The 17β-hydroxysteroid dehydrogenase (17β-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 17β-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 17β-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 17β-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 17β-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 17β-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 17β-HSD type 1 mRNA at the cellular level has not been reported so far. We therefore used in situ hybridization (ISH) to localize the 17β 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 µm 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 17β-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 17β-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 17β-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).
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 17β-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 17β-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, 17β-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 17β-HSD type 1 mRNA transcripts in the human, rat and mouse granulosa cells (Nokelainen et al. 1996, Akinola et al. 1998).
1997, Mustonen et al. 1997, Luu-The 2001). Using microdissection procedures, Zhang et al. (1996) have reported the expression of 17β-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 17β-HSD type 1 protein was only expressed in granulosa cells (Sawetawan et al. 1994). These results clearly suggest that 17β-HSD type 1 is involved in the formation of ovarian E₂, which is eventually released into the general circulation. Female mice at 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 17β-HSD type 1 mRNA expression. We have recently observed that ovariectomy and estrogen administration to ovariec-tomized mice did not modify 17β-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 17β-HSD type 1 expression at least in the skin.

In the testis, 17β-HSD type 1 mRNA was expressed in germ cells, especially spermatogonia,
spermatocytes and round spermatids, but not in Leydig cells, thus suggesting that the enzyme may contribute to the local production of E₂ in the germinal epithelium. A low level of 17β-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 have 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 E₂ synthesized in luminal cells through the action of 17β-HSD type 1 might exert an intracrine activity. The physiological role of estrogens in the prostate is still unclear. In ERα or ERβ 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 E₂ administrated 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, 17β mRNA type 1 was only detected in sebocytes.

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 E₂ formation. In the rat intermediate lobe, Shugrue et al. (1998) reported that ERβ mRNA but not ERα was expressed. On the other hand, Mitchner et al. (1998) found that both ERα and ERβ mRNAs were expressed in the rat intermediate lobe, with a higher expression of ERα mRNA. By immunocytochemistry, neither ERα nor ERβ 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 E₂ 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, 17β-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 17β-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 ERβ 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 E₂ synthesized in luminal cells through the action of 17β-HSD type 1 might exert an intracrine activity. The physiological role of estrogens in the prostate is still unclear. In ERα or ERβ 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 E₂ administrated 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, 17β mRNA type 1 was only detected in sebocytes.
Sebocytes have been shown to express both ER\(_{\alpha}\) and ER\(_{\beta}\) with a much higher expression of ER\(_{\beta}\) (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\(\beta\)-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\(\beta\)-HSD type 1 mRNA in mouse gonads and peripheral tissues. In the testis and peripheral tissues, the action of E\(_2\) formed through 17\(\beta\)-HSD type 1 is probably limited to the cells involved in the formation of E\(_2\) in an intracrine fashion (Labrie 1991). It is noteworthy that tissues expressing 17\(\beta\)-HSD type 1 are also expressing aromatase (Sharpe 1998, Simpson et al. 2000), thus suggesting that E\(_2\) is produced by aromatization of 4-androstenedione (4-dione) into E\(_1\) followed by the conversion of E\(_1\) into E\(_2\) by 17\(\beta\)-HSD type 1, rather than by aromatization of testosterone into E\(_2\).

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