

Immunoelectron microscopic localization of 3β -hydroxysteroid dehydrogenase and type 5 17β -hydroxysteroid dehydrogenase in the human prostate and mammary gland

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ABSTRACT

The subcellular distribution of steroidogenic enzymes has so far been studied mostly in classical endocrine glands and in the placenta. In the peripheral intracrine organs which synthesize sex steroids there is no indication about the organelles which contain the enzymes involved in steroid biosynthesis. We have thus investigated the subcellular localization of two enzymes involved in the production of sex steroids, namely 3β -hydroxysteroid dehydrogenase (3β -HSD) and type 5 17β -hydroxysteroid dehydrogenase (17β -HSD). Using specific antibodies to these enzymes, we conducted immunoelectron microscopic studies in two peripheral tissues, namely the human prostate and mammary gland. In the prostate, immunolabelling for both 3β -HSD and type 5 17β -HSD was detected in the basal cells of the tube-alveoli as well as in fibroblasts and endothelial cells lining the blood vessels. In all the labelled cell types, the gold particles were distributed throughout the cytoplasm. No obvious association with any specific

organelle could be observed, although some concentration of gold particles was occasionally found over bundles of microfilaments. In mammary gland sections immunolabelled for 3β -HSD or type 5 17β -HSD localization, labelling was observed in the cytoplasm of the secretory epithelial cells in both the acini and terminal ducts. Immunolabelling was also found in the endothelial cells as well as in fibroblasts in stroma and blood vessels. The gold particles were not detected over any organelles, except with the occasional accumulation of gold particles over microfilaments. The present data on the localization of two steroidogenic enzymes leading to the synthesis of testosterone indicate that these enzymes are located not only in epithelial cells but also in stromal and endothelial cells in both tissues studied. The absence of any association of the enzymes with membrane-bound organelles appears as a common finding in the reactive cell types of two peripheral tissues.

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INTRODUCTION

The enzyme 3β -hydroxysteroid dehydrogenase (3β -HSD) type 1 which catalyses the conversion of Δ^5 - 3β -hydroxysteroid precursors into Δ^4 -ketosteroids in all steroidogenic tissues has been purified from human placenta microsomes and mitochondria (Luu-The *et al.* 1989b, 1990). Molecular cloning studies then revealed two types of human 3β -HSD (types 1 and 2) (Luu-The *et al.* 1989b, 1990, Lachance *et al.* 1990, 1991, Rhéaume

et al. 1991). In addition to the gonadal tissues, 3β -HSD activity has been demonstrated in a variety of peripheral intracrine tissues, including the prostate and mammary gland (Lachance *et al.* 1990). At the light microscopic level, immunoreactive type 1 3β -HSD has been detected in the testis, ovary and adrenal cortex (Pelletier *et al.* 1992) and in the prostate (El-Alfy *et al.* 1999). In bovine and rat adrenal cortex, 3β -HSD activity has been shown to be associated with both the mitochondrial and microsomal fractions (Cherradi *et al.* 1993,

1994, Sauer *et al.* 1994). By immunoelectron microscopy, 3 β -HSD immunoreactivity was found to be associated only with the smooth endoplasmic reticulum (Ishimura *et al.* 1988) in bovine adrenal cortex.

The enzyme 17 β -hydroxysteroid dehydrogenase (17 β -HSD) controls the last step in the formation of all androgens and oestrogens. So far, six types of 17 β -HSD have been characterized in the human (Jarabak *et al.* 1962, Peltoketo *et al.* 1988, Luu-The *et al.* 1989a, Wu *et al.* 1993, Geissler *et al.* 1994, Adamski *et al.* 1995, Lin *et al.* 1997, Nokelainen *et al.* 1998, Dufort *et al.* 1999, Krazeisen *et al.* 1999). Type 1 17 β -HSD is a soluble protein originally purified from human placenta (Jarabak *et al.* 1962), while types 2 and 3 17 β -HSD are microsomal enzymes cloned from human prostate and testis cDNA libraries respectively (Wu *et al.* 1993, Geissler *et al.* 1994). Type 4 17 β -HSD (Adamski *et al.* 1995) is a peroxisomal enzyme expressed in virtually all human tissues. The recently characterized human type 5 17 β -HSD (Lin *et al.* 1997, Dufort *et al.* 1999) is a soluble enzyme catalysing the reduction of 4-dione to testosterone in peripheral intracrine tissues. This enzyme plays a role in the formation of active androgens in peripheral tissues. Type 7 17 β -HSD first cloned in a rat corpus luteum catalyses the transformation of oestrone (OE₁) to oestradiol (OE₂) (Nokelainen *et al.* 1998). Human type 7 17 β -HSD is a 37 kDa protein which has been found in the ovary, breast, placenta, testis, prostate and liver (Krazeisen *et al.* 1999).

Most of the data on the subcellular localization of steroidogenic enzymes have been obtained following subcellular fractionation studies (Pudney *et al.* 1985, Haniu *et al.* 1987, Luu-The *et al.* 1990, Cherradi *et al.* 1993, 1994, Sauer *et al.* 1994). In fact, the ultrastructural localization of steroidogenic enzymes obtained by immunoelectron microscopy has not so far been extensively investigated and the few reports on the subject are related to classical steroidogenic glands such as the testis, ovary and adrenal cortex (Ishimura *et al.* 1988, Shinzawa *et al.* 1988, Whitnall *et al.* 1993). We have recently developed antibodies against type 5 17 β -HSD and shown by immunocytochemistry that this enzyme could be detected in reproductive organs, including the prostate, uterus and mammary gland (El-Alfy *et al.* 1999, Pelletier *et al.* 1999). So far, the ultrastructural localization of type 5 17 β -HSD has not been reported. The purpose of the present study was to investigate the electron microscopic localization of two enzymes involved in sex steroid biosynthesis, 3 β -HSD and type 5 17 β -HSD, in two intracrine human

reproductive tissues, namely the prostate and mammary gland. As a positive control, the localization of 3 β -HSD was also studied in the interstitial Leydig cells of the human testis.

MATERIALS AND METHODS

Tissue preparation

The mammary gland tissue specimens were obtained from pre-menopausal patients (25–40 years of age) who had breast reduction, while the prostatic specimens were obtained from patients (55–65 years of age) with benign prostatic hyperplasia undergoing transurethral prostatectomy. Testes were obtained from patients (50–60 years of age) who were castrated for prostate cancer treatment.

The specimens were fixed in 3% paraformaldehyde and 1% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) within 15 min after they had been dissected out. After 12 h, the tissues were post-fixed in 0.2 or 0.5% osmium tetroxide, embedded in Araldite and cut at 0.1 μ m with an ultramicrotome. For each organ under study, five separate tissue specimens were studied and the results were shown to be consistent.

Immunocytochemistry

The ultrathin sections were immunostained using the protein A–gold complex (10 nm; British Biocell International, Cardiff, UK), as described (Roth *et al.* 1978). The antiserum to 3 β -HSD and type 5 17 β -HSD were both used at a 1:500 dilution. The 3 β -HSD antiserum used was raised by immunizing rabbits with purified human placental type 1 3 β -HSD (Luu-The *et al.* 1989b, 1990). This antiserum has been widely used to localize 3 β -HSD in several species, including the human (Pelletier *et al.* 1992). The antiserum to type 5 17 β -HSD was raised by immunizing rabbits against a peptide sequence located at amino acid positions 297 to 320 of the human type 5 17 β -HSD. The characteristics of the antiserum have been reported recently (El-Alfy *et al.* 1999, Pelletier *et al.* 1999). This antiserum has been successfully used for immunocytochemical localization in several human tissues, including the testis, prostate, breast, ovary and uterus (El-Alfy *et al.* 1999, Pelletier *et al.* 1999). Control experiments were performed by substituting non-immunized rabbit serum (1:500) or the antiserum (1:500) absorbed with an excess (10⁻⁶ M) of their respective antigen.

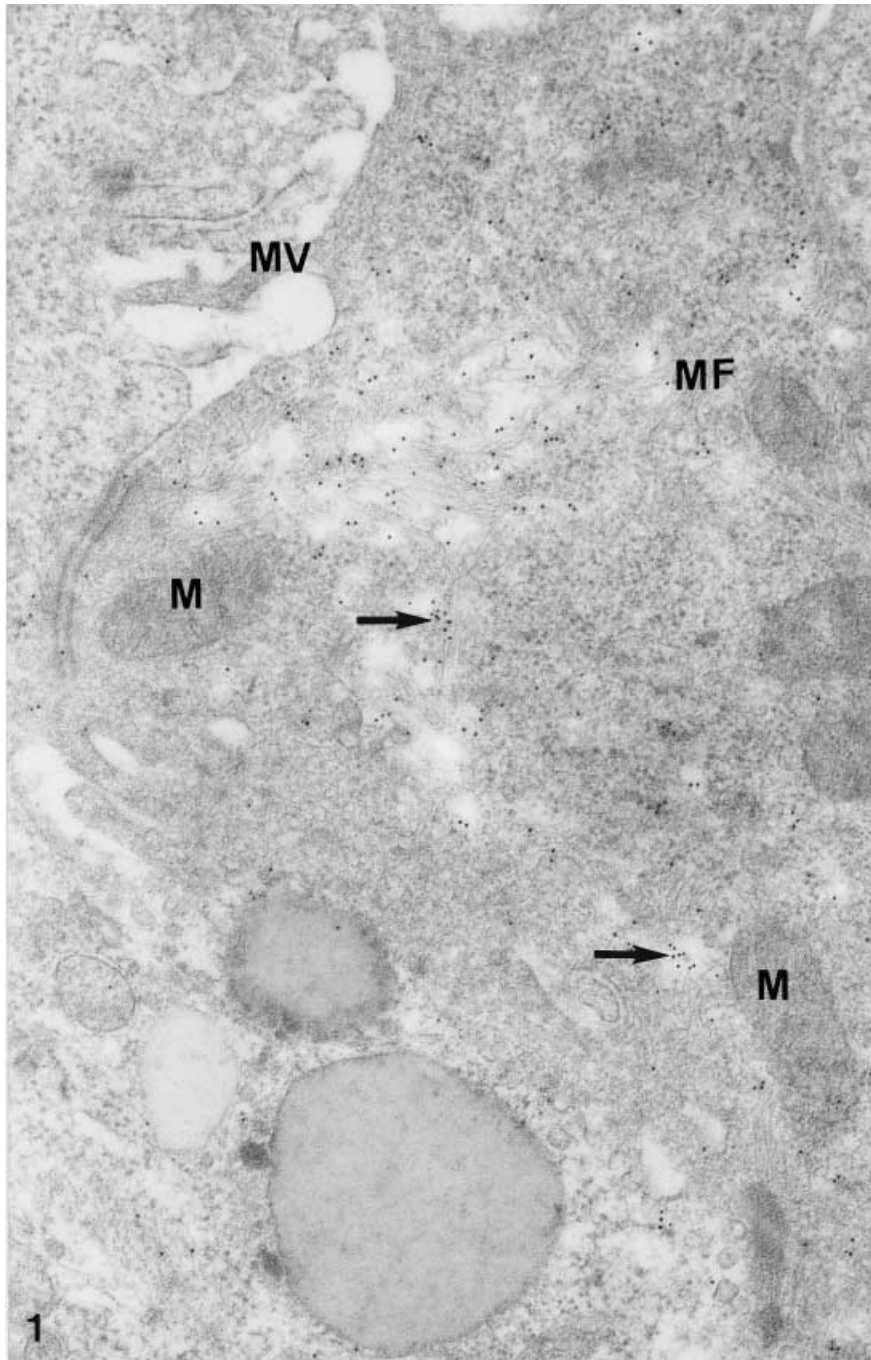


FIGURE 1. Part of an epithelial secretory cell of a mammary gland stained for 3 β -HSD localization. Immunogold labelling (→) can be detected throughout the cytoplasm. Some gold particles are located over microfilament (MF) bundles. M: mitochondria; MV: microvilli projecting to the acinar lumen. $\times 64\,000$.

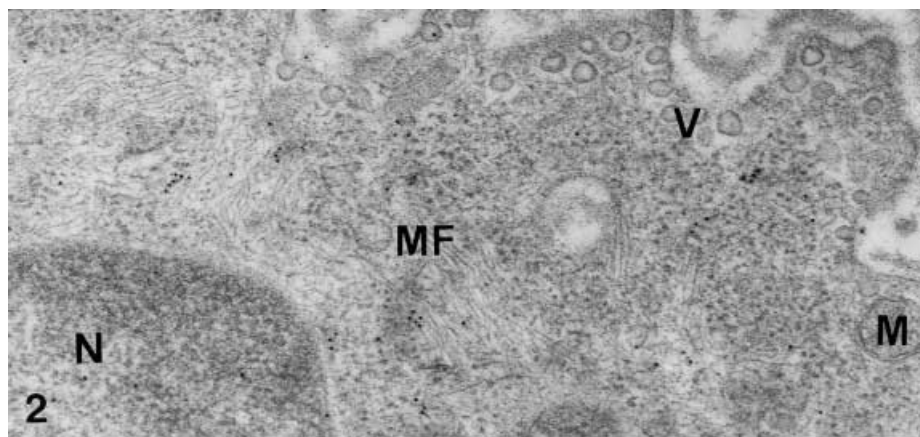


FIGURE 2. Part of a perivascular fibroblast of a mammary gland stained for type 5 17β -HSD. Several gold particles are distributed throughout the cytoplasm. Some particles are located over microfilament (MF) bundles. M: mitochondria; V: endocytotic vesicles; N: nucleus. $\times 64\,000$.

RESULTS

Mammary gland

The normal human mammary gland consists of ducts, acini and surrounding connective tissue. The epithelium lining the acini and ducts is composed of two layers: an inner columnar or cuboidal glandular epithelial layer and an outer discontinuous layer of myoepithelial cells. The myoepithelial cells are surrounded by a basement membrane. The epithelial cells contain free ribosomes, cisternae of rough endoplasmic reticulum as well as Golgi complexes which are not associated with secretory vesicles when the gland is resting. The epithelial cells show luminal microvilli. The contractile myoepithelial cells are characterized by abundant plasmalemmal vesicles and bundles of actin microfilaments. The cells also contain poorly developed cisternae of rough and smooth endoplasmic reticulum and small Golgi complexes.

In sections immunolabelled with 3β -HSD antibodies, the gold particles were localized throughout the cytoplasm of the epithelial cells in both the acini and terminal ducts. No organelles appeared to be specifically labelled (Fig. 1). A few gold particles were occasionally observed over bundles of microfilaments. No significant labelling could be detected over the myoepithelial cells. As previously observed at the light microscopic level (Pelletier *et al.* 1999), immunolabelling was found in the endothelial cells and fibroblasts lining blood vessels as well as in stromal fibroblasts. In these two cell types, immunolabelling did not generally appear to be associated with any specific organelle. As in

epithelial cells, the association of gold particles with bundles of microfilaments was occasionally observed in the endothelial cells and fibroblasts. When the sections were immunolabelled for type 5 17β -HSD, similar results were obtained, no labelling being associated with specific organelles in the three labelled cell types (Fig. 2).

Prostate

The stratified epithelium lining the tube-alveoli is divided into two layers: the basal layer made of low cuboidal cells which are separated from the stroma by a basement membrane and the layer of columnar secretory cells (luminal cells). The secretory cells possess luminal microvilli, well developed rough endoplasmic reticulum and numerous large secretory granules. The basal cells are devoid of any secretory granules.

In the tubulo-alveoli of human prostate specimens, immunolabelling for type 3β -HSD was almost exclusively observed in the basal cells, as recently found at the light microscopic level (El-Alfy *et al.* 1999, Pelletier *et al.* 1999). Immunolabelling was rather diffuse and did not appear to be restricted to specific organelles (Fig. 3).

As in the mammary gland, immunolabelling was also detected in the endothelial cells and fibroblasts of blood vessels as well as in the fibroblasts of stroma. The intracellular distribution of gold particles was rather diffuse throughout the cytoplasm. No obvious association with organelles was detected, except those bundles of microfilaments which occasionally appeared to be more labelled

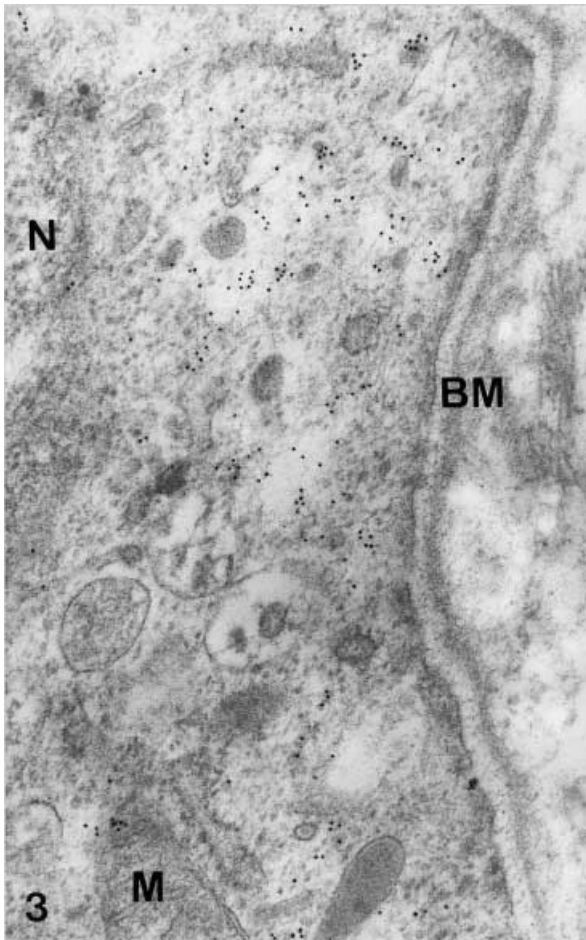


FIGURE 3. Part of a basal cell of the prostate epithelium stained for 3 β -HSD localization. Numerous gold particles are overlying the cytoplasm. No association of gold particles with organelles is observed. M: mitochondria; N: nucleus; BM: basement membrane. $\times 64\,000$.

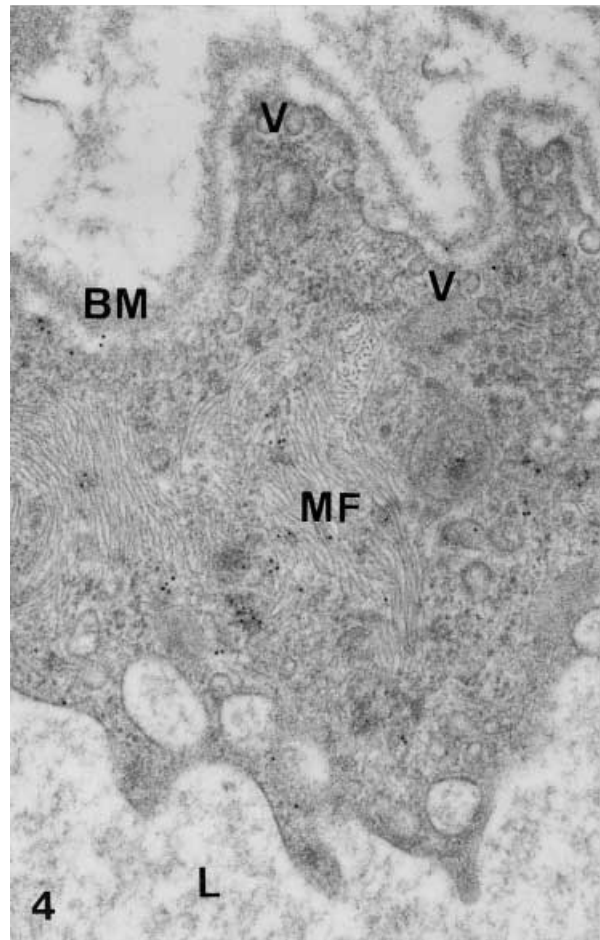


FIGURE 4. Prostate section stained for type 5 17 β -HSD localization. Part of an endothelial cell of a capillary. Gold particles are distributed throughout the cytoplasm. V: endocytotic vesicles; MF: microfilaments; BM: basement membrane; L: capillary lumen. $\times 64\,000$.

that the other cellular components. Very similar results were obtained with the anti-type 5 17 β -HSD (Fig. 4).

In the testis, as previously observed by light microscope studies (Pelletier *et al.* 1992), immunolabelling for the membrane-bound 3 β -HSD was restricted to the interstitial Leydig cells. The gold particles were predominantly detected over the numerous mitochondria and to a lesser extent over cisternae of the smooth endoplasmic reticulum (data not shown). When the antisera were immunoadsorbed with their respective antigens, no association of gold particles with any cell type could be observed. Only very few dispersed gold particles could be detected throughout the sections (Figs 5 and 6).

DISCUSSION

In the prostate, the ultrastructural localization of 3 β -HSD and type 5 17 β -HSD immunoreactivity in basal cells of tube-alveoli, endothelial cells and fibroblasts confirm recent studies conducted at the light microscopic level (El-Alfy *et al.* 1999, Pelletier *et al.* 1999). Since type 5 17 β -HSD and 3 β -HSD are both expressed in basal cells, it may be suggested that testosterone synthesized in basal cells from circulating dehydroepiandrosterone (DHEA) reaches the luminal cells to be ultimately transformed into dihydrotestosterone (DHT) in the luminal cells by the action of 5 α -reductase (Levine *et al.* 1996, Pelletier *et al.* 1998). DHT would then exert its androgenic action in the luminal cells which contain

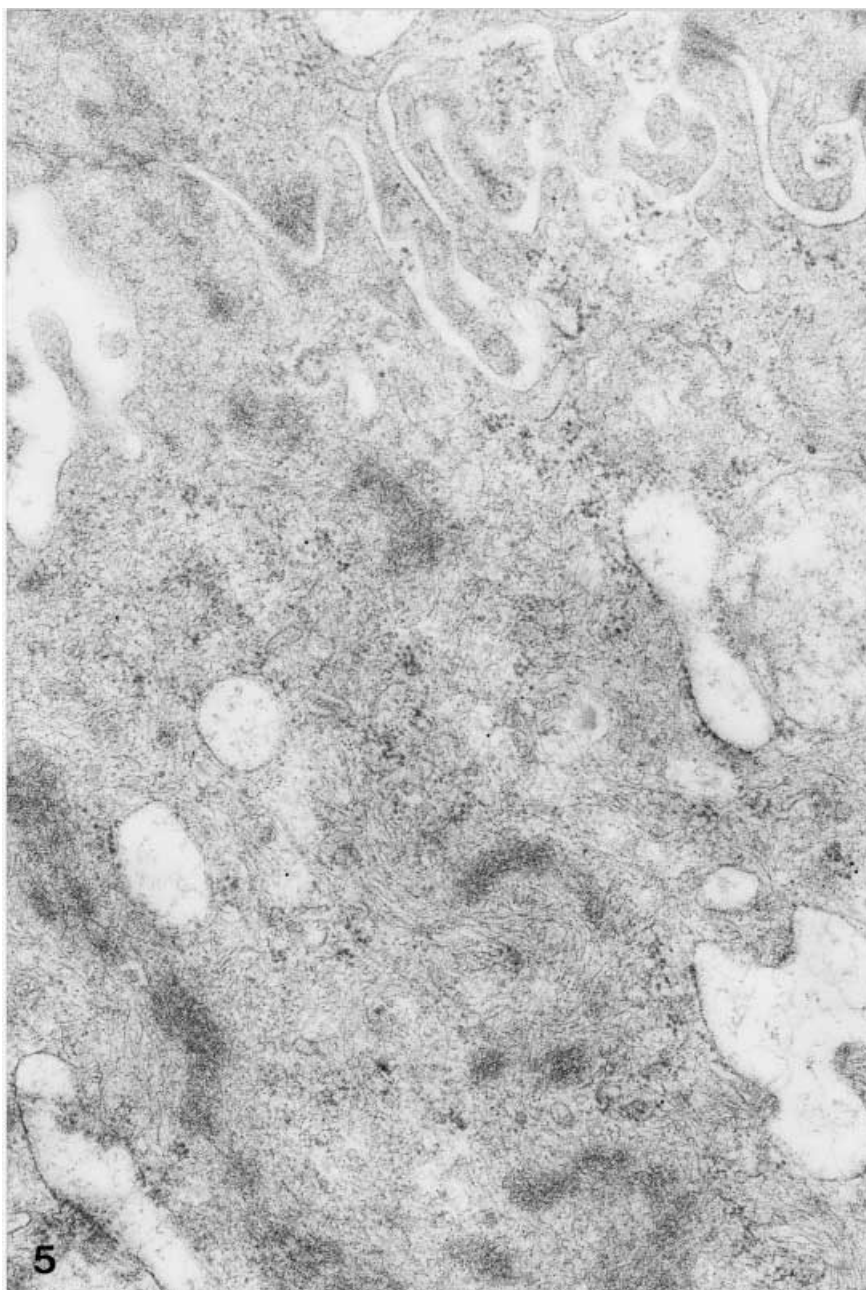


FIGURE 5. Control mammary gland section. Part of an epithelial secretory cell. Immunoabsorption of the antiserum to type 5 17β -HSD with the antigen has completely prevented any labelling. Only a very few dispersed gold particles can be detected. $\times 64\ 000$.

androgen receptors (Ruizeveld de Winter *et al.* 1991, El-Alfy *et al.* 1999).

In the mammary gland, the present data demonstrate for the very first time the localization of 3β -HSD in the epithelial secretory cells in acini as well as in intralobular ducts. As observed in the

prostate, the enzyme was also detected in stromal fibroblasts and in endothelial cells and fibroblasts of blood vessels. The present results also confirm previous data obtained at the light microscopic level indicating the presence of immunoreactive type 5 17β -HSD in the mammary cell types (Pelletier *et al.*

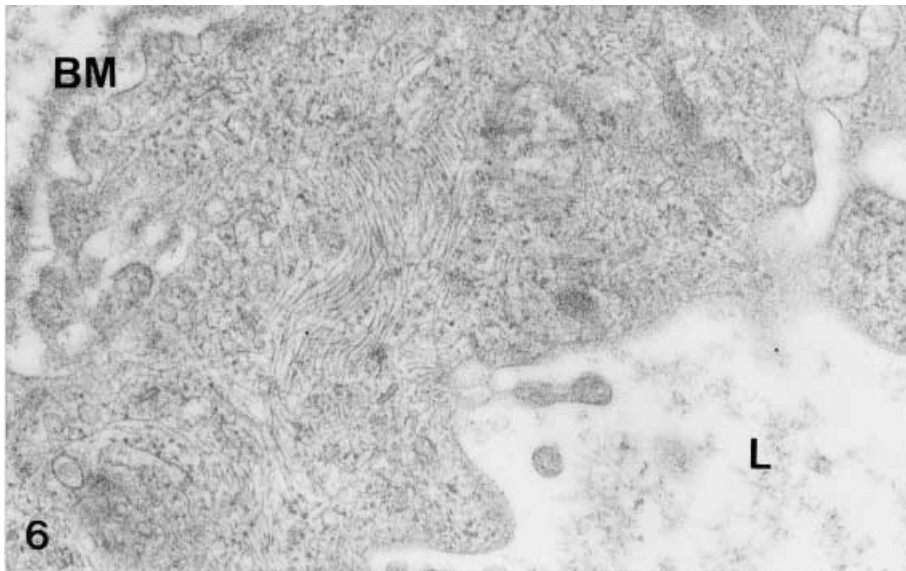


FIGURE 6. Control prostate section. Part of an endothelial cell of a capillary. Immunoabsorption of the antiserum to 3 β -HSD with the purified human 3 β -HSD has completely prevented any staining. Only a very few gold particles can be seen. BM: basement membrane; L: capillary lumen. $\times 64\,000$.

1999) which also contain 3 β -HSD. It can therefore be suggested that these enzymes in the mammary gland are involved in testosterone biosynthesis following transformation of circulating DHEA into androstenedione. These cell types have also been shown to contain androgen receptors (Ruizeveld de Winter *et al.* 1991, Kimura *et al.* 1993). Therefore, it may be suggested that the intracellularly synthesized androgens directly influence the activity of the same cells (intracrine activity) (Labrie *et al.* 1988).

The subcellular distribution of different steroidogenic enzymes has been almost exclusively studied by subcellular fractionation experiments performed in steroid-secreting glands and placenta (Pudney *et al.* 1985, Haniu *et al.* 1987, Luu-The *et al.* 1989b, 1990, Rhéaume *et al.* 1991, Cherradi *et al.* 1993, 1994, Sauer *et al.* 1994). So far, the subcellular localization of steroidogenic enzymes in peripheral organs has not been reported. The present data obtained by immunoelectron microscopy clearly demonstrate that in two peripheral tissues, the prostate and mammary gland, 3 β -HSD and type 5 17 β -HSD are not confined to specific intracellular compartments in epithelial cells, fibroblasts of the stroma as well as endothelial cells and fibroblasts of blood vessels. The detection of 3 β -HSD in mitochondria and smooth endoplasmic reticulum of the Leydig cells indicate that this membrane-bound enzyme is associated with specific organelles in a steroid-secreting cell.

In steroid-secreting glands and in the placenta, 3 β -HSD activity has been found to be associated with microsomal and mitochondrial fractions (Luu-The *et al.* 1989b, 1990, Rhéaume *et al.* 1991, Cherradi *et al.* 1993, 1994, Sauer *et al.* 1994). At the electron microscopic level, association of the enzyme with the smooth endoplasmic reticulum in the adrenal cortical cells has been confirmed (Ishimura *et al.* 1988). In peripheral organs where there is no typical steroid-secreting cell, the subcellular distribution of 3 β -HSD has not so far been reported. It is noteworthy that immunolabelling for 3 β -HSD was not associated with any organelles in the human prostate and mammary gland. The significance of the association of gold particles with microfilaments in many of the examined cells of the prostate and mammary gland remains to be clarified.

Type 5 17 β -HSD is a soluble enzyme which has been purified from mouse liver (Deyashiki *et al.* 1995). In both the human prostate and mammary gland, type 5 17 β -HSD immunoreactivity did not show any specific association with organelles, except with an occasional localization over bundles of microfilaments. In the rat central nervous system, it has been shown by immunoelectron microscopy that type 1 17 β -HSD, which is also a soluble enzyme (Jarabak *et al.* 1962), was exclusively located in astrocytes (Pelletier *et al.* 1995). In agreement with the present results, the gold particles were observed throughout the cytoplasm of

glial cells without any specific association with organelles, such as smooth endoplasmic reticulum and mitochondria. In the mammary gland, it is noteworthy that the myoepithelial cells which contain a large number of microfilaments do not seem to contain any immunoreactivity for either enzyme.

A finding of interest was the localization of both β -HSD and type 5 17 β -HSD in endothelial cells in both prostate and mammary gland tissues. This confirms previous observations at the light microscopic level indicating the presence of β -HSD and type 5 17 β -HSD in endothelial cells in the prostate (El-Alfy *et al.* 1999, Pelletier *et al.* 1999) and that of type 5 17 β -HSD in endothelial cells in the mammary gland, ovary and uterus (Pelletier *et al.* 1999). On the other hand, these enzymes were not detected in several other tissues including testis (Pelletier *et al.* 1999), brain (Dupont *et al.* 1994), pituitary and liver (G Pelletier, V Luu-The, M El-Alfy, S Li & F Labrie, unpublished data). It thus appears that the expression of the enzymes in endothelial cells is well restricted to some tissues.

Even though both β -HSD (Lachance *et al.* 1990) and type 5 17 β -HSD (Labrie *et al.* 1997) activities have been reported in prostate and mammary gland tissues, it is not possible on the basis of the present data to draw conclusions about the respective involvement of the different cell types in the synthesis of androgens from the precursor DHEA. Clearly, other studies involving quantitative approaches at the cellular level such as *in situ* hybridization are required for this information.

In summary, the present data on the ultrastructural localization of two steroidogenic enzymes which catalyse the synthesis of testosterone indicate that these enzymes are located not only in epithelial cells but also in stromal and endothelial cells in both the human prostate and mammary gland. In all these cell types there was no association of the enzymes with membranes or mitochondria. The occasional association of immunoreactive material with microfilaments remains to be clarified. Before generalizing the concept that in intracrine cells which do not possess the organelles found in cells specialized in endocrine steroidogenesis, the steroidogenic enzymes are not associated with any organelles, studies involving cell fractionation and immunoelectron microscopic studies on other tissues are required.

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