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Invited Review

Localization of androgen and estrogen receptors in rat and primate tissues

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Summary. There is now evidence that estrogens and androgens are exerting their effects in different tissues throughout the body. In order to determine the sites of action of these steroids, studies have been performed to identify at the cellular level the localization of androgen receptor (AR) and the two estrogen receptor (ER) subtypes, ERα and ERß, specially in the rat, monkey and human. In the prostate, AR was observed in the secretory and stromal cells. In the testis, Sertoli, Leydig and myoid cells were labelled. In the epididymis and seminal vesicles, both epithelial and stromal cells contained AR. In the ovary, AR was detected in granulosa and interstitial cells. In the uterus, epithelial, stromal and muscle cells were all immunopositive for AR. In the central nervous system, AR-containing neurons were found to be widely distributed throughout the brain. In the mammary gland, epithelial cells in acini and ducts and stromal cells were demonstrated to express AR. In the skin, AR was detected in keratinocytes, sebaceous and sweat glands, and hair follicles. In addition, AR was also found in anterior pituitary, thyroid, adrenal cortex, liver, kidney tubules, urinary bladder, cardiac and striated muscle, and bone. The ER subtypes are in general differentially expressed. While ERα has been predominantly found in anterior pituitary, uterus, vagina, testis, liver and kidney, ERβ is predominant in thyroid, ovary, prostate, skin, bladder, lungs, gastro-intestinal tract, cartilage and bone. In tissues which contain both receptor subtypes, such as ovary, testis and various regions of the brain, a cell-specific localization for each ER subtype has been generally observed. Altogether, the recent results on the cellular localization of sex steroid receptors will certainly contribute to a better understanding of the specific role of these steroids in different target organs.

Key words: Sex steroid receptors, Pituitary gland, Testis, Ovary, Prostate, Uterus

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Introduction

The receptors for steroid hormones belong to a single receptor superfamily, which includes receptors for androgens, estrogens, progesterone, glucocorticoids and mineralocorticoids as well as thyroid hormone, retinoic acid and vitamin D (Evans, 1988; Carson-Jurica et al., 1990; Tsai and O'Malley, 1994). The steroid hormones are exerting effects that are delayed in onset and are called "genomic" effects. These effects are mediated by an intracellular receptor. Other steroid hormone actions which have been recently described are rapid in onset and short in duration and are called "non-genomic". Since the non-genomic mechanisms through which steroids act to influence cellular activity is elusive, this chapter will focus on the localization of the classical intracellular receptor. After being activated by a specific ligand, this receptor can bind to specific target sites on nuclear DNA (termed hormone response elements) with high affinity. The binding of receptor dimers in the promoter region of steroid sensitive target gene is acting to enhance or repress transcription.

The androgen receptor (AR) and two estrogen receptor (ER) subtypes, ERα and ERβ, have been cloned and fully characterized (Chang et al., 1988; Kuiper et al., 1996; Mosselman et al., 1996). These findings have led to the development of molecular probes to detect ERα, ERβ and AR mRNA and specific antibodies to localize the receptor proteins. In order to determine the differential effects of estrogens and androgens, it is imperative to identify their target sites. The identification of tissues and cells expressing the genomic steroid receptors can be achieved by binding, *in situ* hybridization or immunocytochemical studies. In the present review, we will summarize the available data on the localization of AR, ERα and ERβ as achieved by in situ hybridization and immunocytochemistry in rat, monkey and human tissues.

Androgen receptors

The AR has been cloned in rat and human (Chang et al., 1988; Lubahn et al., 1988; Tan et al., 1988). AR

mRNA exists in two isoforms (McLachlan et al., 1991). Since both mRNAs have the same coding sequence, it is likely that they produce a single AR protein. So far, most of the localization studies on AR have been performed by immunocytochemical means. In these studies, monoclonal or polyclonal antibodies to synthetic peptides with sequences only found in human and rat AR have been used. Nuclear immunostaining was detected in large variety of tissues in rat, monkey and human (Tan et al., 1988; Sar et al., 1990; Simerley et al., 1990; Ruizeveld de Winter et al., 1991; Kimura et al., 1993; El-Alfy et al., 1999; Pelletier et al., 2000).

Reproduction organs

In the rat, monkey and human testis, AR was located in nuclei of Sertoli cells as well as of peritubular myoid cells and Leydig cells (Fig. 1) (Kimura et al., 1993). In the epididymis and seminal vesicles, both epithelial and stromal cells exhibited strong nuclear staining for AR. In the human and rat prostate, nuclear staining for AR was found in luminal cells of the alveoli as well as in the stroma and endothelial cells of capillaries, arterioles and veins (Fig. 2) (Takeda et al., 1985; Sar et al., 1990; Kimura et al., 1993; El-Alfy et al., 1999; Pelletier et al., 2000). In the basal cells of alveoli and neuroendocrine cells, no AR immunoactivity could be observed.

In the rat, monkey and human ovary, specific nuclear labelling for AR was found in granulosa cells of growing follicles at all stages from primary to mature follicles, including pre-ovulatory follicles (Fig. 3) (Ruizeveld de Winter et al., 1991; Kimura et al., 1993; Hirai et al., 1994). The primordial follicles and corpora lutea were devoid of immunoreactivity, but scattered interstitial cells were immunopositive. In the human and rat uterus, AR immunoreactivity was detected in nuclei of both epithelial cells lining the glands and those covering the surface (Fig. 4) (Kimura et al., 1993). A large number of stroma cells in the endometrium and muscle cells also exhibited nuclear staining. Similarly in the human Fallopian tube and rat oviduct, AR staining was detected in epithelial, stromal and muscle cells. In the human vagina, strong immunostaining was detected in the epithelial cells as well as stromal and muscle cells.

Endocrine glands

In the anterior lobe of the rat pituitary gland, about 50% of secretory cells showed nuclear labelling. The nuclei of the epithelial cells lining the pituitary cleft were also stained, while no labelling was seen in the intermediate and posterior lobes. Double immunostaining revealed that all the gonadotrophs contain AR (Fig. 5). In the human pituitary, Kimura et al. (1993) have reported that most gonadotrophs and some growth hormone secreting cells were immunopositive for AR. In the monkey and human adrenal glands, AR immunoreactivity was observed in the cortex but not in the medulla (Kimura et al., 1993). In the rat and monkey thyroid gland, nuclear staining was found in both follicular and parafollicular cells. No AR was found in the parathyroid glands. In the pancreas, nuclear staining was detected in a large number of cells found in the islets of Langerhans. These AR expressing cells might correspond to ß-cells, although it cannot be excluded that more than one cell type might express AR.

Central nervous system

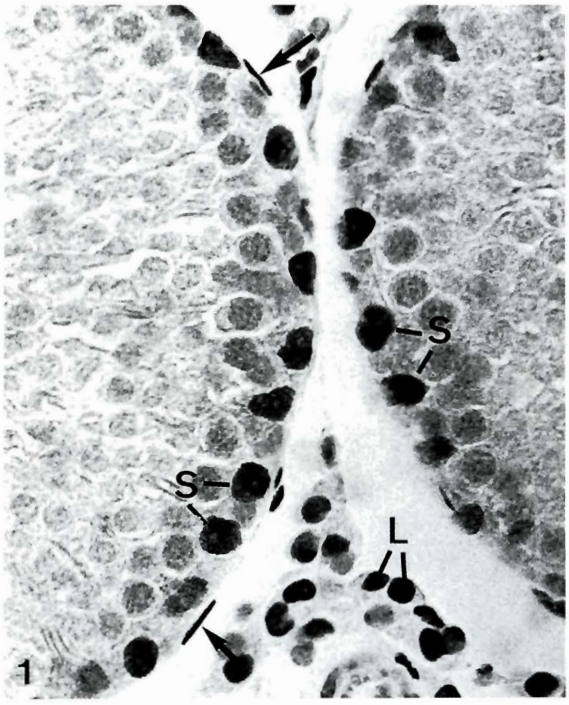
Neurons expressing AR were found throughout the central nervous system. The highest concentration of AR-containing neurons was observed in the hypothalamus and regions of the telencephalon that provide input to the hypothalamus (Simerley et al., 1990; Greco et al., 1996; Baker et al., 1997). Thus in the hypothalamus large numbers of labelled cells were found in the medial preoptic, ventromedial, periventricular and arcuate nuclei (Fig. 6). In the medial preoptic area using double immunostaining procedure, we observed that AR was localized in about 50% of LHRH-containing neurons. AR-containing cells were also found in relatively large number in the lateral septal nucleus, the medial and cortical nuclei of the amygdala, and the bed nucleus of the stria terminalis. Labelled cells were also observed in the lateral hypothalamus, CA-1 region of the hippocampus and deep layers of the cerebral cortex. Moreover, AR-expressing cells were detected in the olfactory regions of the cortex and olfactory bulbs. They were also found in the vestibular nuclei, the cochlear nuclei, the medial geniculate nucleus and the nucleus of the lateral lemniscus as well as in periventricular organs such as the area prostrema (Fig. 7) and the subfornical organ. AR-containing cells were also localized in motor nuclei associated with the fifth, seventh, tenth and twelfth cranial nerves as well as in spinal motoneurons. In the cerebellum, AR was detected in Purkinje cells.

Fig. 1. Immunolocalization of AR. Rat testis. Staining is present in nuclei of Sertoli cells (S), peritubular myoid cells (arrows) as well as Leydig cells (L). x 670

Fig. 2. Immunolocalization of AR. Rat prostate. **A.** Nuclear labelling is observed in the majority of epithelial secretory cells (E) of alveoli and in some stromal cells (arrows). **B.** Immunoabsorption control. No reaction can be detected. L : lumen. x 560

Fig. 3. Immunolocalization of AR. Human ovary. Nuclei of granulosa cells (G) of a growing follicle are strongly stained, while weaker nuclear staining can be detected in theca interna (TI) and stromal cells (arrows). x 560

Fig. 4. Immunolocalization of AR. Human uterus. Nuclear labelling is present in luminal (L) and glandular (G) epithelial cells as well as stromal cells (arrows). x 560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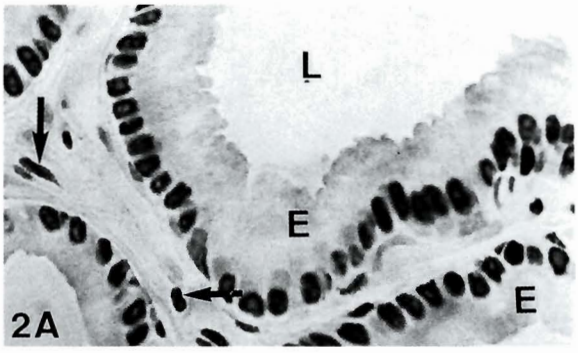
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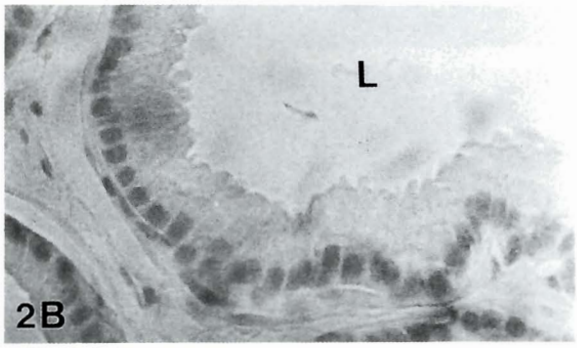
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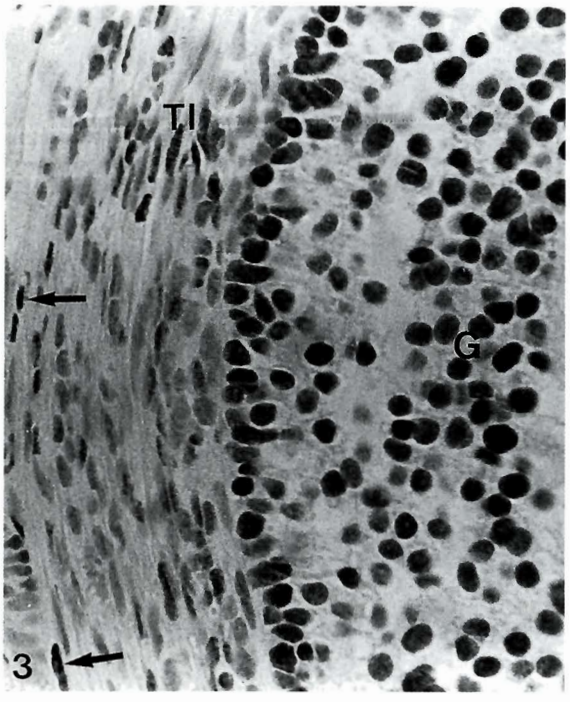
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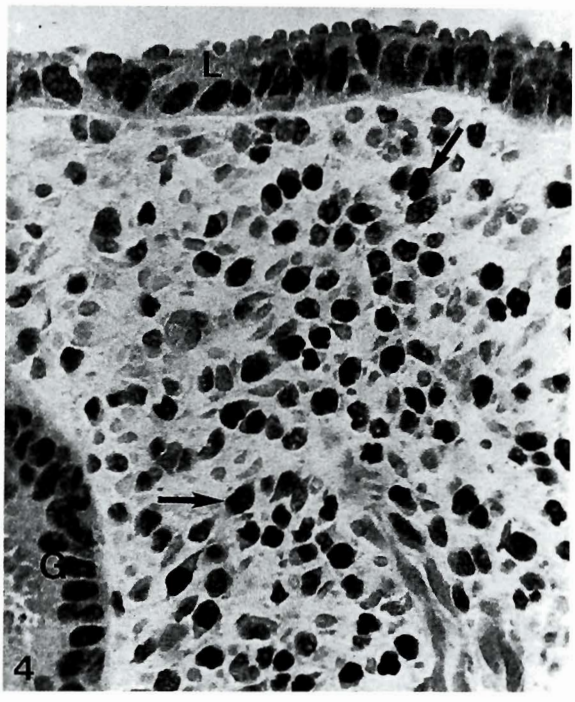
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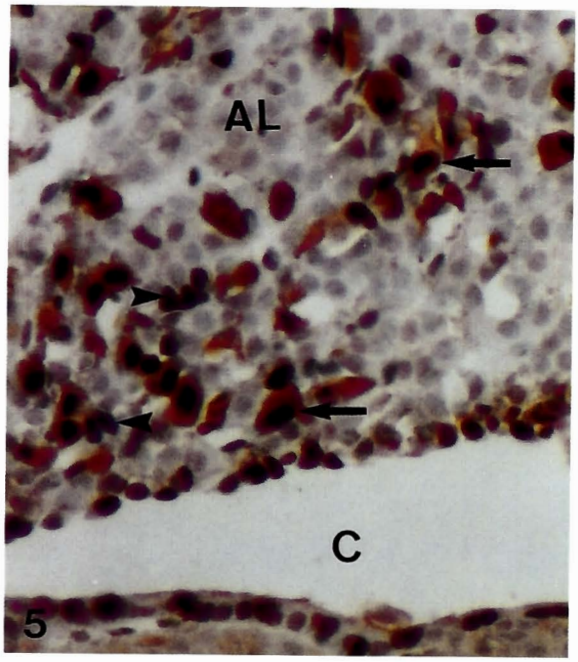

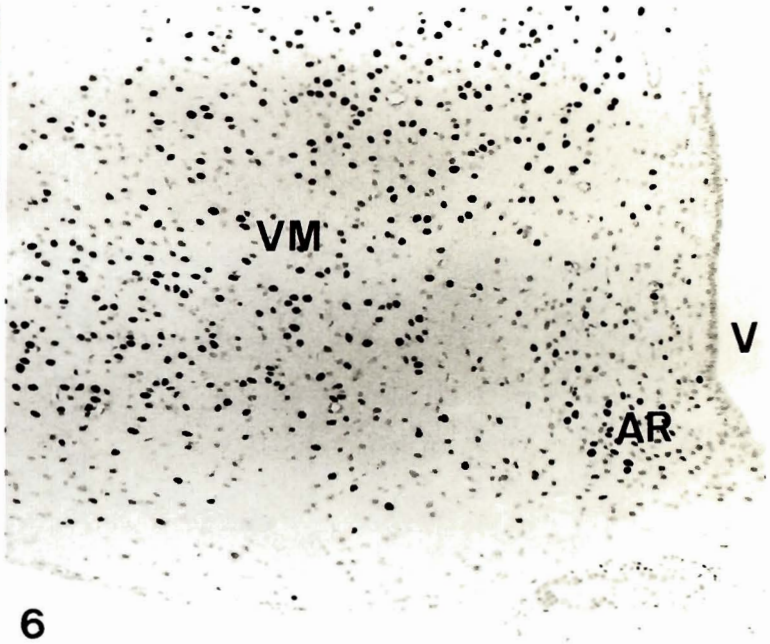

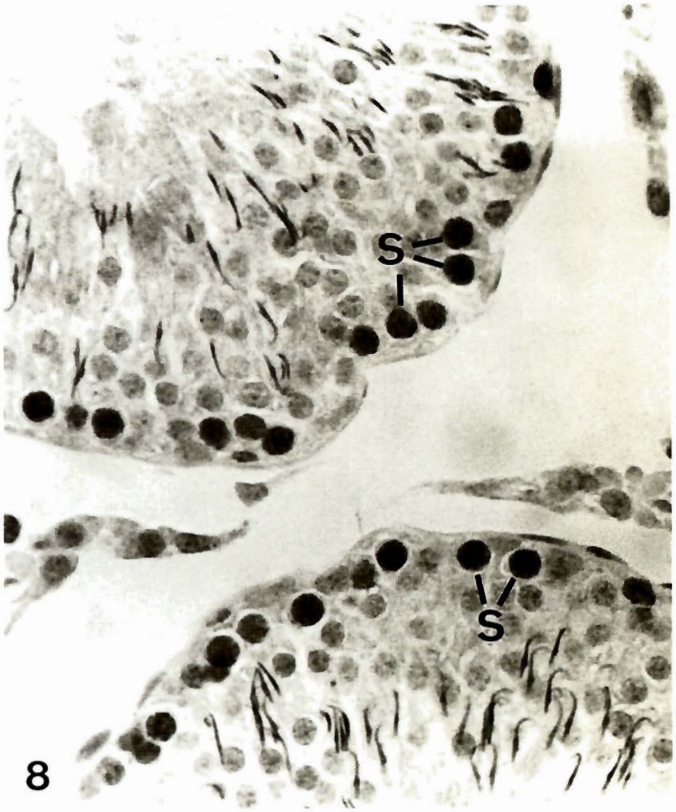

Fig. 5. Immunolocalization of AR. Rat pituitary. Double immunolabelling for AR (black nuclear staining) and LH (pink cytoplasmic staining). In the anterior lobe (AL) AR immunoreactivity is observed in nuclei of LH cells (arrows). Labelled nuclei are also observed in cells which do not stain for LH (arrowheads) and in epithelial cells lining the pituitary cleft (C). x 500

Fig. 6. Immunolocalization of AR. Section through the hypothalamus of a rat brain. Immunostained nuclei are located in the arcuate (AR) and ventromedial (VM) hypothalamic nuclei. V: Third ventricle. x 120

Fig. 7. Immunolocalization of AR. Section through the area prostrema of the rat brain. A large number of immunostained nuclei are observed. V: Fourth ventricle. x 320

Fig. 8. Rat testis. Immunolocalization of ERß. Nuclear staining can only be detected in Sertoli cells (S). x 560

Mammary glands and skin appendages

In the mammary gland of several species including rat and human, AR was localized in nuclei of both epithelial cells of acini and ducts and stromal cells (Ruizeveld de Winter et al., 1991). In acini, there was a wide variation in the intensity of staining and a few cells were consistently unstained. The myoepithelial cells did not express AR. In the skin, epidermal keratinocytes and cells of sebaceous and sweat glands and ducts were all AR immunopositive. Hair follicle cells were also immunolabelled (Ruizeveld de Winter et al., 1991).

Other tissues

In the rat kidney, cells of both proxinal and distal tubules were shown to contain AR (Takeda et al., 1985). In the human and monkey bladder, epithelial cells exhibited strong AR immunostaining. Cardiac muscle and striated muscle cells were consistently found to exhibit nuclear staining for AR in the human (Kimura et al., 1993). Finally, nuclei of rat and human hepatocytes have also been shown to contain immunoreactivity AR (Kimura et al., 1993).

Estrogen receptors

Since the recent discovery of a second ER subtype ER-ß (Kuiper et al., 1996; Mosselman et al., 1996) specific antibodies and molecular probes have been developed to specifically detect α-ER and ß-ER. It is likely that some of the antibodies to ER previously used for localization studies could recognize both ER subtypes because of the close structural analogy between ER-α and ER-β. For the same reason, *in situ* hybridization studies performed prior to the discovery of ER-b should be interpreted with caution. During the last few years, the histological localization of both ER-α and ER-β has been performed by *in situ* hybridization as well immunocytochemistry.

Reproduction organs

In the rat testis, *in situ* hybridization demonstrated high levels of ERα mRNA, while the hybridization signal for ERβ was weak (Pelletier et al., 2000). At the cellular level, both the ERα mRNA and the ERα protein were found in spermatids and spermatocytes as well as Leydig cells. In the Leydig cells, immunoreactivity was detected in nuclei, while in the spermatids and spermatocytes the staining was cytoplasmic. ERβ could be detected only in the nuclei of Sertoli cells in the rat, monkey and human testis (Fig. 8). In the rat prostate, ERβ mRNA was found in the epithelial cells of alveoli as well as stromal cells (Fig. 9A). By immunocytochemistry similar results were obtained. In human prostate, ERβ immunostaining was detected in nuclei of secretory and basal cells of alveoli (Fig. 10). Stromal cells and endothelial cells of capillaries, arteries and veins were also immunopositive for ERβ. ERα could not be detected by either *in situ* hybridization or immunocytochemistry in rat or human prostatic tissue (Fig. 9B). In the rat and monkey seminal vesicles both ERα and ERβ were found to be expressed in the epithelial cells as well as in some stromal cells (Saunders et al., 1997; Pelletier et al., 1999).

In the rat and human ovary, ERα was found to be expressed the theca interna cells, interstitial gland cells and germinal epithelium (Fig. 11) (Hiroi et al., 1999; Sar and Welsch, 1999). No ERα expression could be detected in the granulosa and luteal cells. In contrast, in growing follicles, ERβ could only be detected by immunocytochemistry in nuclei of granulosa cells, the other structures being totally negative (Fitzpatrick et al., 1999). *In situ* hybridization studies generated the same results (Fig. 12). In the rat, monkey and human uterus, ERα was localized by immunocytochemistry and *in situ* hybridization in both glandular and luminal epithelial cells (Hiroi et al., 1999). In the stroma and muscular layers, a large number of cells appeared also positive for ERα. In rat oviduct, the epithelial, stromal and smooth muscle cells were all shown to express ERα. Similarly in human Fallopian tubes, ERα was found to be expressed in epithelial, stromal and muscular cells. ERβ could not be detected in the uterus. In the human vagina, only ERα immunoreactivity could be detected in deep layers of the epithelium as well as in stromal cells of the lamina propria (Fig. 13).

Endocrine glands

In the rat pituitary gland, by both *in situ* hybridization and immunocytochemistry it has been demonstrated that, in the anterior lobe, the vast majority of the secretory cells contain ERα while in the intermediate and posterior lobes no ERα expression was found. No ERβ expression could be demonstrated in any of the three lobes. On the other hand, Mitchner et al. (1998) have shown by *in situ* hybridization that ERβ was expressed although to a lesser dengue than ERα in some cell types in the rat anterior pituitary.

In the adrenal glands, both ERα and ERβ was detected in the cortex as well as the medulla (Enmark et al., 1997; Kuiper et al., 1997; Saunders et al., 1997). In the rat and monkey thyroid gland, we have observed immunolabelling for ERβ in nuclei of follicular cells, while ERα was not found in the thyroid. In the parathyroid glands and endocrine pancreas, the presence of either ERα or ERβ has not been reported.

Central nervous system

The distribution of ERα and ERβ has been studied in the rat central nervous system by in situ hybridization and immunocytochemistry (Li et al., 1997; Shughrue et al., 1997; Alves et al., 1998; Laflamme et al., 1998). The presence of both ERα and ERβ has been detected throughout the rostro-caudal extent of the rat brain and

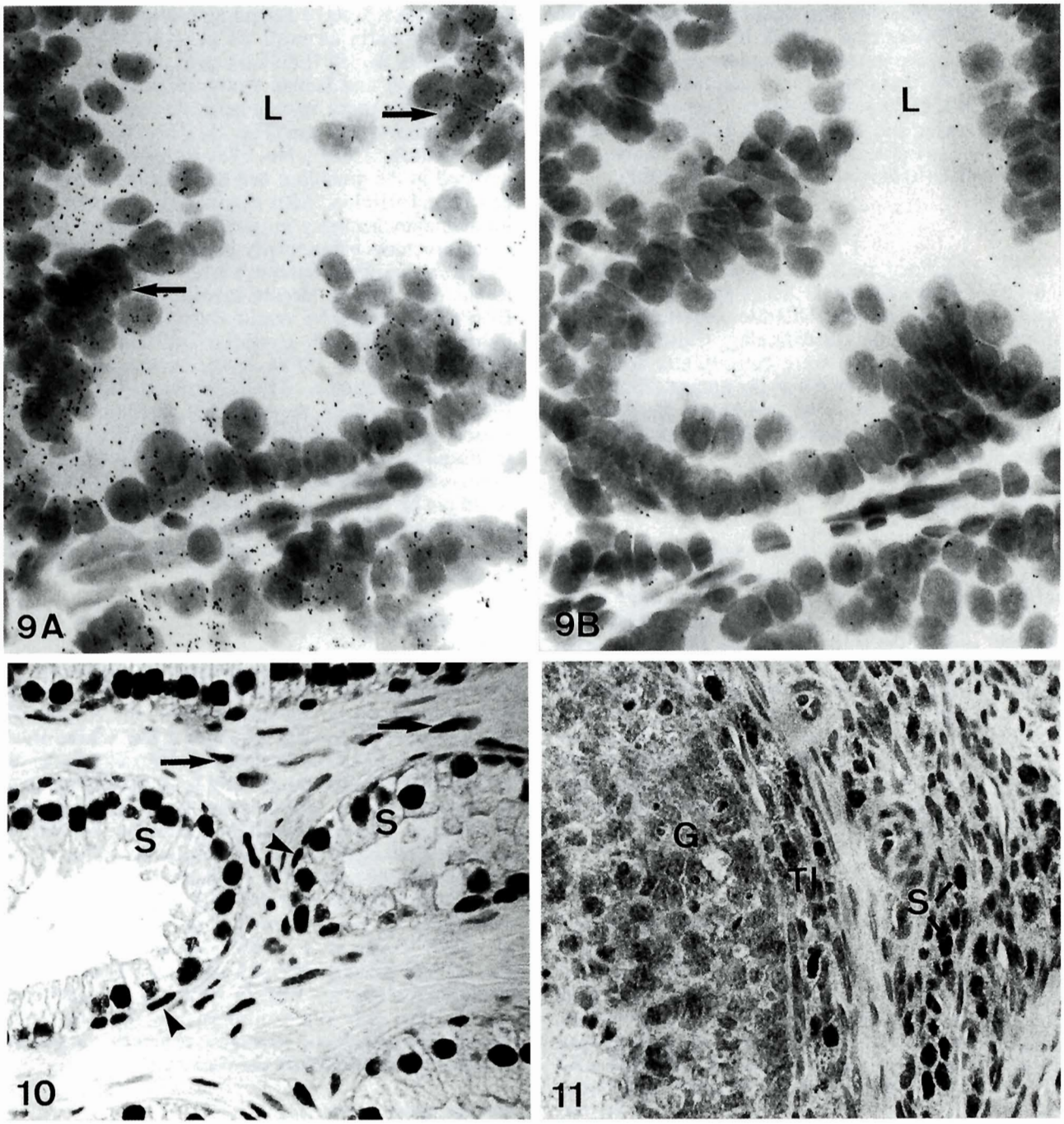

Fig. 9. Rat prostate. **A.** Localization of ERß mRNA by in situ hybridization. Silver grains are overlying the epithelial cells (arrows) of an alveolus. **B.** Localization of ERα mRNA. No significant labelling can be detected. L: lumen. x 560

Fig. 10. Human prostate. Immunolocalization of ERß. Nuclear staining is present in secretory (S) and basal cells (arrowheads) of alveoli as well as in stromal cells (arrows). x 560

Fig. 11. Rat ovary. Immunolocalization of ERα. The nuclei of granulosa cells (G) in growing follicle are unstained. Nuclear staining can be detected in a few cells in the theca interna (TI) and adjacent stroma (S). x 560

also in the spinal cord. Neurons of the supraoptic, paraventricular, suprachiasmatic and tuberal hypothalamic nuclei, zona incerta, ventral tegmental area, cerebellum (Purkinje cells) laminae III-V, VIII and IX of the spinal cord and pineal gland were shown to contain almost exclusively ERβ. In the other hand, only ERα was detected in the medial septal nucleus, the ventro-medial hypothalamic nucleus, the lateral

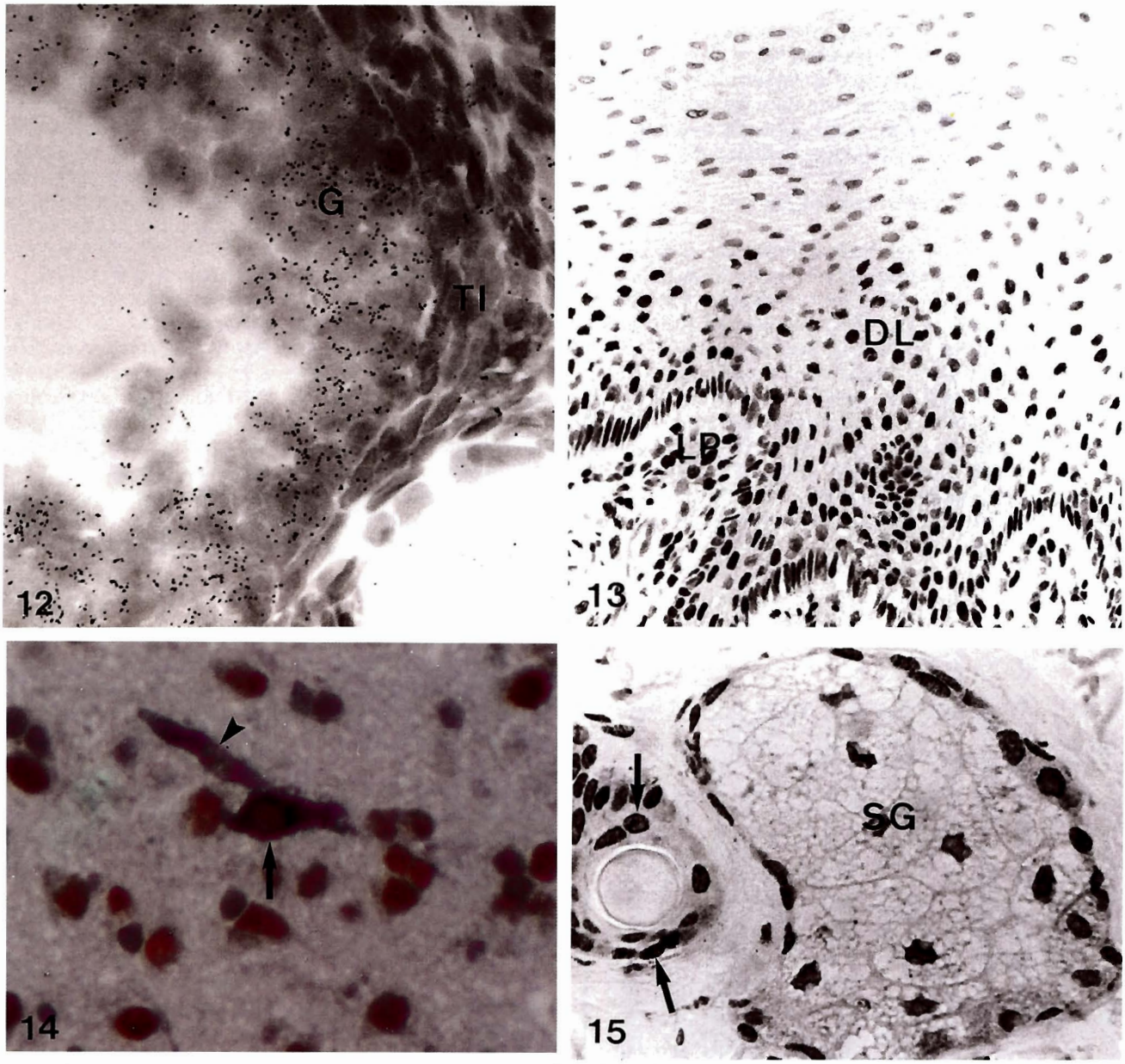

Fig. 12. Rat ovary. Hybridization with a labelled ERß antisense probe. Granulosa cells (G) of a large follicle exhibit hybridization signal. The theca interna (TI) and stromal cells are unlabelled. x 560

Fig. 13. Human vagina. Immunolocalization of ERα. Nuclear staining is prominent in the deep layers (DL) of the stratified epithelium as well as in the stromal cells of the lamina propria (LP). x 260

Fig. 14. Double-immunolabelling for ERα (brown nuclear staining) and LHRH (dark blue cytoplasmic staining). An LHRH neuron is ERα immunoreactive (arrow) while another does not exhibit any nuclear staining (arrowhead). Several ERα positive nuclei are in proximity of the LHRH neurons. x 640

Fig. 15. Human skin. Immunostaining for ERß. Immunostained nuclei are observed in hair follicle cells (arrows) and secretory cells in a sebaceous gland (SG). x 560

hypothalamic area, the subformical organ and the area postrema. In several other brain areas, including some hypothalamic nuclei such as the arcuate and periventricular nuclei, medial nuclei of the amygdala, bed nucleus of the stria terminalis, lateral habenula, olfactory cortex and superficial laminae of the spinal chord, both ER subtypes were shown to be expressed. In the hippocampus, only few neurons containing ERα were detected in the CA3 pyramidal layer. Using double immunostaining procedures we demonstrated that about 20% of LHRH-containing neurons were immunopositive for ERα in the medial preoptic area surrounding the organum vasculosum of the lamina terminalis (Fig. 14).

Mammary glands and skin appendages

In the rat, monkey and human mammary gland, both ER-α and ER-β were observed in acinar and ductal cells as well as in stromal cells in close proximity of the acini and ducts (Dotzlaw et al., 1997). In these positive cells, strong staining was found in nuclei, while a weak cytoplasmic staining could also be detected. The myoepithelial cells did not appear to express any ERs. In the rat and human skin, nuclear immunostaining for ERβ was observed in keratinocytes as well as in cells of sebaceous glands, sweat glands and hair follicles (Fig. 15). This localization appears very similar to that observed for of AR. ERα was found to be poorly expressed in skin.

Other tissues

In the rat kidney, ERα mRNA was found to be highly and ERβ poorly expressed (Kuiper et al., 1997). In contrast, in human kidney, ERβ was the predominant ER subtype (Enmark et al., 1997). In rat, monkey and human bladder, ERβ was localized by immunocytochemistry in nuclei of epithelial, stroma and smooth muscle cells, while no ERα could be detected (Saunders et al., 1997). In the human ERβ has been found by *in situ* hybridization to be highly expressed in the mucosa of the stomach, duodenum, colon and rectum, whereas the muscle layer is devoid of labelling (Enmark et al., 1997). ER-α exhibited low expression in the gastrointestinal tract. In the human and rat liver, ERα was predominant, being immunolocated in hepatocyte nuclei. In the human and mouse lung, although transcripts for each type of ER were present, ERβ was clearly predominant (Couse et al., 1997). By *in situ* hybridization, ERβ mRNA was observed in the lung parenchyma and blood vessels (Enmark et al., 1997).

In the rat heart ERβ was detected by immunocytochemistry in the nuclei of cardiac muscle cells (Saunders et al., 1997) while ERα mRNA was detected in the heart of mice (Couse et al., 1997). In blood vessels of several organs, both ERα and ERβ have been found in endothelium and smooth muscle layer (Pelletier et al., 2000). In the striated muscle, cartilage (Fig. 16), bone and thymus, ERβ is the predominant ER subtype. In the adipose tissue, both ERα and ERβ have been detected (Kuiper et al., 1997). In several tissues, the identification of the cells expressing ERα and ERβ remains to be clarified.

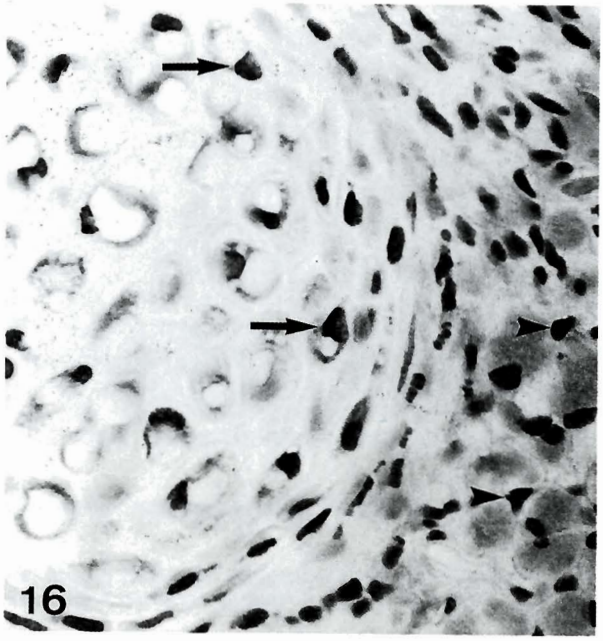

Fig. 16. Monkey tracheal cartilage. Immunolocalization of ERß. Nuclei of chondrocytes (arrows) are labelled as well as are nuclei of adjacent smooth muscle cells (arrowheads). x 560

Conclusion

The recent studies on the localization of sex steroid receptors indicated that these receptors are basically localized in the nucleus of the target cells, regardless of hormonal status (for a review Yamashita, 1998). Most interesting results have been obtained on the identification of the sites of action of these sex steroids. It then appears that AR is not confined to well known target tissues of androgens such as testis, male accessory sex organs, skin, pituitary and hypothalamus. In fact, AR has been found in female reproductive organs including ovaries (granulosa cells of growing follicles), uterus, vagina and breast as well as several other tissues such as the uro-genital tract, heart, lung, thyroid gland, adrenal glands, bone and several brain areas not directly involved in the regulation of reproductive functions.

The role of androgens in several tissues is still largely unknown. The precise localization of AR has recently led to further investigation on the effects of androgens even in the well-known targets for these steroids. For example, the presence of AR in endothelial and muscle cells in blood vessels in prostate has been recently correlated by a stimulating effect of testosterone on the vasculature in the prostate of castrated rat (Franck et al., 1998). In the female, the local production of androgens may be important for the activation of AR in several tissues. In the ovary, it has been shown that locally produced androgens act via granulosa cell AR to

modulate follicular responsiveness to gonadotropins and thereby contribute to the paracrine regulation of ovarian functions (Tetsuka et al., 1995).

The recent findings indicating a broad expression of ER-β in several male and female tissues which display little on no evidence of ERα strongly suggest that estrogens can exert some influence at multiple sites. It also now appears that there is generally a cell-specific localization for ERα and ERβ in tissues that express both types of ER. As an example, in the rat and human ovary, ERβ was found to be expressed in the granulosa cells of growing follicles while ERα expression was restricted to theca interna cells, interstitial gland cells and germinal epithelium (Pelletier et al., 1999, 2000; Sar and Welsch, 1999). Also in the different brain regions which contain both ERα and ERβ, there is no or very few cells which express both types of ER (Laflamme et al., 1998). It has been suggested that each subtype of ER might exert a different function: ERα being involved in activation and ERβ in suppression of cellular functions including cellular division. In ERβ knockout mice, hyperplasia of prostate epithelial cells which express ERβ but not ERα has been observed (Krege et al., 1998). In tissues such as anterior pituitary gland and uterus which are positively regulated by estrogens (Labrie et al., 1983) ERα is predominantly expressed. On the other hand, the presence of ERβ in cells which are producing estrogens such as granulosa cells in the ovary and Sertoli cells in the testis, might be related to an autoregulation of these steroidogenic cells.

Finally, it is noteworthy that AR is expressed at the majority of sites at which ERs are also expressed. Since aromatase, the enzyme which converts testosterone to estradiol, is also found at many of the same sites (Sharpe, 1998) it may be suggested that local balance between estrogen and androgen action could lead to a fine regulation of target cells.

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