

G. Pelletier · V. Luu-The · S. Li · F. Labrie

Localization of type 5 17 β -hydroxysteroid dehydrogenase mRNA in mouse tissues as studied by in situ hybridization

Received: 9 November 2004 / Accepted: 21 February 2005 / Published online: 22 April 2005
© Springer-Verlag 2005

Abstract The mouse enzyme type 5 17 β -hydroxysteroid dehydrogenase (17 β -HSD) catalyzes the conversion of androstenedione to testosterone and, to a lesser degree, the conversion of estrone to estradiol. In order to determine the exact sites of action of type 5 17 β -HSD, we studied the cellular localization of the mRNA of the enzyme in mouse tissues by using in situ hybridization. Specific hybridization signal was found in the liver, ovary, adrenal cortex, and kidney. In the liver of mice of both sexes, a strong signal was observed in all hepatocytes. In the ovary, specific labeling was detected in the granulosa and theca interna cells in growing follicles and in luteal cells. In the female adrenal cortex, intense labeling was restricted to the zona reticularis, whereas no type 5 17 β -HSD mRNA expression could be found in the male adrenal cortex. In the kidney of mice of both sexes, type 5 17 β -HSD mRNA was expressed in epithelial cells in both the proximal and distal convoluted tubules. The data indicate that androgens and estrogens are formed via the action of type 5 17 β -HSD in specific cell types in the liver, ovary, adrenal cortex, and kidney.

Keywords Type 5 17 β -hydroxysteroid dehydrogenase · Androgens · Estrogens · In situ hybridization · Intracrinology · Mouse (C57BL6)

Introduction

The 17 β -hydroxysteroid dehydrogenase (17 β -HSD) enzymes play pivotal roles in the biosynthesis and metabolism of active sex steroids. They can be classified in two functional groups: those represented by types 1, 3, 5, and 7

17 β -HSD, which catalyze the formation of sex steroids, and those represented by types 2, 4, 6, 8, 9, 10, and 11 17 β -HSD, which inactivate them (Labrie et al. 2000; Luu-The 2001; Mindnich et al. 2004; Vihko et al. 2004). Type 5 17 β -HSD is unique among the 17 β -HSDs because it belongs to the aldo-keto reductase family, whereas the others are members of the short-chain alcohol dehydrogenases (Labrie et al. 2000; Luu-The 2001; Mindnich et al. 2004; Peltoketo et al. 1999). Both human and mouse type 5 17 β -HSD catalyze the conversion of androstenedione to testosterone (Deyashiki et al. 1995; Dufort et al. 1999). In addition, the mouse type 5 17 β -HSD efficiently catalyzes the transformation of estrone (E₁) to estradiol (E₂), which corresponds to 60% of the transformation of androstenedione to testosterone (Dufort et al. 1999). Northern blot analysis of tissues from 5-week-old male mice has revealed that type 5 17 β -HSD mRNA is expressed in the liver, kidney, testis, and stomach, the liver mRNA content being considerably higher than those observed in the other tissues (Deyashiki et al. 1995). Mustonen et al. (1997) have also reported that type 5 17 β -HSD mRNA is expressed in the testis, liver, kidney, and adrenals of both male and female adult mice. In humans we have shown, using specific ribonuclease protection assay, that the mRNA of the enzyme is expressed in the prostate and testis and in the liver and adrenals in both sexes (Dufort et al. 1999).

So far, the cellular localization of type 5 17 β -HSD has not been reported in mouse tissues. Therefore, in order to gain information about the exact sites of formation of type 5 17 β -HSD, we have determined the cellular localization of type 5 17 β -HSD mRNA in tissues of adult mice of both sexes by using in situ hybridization (ISH).

This work was supported by Genome Canada and Genome Québec.

G. Pelletier (✉) · V. Luu-The · S. Li · F. Labrie
Oncology and Molecular Endocrinology Research Center,
Le Centre Hospitalier de l'Université Laval (CHUL),
2705, Laurier Boulevard,
Québec, G1V 4G2, Canada
e-mail: georges.pelletier@crchul.ulaval.ca
Tel.: +1-418-6542296
Fax: +1-418-6542761

Materials and methods

Animals and histological procedures

Four adult male (26–30 g) and female (24–27 g) C57BL6 mice were housed under constant temperature (21±1°C) and a light regimen of lights on from 0600 to 2000 hours.

The animals received Purina Chow (Ralston-Purina, St-Louis, Mo.) and tapwater ad libitum. Experiments were 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 hours for histological procedures as described below. The females were in proestrous.

All the animals were perfused transcardially with 50 ml 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Various tissues, i.e., liver, stomach, jejunum, colon, kidney, lung, skin, adrenal, pituitary, brain, testis, prostate, ovary, uterus, vagina, and mammary gland were excised and postfixed in the same fixative for 24 h at 4°C and placed in 15% sucrose in 0.1 M phosphate buffer before being quickly frozen in isopentane chilled in liquid nitrogen. Frozen sections (10 µm thick) were serially cut at -20°C and mounted on gelatin- and poly-L-lysine-coated slides.

ISH procedure

The vector used for the production of the cRNA probe was constructed by the insertion of a cDNA fragment of

455 bp of mouse type 5 17β-HSD (GenBank accession no. AH007907) into a pBSKSII+vector (Stratagene, La Jolla, Calif.). The cDNA fragment located at position 511–1,026 downstream from the ATG start codon was obtained by polymerase chain reaction amplification. The selected fragment had 65% and 68% homology with 20α-HSD and 3α-HSD cDNAs, respectively. Moreover, with the type 5 17β-HSD probe, no hybridization signal was found in tissues such as skin and testis, which exhibit marked expression of mRNAs for 20α-HSD (Pelletier et al. 2003) and 3α-HSD (G. Pelletier et al., unpublished). ISH with the antisense and sense ³⁵S-labeled cRNA probes was performed as previously described (Givalois et al. 1997). Briefly, the sections were prehybridized at room temperature (RT) in a humid chamber for 2 h in 450 µl (per slide) prehybridization buffer containing 50% formamide, 5×SSPE (1×SSPE = 0.1 M NaCl, 10 mM NaH₂PO₄ pH 7.4, 1 mM EDTA), 5×Denhardt's buffer, 200 mg/ml denatured salmon testis DNA (Sigma), 200 µg/ml yeast tRNA, 2 µg/ml Poly A (Boehringer-Mannheim, Montreal, Canada), and 4% dextran sulfate. After prehybridization, 100 µl hybridization mixture (prehybridization buffer containing, in addition, 10 mM dithiothreitol and ³⁵S-labeled cRNA probe at a concentration of 10×10⁶ cpm/ml) was spotted onto each slide, sealed under a coverslip, and incubated at 37°C overnight (15–20 h) in a humid chamber.

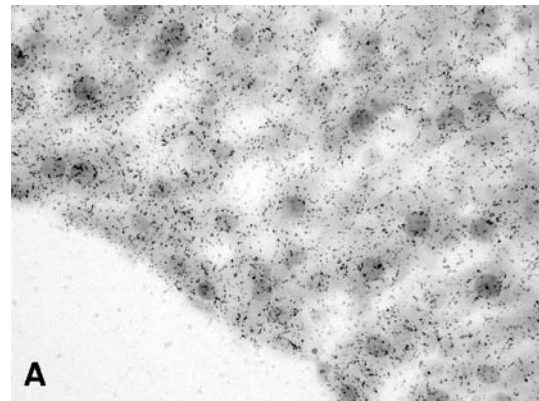


A

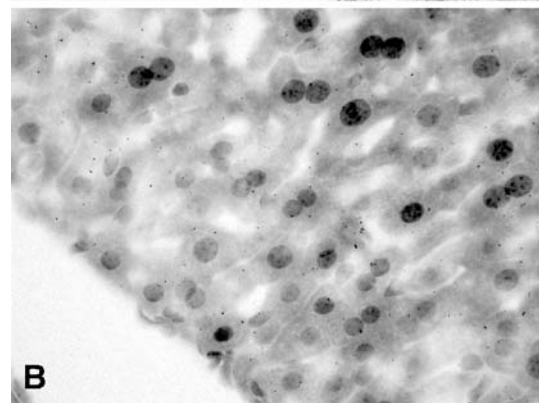


B

Fig. 1 X-ray film autoradiographs illustrating the expression of type 5 17β-HSD mRNA in sections from a male mouse liver. **a** Section hybridized with the antisense riboprobe. Strong hybridization signal is observed throughout the tissue. **b** Consecutive section hybridized with the sense probe. Only weak background can be detected. Exposure time: 0.5 day. ×40



A



B

Fig. 2 **a** Micrographs of the liver section in Fig. 1 showing strong labeling of hepatocytes after hybridization with the anti-sense probe. **b** Adjacent control section obtained following hybridization with the sense probe. Only diffuse background can be observed. Exposure time: 1 day. ×700

After hybridization, coverslips were removed, and the slides were rinsed in $2\times$ SSC ($1\times$ SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at RT for 30 min. Sections were digested by RNase A (20 μ g/ml in $2\times$ SSC) at 37°C for 30 min, rinsed in decreasing concentrations of SSC ($2\times$ SSC and $1\times$ SSC) for 30 min at RT, washed in $0.5\times$ SSC for 30 min at 37°C , followed by 90 min in $0.5\times$ SSC at RT and $0.1\times$ SSC at 60°C , and finally for 30 min in $0.1\times$ SSC at RT. The sections were then dehydrated and exposed to Kodak Biomax MR films for 0.5–8 days before being coated with liquid photographic emulsion (Kodak-NTB2; diluted 1:1 with water). Slides were exposed for 1–35 days, developed in Dektol developer (Kodak, Rochester, N.Y.) for 2 min, and fixed in rapid fixer (Kodak) for 4 min. Thereafter, tissues were rinsed in running water for 30 min, counterstained with hematoxylin, and rapidly dehydrated through graded concentrations of ethanol, cleared in toluene, and coverslipped with Permount (Fisher Scientific, Montreal, Canada).

Results

After 0.5–8 day exposure of the films, specific radiolabeling was observed in the liver, ovary, adrenal gland, and kidney (Figs. 1, 3, 6, 8). At the shortest time interval studied

(0.5 day), strong autoradiographic reaction was seen in the liver (Fig. 1). No specific hybridization signal could be detected in the brain, pituitary, uterus, vagina, mammary gland, testis, prostate, lung, skin, stomach, jejunum, or colon. The photographic emulsion-coated sections revealed precise information about the cell types expressing type 5 17β -HSD in the various positive tissues.

In the liver, strong hybridization signal was observed after only 1 day exposure time (Fig. 2). All the hepatocytes appeared uniformly labeled (Fig. 3). No sex difference could be recorded. In the ovary, after a 35-day exposure, specific labeling was observed in granulosa and theca interna cells in growing follicles at all stages of development (Fig. 4). Corpora lutea cells also appeared to be specifically labeled (Fig. 5). Variations in the intensity of labeling between luteal cells were consistently observed.

In the female adrenal glands, X-ray autoradiographs revealed that the labeling was restricted to the zona reticularis of the cortex (Fig. 6). After 14 days of exposure, strong labeling was observed over the zona reticularis, the other zones of the cortex and the medulla being totally negative (Fig. 7). In the male adrenal glands, no labeling could be observed, even at the longest time interval studied (45 days). In the kidney, specific labeling was present in the cortical regions (Fig. 8). At the light-microscope level, specific hybridization signal was found in the epithelial

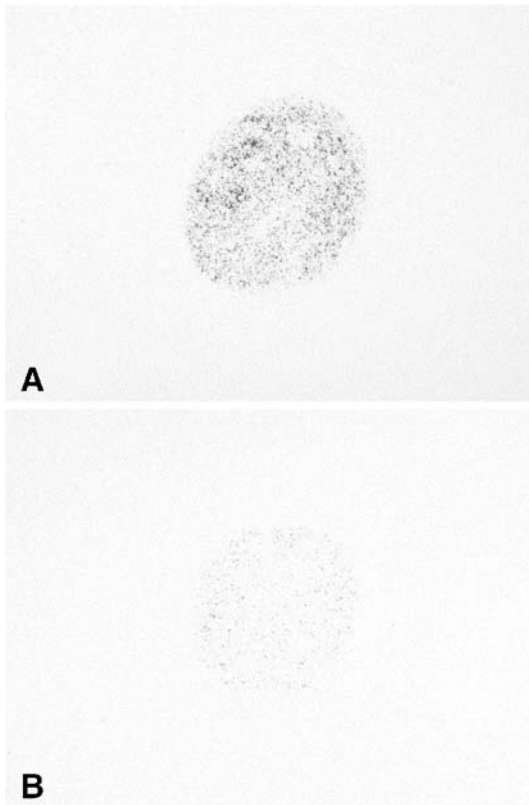


Fig. 3 X-ray film autoradiographs illustrating the expression of type 5 17β -HSD mRNA in ovarian sections. **a** Section hybridized with the antisense probe. Diffuse reaction can be observed. **b** Adjacent control section hybridized with the sense probe. Only weak uniform labeling can be detected. Exposure time: 7 days. $\times 40$

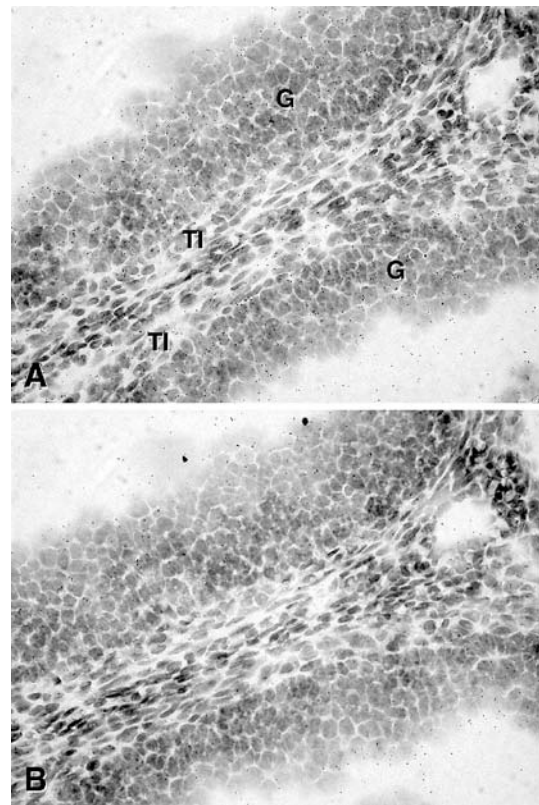


Fig. 4 a Sections through ovarian follicles. Labeling can be seen over granulosa cells (G) and theca interna cells (TI) from two follicles after hybridization with the anti-sense probe. **b** Consecutive section hybridized with the sense probe. Note the presence of non-specific labeling over the follicular fluid. Exposure time: 35 days. $\times 700$

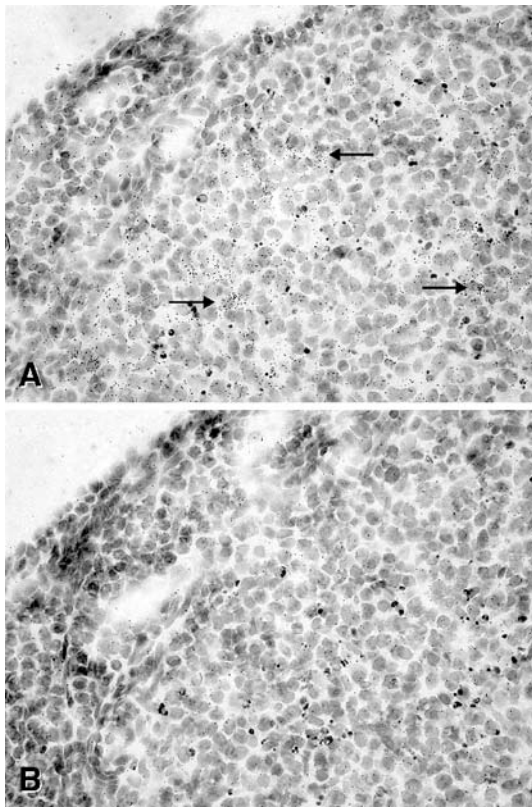


Fig. 5 **a** Section through a corpus luteum. Radiolabeled luteal cells can be observed (*arrows*) after hybridization with the anti-sense probe. **b** Consecutive section hybridized with the sense probe. Only weak labeling can be detected. Exposure time: 35 days. $\times 700$

cells in both the proximal and distal convoluted tubules, whereas the glomeruli, collecting tubules, and blood vessels were unlabeled (Fig. 9).

Discussion

The mouse enzyme type 5 17β -HSD converts androstenedione to testosterone and also, to a lesser extent, E_1 to E_2 (Deyashiki et al. 1995; Dufort et al. 1999). The cloning of the cDNA encoding mouse type 5 17β -HSD (Deyashiki et al. 1995) has allowed us to study the cellular localization of the mRNA of the enzyme by using ISH. From the present results, we cannot conclude that the enzyme protein is present in the cells expressing the enzyme transcripts. To date, the immunocytochemical localization of type 5 17β -HSD has not been reported in the mouse. On the other hand, type 5 17β -HSD enzymatic activities have been shown in the liver, ovaries, and adrenals (Dufort et al. 1999; Luu-The 2001; Mindnich et al. 2004). In the liver, notable expression of type 5 17β -HSD has been found in the hepatocytes. The present data are in agreement with previous reports indicating high type 5 17β -HSD mRNA expression in mouse liver as determined by Northern blot analysis (Deyashiki et al. 1995) and human liver as evaluated by ribonuclease protection assay (Dufort et al. 1999). Marked expression of androgen receptors and estrogen

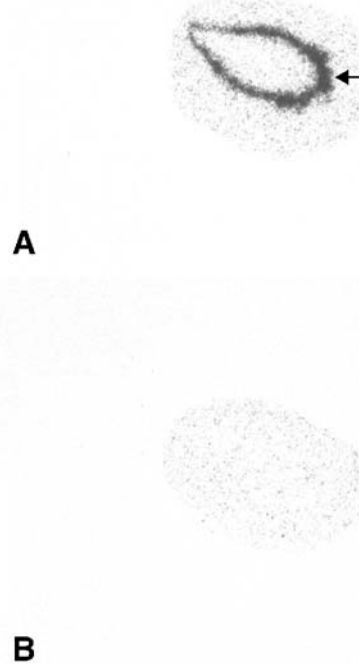


Fig. 6 **a** X-ray film autoradiograph showing a section through the adrenal gland of a female mouse. Labeling can be observed in the zona reticularis (*arrow*) after hybridization with the anti-sense probe. The other layers of the cortex and the medulla are totally unlabeled. **b** Adjacent control section. Only diffuse background is present. Exposure time: 7 days. $\times 40$

receptor α has also been reported in hepatocytes (Kuiper et al. 1997; Pelletier 2000). Two enzymes involved in the synthesis of androstenedione, the precursor of testosterone, viz., 3 β -HSD and cytochrome P450c17, are expressed in hepatocytes (Miller 1988; Penning 1997). Type 7 17β -HSD, which converts E_1 to E_2 , is also expressed in hepatocytes (Nokelainen et al. 1998). These previous observations, together with the present findings, suggest that locally produced androgens and/or estrogens exert an influence on hepatocyte function by an intracrine mechanism. In ovary, type 5 17β -HSD mRNA has been detected in the granulosa and theca cells in growing follicles and in luteal cells. In human ovary, type 5 17β -HSD has been found to be expressed in theca interna cells (Pelletier et al. 1999), the layer that produces androgen (McNatty et al. 1979). Using frozen sections, we have not been able to establish which of the luteal cell types (luteinizing granulosa or theca lutein cells) of the corpora lutea strongly express type 5 17β -HSD mRNA. In the mouse, the enzyme can be hypothesized to be involved in the formation of testosterone in the theca cell layer but in E_2 formation in the granulosa and luteal cells.

Northern blot analysis has detected type 5 17β -HSD mRNA in mouse testis (Deyashiki et al. 1995; Mustonen et al. 1997). In the present experiments, we have been unable to detect any hybridization signal in this organ. This dis-

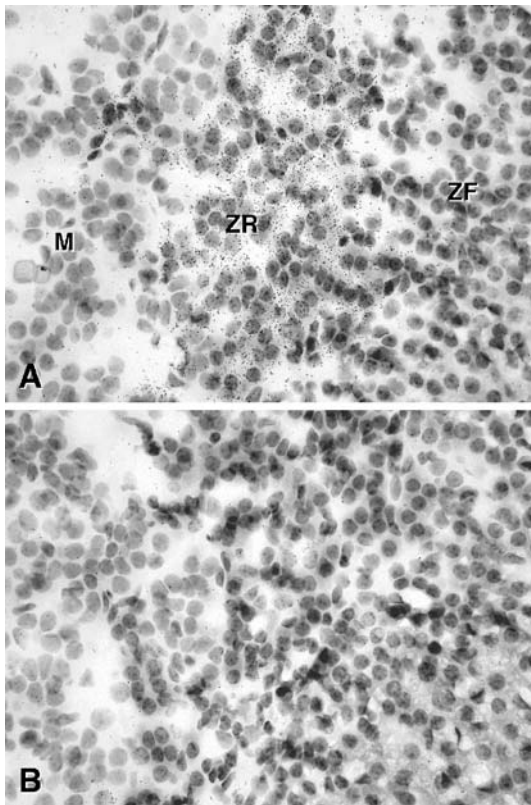


Fig. 7 Micrographs of the adrenal gland sections shown in Fig. 6 (ZF zona fasciculata, M medulla). **a** Labeling is observed over the zona reticularis (ZR) **b** In the control section, only a few dispersed silver grains can be detected. Exposure time: 14 days. $\times 700$

crepancy might simply be explained by the expression of the mRNA of the enzyme being too low to be detected by ISH. On the other hand, since type 3 17β -HSD, the enzyme involved in testosterone synthesis in testis, is highly expressed in mouse Leydig cells (G. Pelletier et al., unpublished), type 5 17β -HSD might play a minor role in testicular androgen formation.

The female adrenal cortex exhibits high expression of type 5 17β -HSD mRNA in the zona reticularis, whereas no expression of the enzyme has been detected in the male. Northern blot analysis has also revealed the same sex-related expression of type 5 17β -HSD in mouse adrenal glands, the mRNA expression being high in the female and almost absent in the male (Mustonen et al. 1997). Using a ribonuclease protection assay, we have previously observed low mRNA expression of type 5 17β -HSD in whole human adrenal glands (Dufort et al. 1999). This low expression of type 5 17β -HSD mRNA might be explained by the restricted expression of the enzyme to the zona reticularis, which consists in a few layers of cells and which represents a small proportion of the total number of adrenal cells. The physiological role of type 5 17β -HSD in the adrenal cortex remains to be fully clarified. We have previously demonstrated that mouse type 5 17β -HSD has some 20α -HSD activity (Dufort et al. 1999). Since, in the mouse adrenal, cytochrome P450c17 is absent (Miller 1988; Pelletier et al. 2001), progesterone cannot be converted into

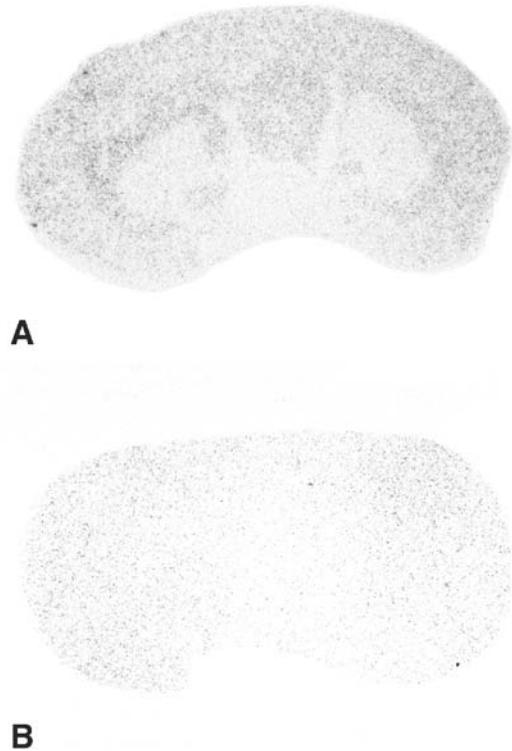


Fig. 8 X-ray film autoradiographs showing kidney sections from a male mouse (**a**). Diffuse labeling of the cortex can be observed after hybridization with the anti-sense probe. In the consecutive control section, only weak labeling is present throughout the section (**b**). Exposure time: 7 days. $\times 40$

androgen precursors (dehydroepiandrosterone and androstenedione). Thus, excess progesterone produced by the zona reticularis could be partly inactivated by type 5 17β -HSD, most of the progesterone being degraded by 20α -HSD. In the male adrenal cortex, excess progesterone could be inactivated by 20α -HSD and 5α -reductase, which is highly expressed in the zona reticularis of the male mouse (V. Luu-The et al., unpublished).

In the kidney, type 5 17β -HSD mRNA is expressed in the epithelial cells in the proximal and distal convoluted tubules. Type 5 17β -HSD mRNA has been detected by Northern blot analysis in the mouse kidney (Deyashiki et al. 1995), but no indication about the cell types involved in the expression of the enzyme has previously been available. Both 3β -HSD and cytochrome P450c17 have been reported to be expressed in the kidney (Miller 1988; Penning 1997) but without any indication of their cellular localization. Thus, that androstenedione might be synthesized by the combined action of these two steroidogenic enzymes and subsequently converted into testosterone through the action of type 5 17β -HSD. The presence of androgen receptors in epithelial cells in both proximal and distal convoluted tubules (Pelletier 2000; Sar et al. 1990) suggests that locally produced androgen and estrogen could directly influence the activity of both types of tubule. However, no indication concerning a direct influence of estro-

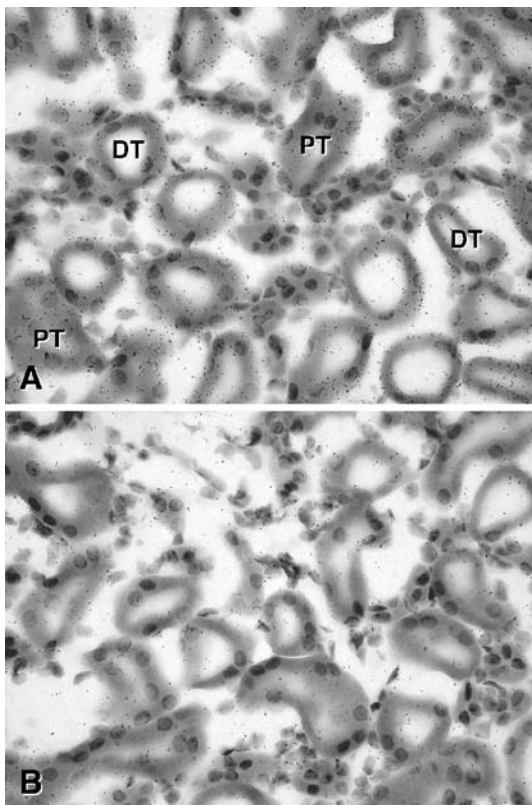


Fig. 9 Micrographs of the sections shown in Fig. 8. **a** Silver grains are seen overlying the epithelial cells of both proximal (*PT*) and distal (*DT*) convoluted tubules after hybridization with the anti-sense probe. **b** Control section hybridized with the sense probe. Few dispersed silver grains are present throughout the section. Exposure time: 35 days. $\times 700$

gens or androgens on convoluted tubule function has been found to date.

In summary, we have identified, by using ISH, the cell types expressing type 5 17β -HSD mRNA in the ovary, adrenal cortex, liver, and kidney in mouse. In the ovary and adrenal cortex, the enzyme might contribute to the formation of androgens and the estrogens subsequently released into the circulation. In the liver and kidney, the influence of androgens and estrogens formed through the action of type 5 17β -HSD is likely to be limited to the cells involved in the formation of sex steroids in an intracrine fashion.

References

- Deyashiki Y, Ohshima K, Nakanishi M, Satao K, Matsuura K, Hara A (1995) Molecular cloning and characterization of mouse estradiol 17β -dehydrogenase (A-specific), a member of the aldo-keto reductase family. *J Biol Chem* 270:10461–10467
- Dufort I, Rheault P, Huang XF, Soucy P, Luu-The V (1999) Characteristics of a highly labile human type 5 17β -hydroxysteroid dehydrogenase. *Endocrinology* 140:1–7
- Givalois L, Li S, Pelletier G (1997) Age-related decrease in the hypothalamic CRH mRNA expression is reduced by dehydroepiandrosterone (DHEA) treatment in male and female rats. *Mol Brain Res* 48:107–114
- Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 138:863–870
- Labrie F, Luu-The V, Lin S-X, Simard J, Labrie C (2000) Role of 17β -hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. *Trends Endocrinol Metab* 11:421–427
- Luu-The V (2001) Analysis and characteristics of multiple types of human 17β -hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* 76:143–151
- McNatty KP, Makris A, DeGrazia C, Osathanondh R, Ryan, KJ (1979) The production of progesterone androgens and estrogens by granulosa cells. Theca tissue and stroma tissue from human ovaries in vitro. *J Clin Endocrinol Metab* 49:687–699
- Miller WL (1988) Molecular biology of steroid hormone synthesis. *Endocr Rev* 9:295–318
- Mindnich R, Müller G, Adamski J (2004) The role of 17β -hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* 218:7–20
- Mustonen MVJ, Poutanen MH, Isomaa W, Vihko RK (1997) Cloning of mouse 17β -hydroxysteroid dehydrogenase, type 2, and analysing expression of the mRNA for types 1, 2, 3, 4 and 5 in mouse embryos and adult tissues. *Biochem J* 325:199–205
- Nokelainen P, Peltoketo H, Vihko R, Vihko P (1998) Expression cloning of a novel estrogenic mouse 17β -hydroxysteroid dehydrogenase/ 17 -ketosteroid reductase (m 17β -HSD), previously described as a prolactin receptor-associated protein (PRAP) in rat. *Mol Endocrinol* 12:1048–1059
- Pelletier G (2000) Localization of androgen and estrogen receptors in rat and primate tissues. *Histol Histopathol* 15:1261–1270
- Pelletier G, Luu-The V, Têtu B, Labrie F (1999) Immunocytochemical localization of type 5 17β -hydroxysteroid dehydrogenase in human reproductive tissues. *J Histochem Cytochem* 47:731–737
- Pelletier G, Li S, Luu-The V, Tremblay Y, Belanger A, Labrie F (2001) Immunoelectron microscopic localization of three key steroidogenic enzymes (cytochrome P450(scc), 3 β -hydroxysteroid dehydrogenase and cytochrome P450(c17)) in rat adrenal cortex and gonads. *J Endocrinol* 171:373–83
- Pelletier G, Luu-The V, Li S, Ren L, Labrie F (2003) Sex-related expression of 20α -hydroxysteroid dehydrogenase mRNA in the adult mouse. *J Histochem Cytochem* 51:1–12
- Peltoketo H, Luu-The V, Simard J, Adamski J (1999) 17β -hydroxysteroid dehydrogenase/ 17 -ketosteroid reductase family; nomenclature and main characteristics of the 17β -HSD/KSR enzymes. *J Mol Endocrinol* 23:1–11
- Penning TM (1997) Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev* 18:281–305
- Sar M, Lubahn DB, French FS, Wilson EM (1990) Immunohistochemical localization of the androgen receptor in rat and human tissues. *Endocrinology* 127:3180–3186
- Vihko P, Harkonen P, Soronen P, Torn S, Herrala A, Kurkela R, Pulkka A, Oduwole O, Isomaa V (2004) 17β -hydroxysteroid dehydrogenases—their role in pathophysiology. *Mol Cell Endocrinol* 215:83–88