

Androgen receptor distribution in the rat prostate gland and seminal vesicles

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ABSTRACT: To better understand the effect of androgen on the growth and function of accessory sex glands, it can be useful to determine the presence or absence of the androgen receptor (AR) in the individual cell types. Five adult intact male rats were sacrificed at 120 days. Their seminal vesicles and prostate glands were removed, fixed in Bouin's fixative and embedded in paraffin wax. The tissues were sectioned at 5 µm and stained using the microwave-stimulated antigen retrieval technique for immunohistochemistry. Positive immunohistochemical staining for the AR was evident in nuclei but not in the cytoplasm of gland cells such as luminal cells, basal cells, periacinar smooth muscle cells, other stromal cells, and smooth muscle cells in the tunica muscularis. The proportions of AR-positive and AR-negative basal cells were similar. The staining intensity of luminal cells was greater than basal cells and stromal cells. The numbers of AR-positive and AR-negative luminal cells in the prostate were almost equal. On the other hand, in the seminal vesicle only a small number of luminal cells were AR-negative. These observations can be interpreted to mean that the epithelium of the seminal vesicle is more sensitive to androgenic stimulation than the prostate epithelium.

Keywords: prostate; seminal vesicle; androgen receptor; immunohistochemistry; rat

The seminal vesicle and prostate are accessory glands of the male reproductive system. They synthesise and release an abundant amount of fluids that facilitate the transport and provide nutrition to spermatozoa. In adult rats these glands are composed of many cell types: luminal and basal epithelial cells, smooth muscle cells, fibroblasts and vascular endothelial cells (Franks 1967; Ross and Pavlina 2006). Androgens are essential for the normal development and functional maintenance of male reproductive organs. The prostate and seminal vesicle, like much of the male reproductive tract, depend on androgen action for normal development and differentiation as well as, subsequently, to adopt proper structure and functional activity (Prins 1989; Luke and Coffey 1994; Thompson 2001). The androgen receptor mediates the actions of androgen on its target cells (Mooradian et al. 1987). This nuclear transcription factor, upon binding to androgen, becomes competent to bind DNA and to stimulate androgen-dependent gene transcription (Zhu et al. 2000). To better understand androgen regulation of accessory sex glands, it is important

to know the AR distribution in these tissues. The presence of AR has been demonstrated in prostate and seminal vesicle cells from several species using biochemical (Sar et al. 1990; Takeda et al. 1990; Prins et al. 1991; Janssen et al. 1994; Yamashita 2004) as well as autoradiographical (Stumpf and Sar 1976; Peters and Barrack 1987) approaches. In these glands, AR has been shown to be localised in the nucleus of luminal epithelial cells (Prins et al. 1991; Prins and Birch 1995), basal cells (Takeda et al. 1990; Prins et al. 1991; Kimura et al. 1993; Iwamura et al. 1994; Janssen et al. 1994; Prins and Birch 1995) smooth muscle cells (Prins et al. 1991; Janssen et al. 1994; Prins and Birch 1995; Chaves et al. 2012; Iwamura et al. 1994; Kimura et al. 1993; Banerjee et al. 2001) and fibroblasts (Prins et al. 1991; Iwamura et al. 1994; Chaves et al. 2012) in immunohistochemical studies. The presence of AR in basal cells is controversial. Some investigators have demonstrated that AR is detectable in basal cells (Takeda et al. 1990; Kimura et al. 1993; Iwamura et al. 1994; Janssen et al. 1994; Prins and Birch 1995) while others failed to observe any evidence of AR

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in these cells (de Winter et al. 1990; Prins et al. 1991; de Winter et al. 1991; Prins and Birch 1995). Androgen action is also believed to be involved in the development of prostatic disease. The immunocytochemical detection of androgen receptor (AR) in the target tissues is of great importance for an understanding of the roles of AR in the activity of steroid hormones and for the simplification and extension of receptor characterisation in various diseases, particularly in hormone-dependent cancers (Perrot-Appanat et al. 1985). The aim of the present study was to determine the distribution of AR immunoreactivity in the rat prostate gland and seminal vesicles. Knowing the AR localisation in these tissues will constitute a valuable resource for future investigations.

MATERIAL AND METHODS

All experimental procedures were approved by Firat University Local Ethics Committee on Animal Experimentation (Approval No. 04.07.2013/216). Five adult male Wistar rats were killed at the age of 120 days after being anaesthetised with ethyl ether. The seminal vesicle and prostate glands were taken out rapidly and fixed in Bouin's fixative for 36 h at 4 °C and then dehydrated in ethanol, cleared in xylene and embedded in paraffin wax.

Immunohistochemistry. Five µm thick sections were cut, mounted on poly-L-lysine-coated slides and heated in an oven at 60 °C for 1 h to promote adherence to the slide. The sections were dewaxed in xylene and then rehydrated in descending grades of ethanol. Endogenous peroxidase was blocked by 10 min incubation in 3% H₂O₂ in methanol.

An antigen retrieval step was performed by heating the sections. Sections were immersed in 0.01M citrate buffer at pH 6.0, and heated four times for 5 min in a 600 W microwave oven. After heating, the material was left to cool down to room temperature, after which the slides were washed in phosphate buffered saline (PBS). Immunohistochemical procedures were performed using the PG21 antibody. Its use as a valid immunological probe for the AR in a variety of species, including human and rat, has been previously established (Prins et al. 1991; Suarez-Quian et al. 1996). Immunocytochemistry was performed using the avidin-biotin (ABC) technique. After antigen retrieval, sections were incubated with 10% non-immune goat serum for 10 min

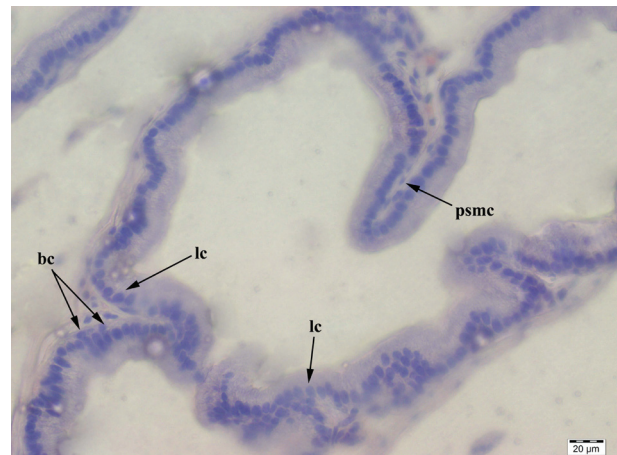


Figure 1. The prostate of 120 day old rat (negative control); magnification × 400

at room temperature to block nonspecific binding. Then, sections were incubated (4 °C, 16–20 h) in a humidified chamber with the AR-specific antibody PG21 [Rabbit Anti-Rat/Human Androgen Receptor Polyclonal Antiserum (Millipore Corporation)] diluted 1 : 50 in PBS. Sections were rinsed with PBS and were subsequently incubated with biotinylated goat anti-rabbit antibody (Zymed 50-235Z) for 30 min, streptavidin horseradish peroxidase (HRPO conjugate, SA 1007, CALTAG) for 30 min, and AEC (3-amino-9-ethyl-carbazole) for 10 min at room temperature. Between incubations, sections were rinsed three times with PBS. Sections incubated without the primary antibody but with PBS were used as negative controls (Figure 1). Counterstaining was performed with Mayer's haematoxylin (3 min). After counterstaining, sections were rinsed in distilled water and coverslips were mounted with mounting medium. Stained tissues were examined with an Olympus BX-51 photomicroscope and photographs were taken.

Table 1. A comparison of obtained findings with the results of similar studies

Prostate and seminal vesicle			
Cell types	This study	Iwamura et al. (1994)	de Winter et al. (1990)
Luminal cells	++/-	++	++
Basal cells	+/-	+/-	-
Smooth muscle cells	+/-	+/-	++/-
Smooth muscle cells in tunica muscle*	++	NE	NE

*seminal vesicle, ++ = strong, + = moderate, - = negative, ++/-, +/- = variable, NE = not examined

Table 2. The numbers of the positive and negative cells in the prostate and seminal vesicle

Prostate cell number	Cells types			
	luminal cell	basal cell	smooth muscle cell	smooth muscle cells in tunica muscle
Positive cells	229	28	45	–
Negative cells	235	42	35	–
Seminal vesicle cell number				
Positive cells	768	40	57	127
Negative cells	116	34	100	50

Immunohistochemically stained sections were reviewed by two independent observers. Only nuclear staining was regarded as positive AR immunoreactivity. The degree of immunostaining was designated in semi-quantitative analysis as strong (++), moderate (+), negative (–) or variable (++, –, +/–) immunoreactivity (Table 1). Nuclei were declared negative if receptor staining intensity did not differ visibly from that of negative control sections on a within-slide basis. The numbers of the positive and negative cells were counted in randomly selected histological slides from the prostate and seminal vesicle (Table 2). In the selected histological slide, 10 different areas were examined using an ocular micrometre at a $\times 1000$ magnification.

RESULTS

To determine AR distribution in the prostate and seminal vesicle glands of adult rats, we performed

immunohistochemical examination of paraffin-embedded sections. In Table 1 the results of this examination are summarised and compared with previous studies.

In the present study immunohistochemical staining for the AR could be detected in prostate and seminal vesicle cells. Positive immunohistochemical staining for the AR was evident in the nuclei but not in the cytoplasm of gland cells (Figure 2, 3, 4, 5, 6, 7, 8).

In the prostate gland, the proportions of AR-positive and AR-negative luminal cells were almost equal (Figure 2, 3, 4). In the stromal area, AR positivity was observed in smooth muscle cells and some other stromal cells. The staining intensity of AR in the periacinar smooth muscle cells was variable. Some of these cells were AR-positive but others were AR-negative. The staining intensity of luminal cells was stronger than periacinar smooth muscle cells and other stromal cells (Figure 2, 3, 4 and 5). In both glands, a proportion of the basal cells were AR-positive, while others were negative.

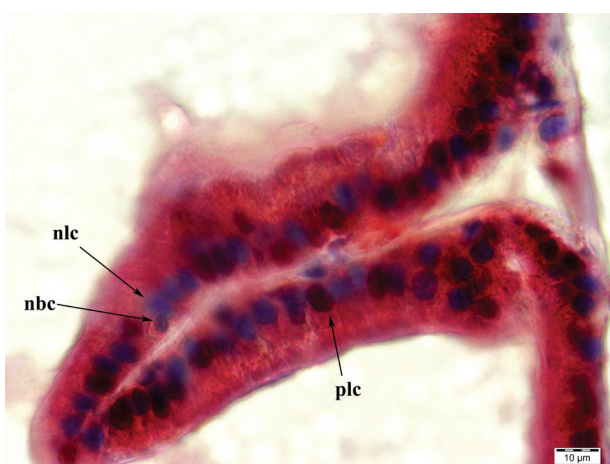


Figure 2. Immunohistochemical localisation of androgen receptor (AR) in prostate; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, nbc = negative basal epithelial cells; magnification $\times 1000$

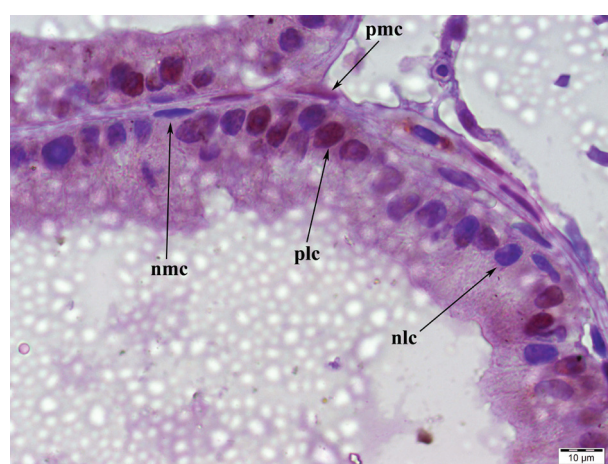


Figure 3. Immunohistochemical localisation of androgen receptor (AR) in prostate; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, pmc = positive periacinar smooth muscle cells, nmc = negative periacinar smooth muscle cells; magnification $\times 1000$

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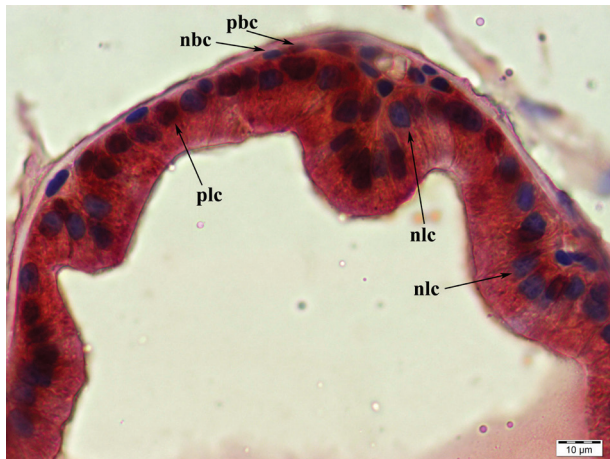


Figure 4. Immunohistochemical localisation of androgen receptor (AR) in prostate; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, pbc = positive basal epithelial cells, nbc = negative basal epithelial cells; magnification $\times 1000$

In seminal vesicles, the majority of luminal cells were AR-positive but a small number were AR-negative. Staining properties of stromal cells in the seminal vesicle was the same as the prostate. The smooth muscle cells in the tunica muscularis were strongly stained. The nuclear staining intensity of these cells was similar to luminal epithelial cells (Figure 6, 7, 8). Staining intensity in luminal cells was similar in both glands, and stronger than in basal cells.

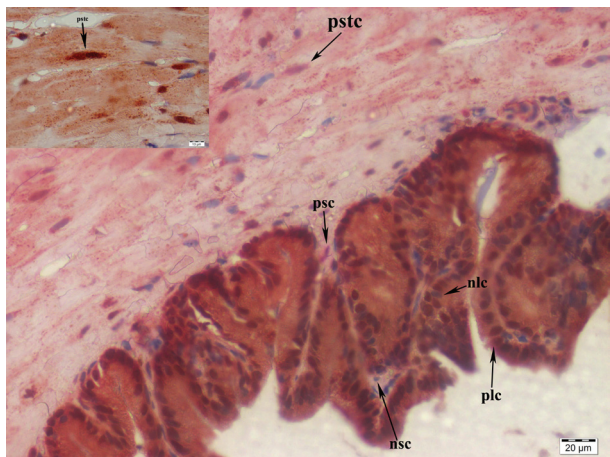


Figure 6. Immunohistochemical localisation of androgen receptor (AR) in seminal vesicle; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, psc = positive stromal cells, nsc = negative stromal cells, pstc = positive smooth muscular cells in tunica mucosa; magnification $\times 400$

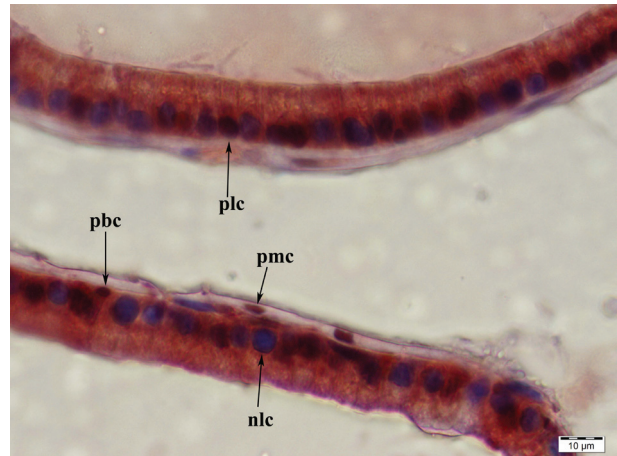


Figure 5. Immunohistochemical localisation of androgen receptor (AR) in prostate; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, pbc = positive basal epithelial cells, pmc = positive periacinar smooth muscle cells; magnification $\times 1000$

DISCUSSION

In this study, immunohistochemistry was used to investigate the specific cellular localisation of the AR in the prostate and seminal glands of adult rats. AR-positive reactions were visible in the nuclei but not in the cytoplasm of cells from these glands. A nuclear immunolocalisation of this receptor is in agreement with previous studies in humans



Figure 7. Immunohistochemical localisation of androgen receptor (AR) in seminal vesicle; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, pbc = positive basal epithelial cells, nbc = negative basal epithelial cells, nsc = negative stromal cells; magnification $\times 1000$

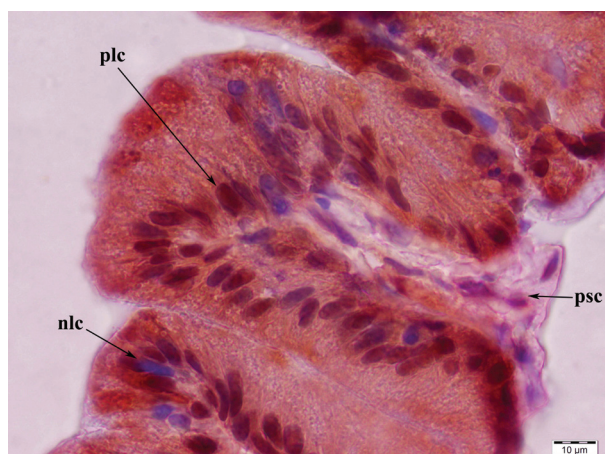


Figure 8. Immunohistochemical localisation of androgen receptor (AR) in seminal vesicle; plc = positive luminal epithelial cells, nlc = negative luminal epithelial cells, psc = positive stromal cells; magnification $\times 1000$

(Sar et al. 1990; Takeda et al. 1990; de Winter et al. 1991; Kimura et al. 1993; Janssen et al. 1994), rats (Sar et al. 1990; Takeda et al. 1990; Yamashita 2004; Gur et al. 2011) and mice (Takeda et al. 1990; Yamashita 2004), and supports the concept that ligand-dependent regulators are mainly found in the nuclei of steroid-sensitive target cells (Malayer and Gorski 1993).

We observed AR-positive reactions in epithelial cells and stromal cells. These findings are consistent with the results of previous reports (de Winter et al. 1990; Takeda et al. 1990; Prins et al. 1991; Kimura et al. 1993). Moreover, in both glands the AR staining intensity in the luminal epithelial cells was stronger than in stromal smooth muscle cells. These observations are in agreement with previous studies (Iwamura et al. 1994; Janssen et al. 1994). Our results showed that the proportions of AR-positive and AR-negative luminal cells in the prostate were almost equal. However in most previous studies it was reported that all of the luminal epithelial cells were AR-positive (Sar et al. 1990; Kimura et al. 1993; Iwamura et al. 1994; Mirosevic 1999; Yamashita 2004). AR-negative luminal epithelial cells have been mentioned in only a few studies (Prins et al. 1991; Janssen et al. 1994).

We observed that most of the luminal cells in the seminal vesicle were AR-positive, while only a few of them were AR-negative. To our knowledge, this is the first study in seminal vesicles that reports AR negativity for luminal cells. In both of these glands, the staining intensity of AR in luminal

cells is controversial. Sar et al. (1990) reported that nuclear staining intensity of prostate luminal cells is stronger than seminal vesicle luminal cells (Sar et al. 1990); however, de Winter et al. (1990) and Iwamura et al. (1994) observed that the staining properties of these cells were similar. The results presented herein are in agreement with previous studies (de Winter et al. 1990; Iwamura et al. 1994).

The staining features of epithelial cells differed in this study. Staining intensity was stronger in luminal cells than in basal cells. This result is in agreement with previous studies (Prins et al. 1991; Kimura et al. 1993; Chaves et al. 2012). The presence of AR in basal cells in the prostate and seminal gland is controversial; some investigators have demonstrated that AR is detectable in basal cells (Takeda et al. 1990; Prins et al. 1991; Kimura et al. 1993; Iwamura et al. 1994; Janssen et al. 1994; Prins and Birch 1995) while others detected no AR in these cells (de Winter et al. 1990; de Winter et al. 1991; Prins et al. 1991; Prins and Birch 1995). In this and other studies (Masai et al. 1990; Brolin et al. 1992; Bonkhof and Remberger 1993; Kimura et al. 1993), variable basal cell staining was observed. Bonkhoff and Remberger (1993) reported that in the rat prostate the basal cells express nuclear AR widely under normal and hyperplastic conditions. Basal cells with increased AR expression were most frequently detected in hyperplastic lesions including post-atrophic and atypical hyperplasia. The presence of nuclear receptors for both androgens and oestrogens or progestins in basal cells may indicate that these cells are targets of the hormonal imbalance which has frequently been implicated in the aetiology of benign prostatic hyperplasia. Previous studies (Wang and Wong 1998; Steiner and Raghov 2003) have also reported that both testosterone and oestrogen are necessary for the development of benign prostatic hyperplasia and prostate cancer. These results indicate that it is important to know the precise distribution of the androgen receptor in accessory sex gland cells.

We observed AR positivity in smooth muscle cells and some other stromal cells in the stromal area of the prostate. These results are in agreement with those reported for humans (Kimura et al. 1993; de Winter et al. 1991) and rats (Prins et al. 1991). Prins et al. (1991) reported that periacinar smooth muscle was strongly positive for AR and that this staining did not vary with the thickness of the muscle layer. In contrast to this earlier report

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(Prins et al 1991), in the present study, the staining properties of AR in the periacinar smooth muscle cells were variable.

In conclusion, in this study AR-positive staining was detected in epithelial cells and stromal cells of both glands. This indicates that only certain cell types can be considered direct targets of androgen action. Also, we observed that the proportions of AR-positive and AR-negative luminal cells in the prostate were almost equal whereas in the seminal vesicle only a small number of luminal cells were AR-negative. This finding can be interpreted to mean that the luminal cells in the seminal vesicle are more sensitive to androgenic stimulation than the luminal cells in the prostate.

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