Effects of Androgen on Androgen Receptor Expression in Rat Testicular and Epididymal Cells: A Quantitative Immunohistochemical Study

Li-Ji Zhu, Matthew P. Hardy, Ivan V. Inigo, Ilpo Huhtaniemi, C. Wayne Bardin, and Alfred J. Moo-Young

The Population Council, Center for Biomedical Research, New York, New York 10021, Department of Physiology, University of Turku, FIN-20520 Turku, Finland

ABSTRACT

Androgen is essential for maintenance of spermatogenesis in the testis and for maturation of spermatozoa in the epididymis. The effects of androgen are mediated through its receptor (AR), the levels of which are, in turn, regulated by androgen. Previous studies have shown that AR concentrations in Leydig and Sertoli cells are differentially regulated during development. The aim of the present study was to determine if cell-type-specific regulation of AR by androgen occurs in testicular and epididymal cells during adulthood. Adult male rats were treated with the LHRH-antagonist Azaline B (100 g/day) by osmotic pump for 1, 2, 3, 4, or 8 wk to suppress endogenous androgen, with identical numbers of intact control animals at each time period. An androgen replacement group was simultaneously treated with the antagonist and a synthetic androgen, 7α-methyl-19-nortestosterone (MENT), during the final 4 wk of the experiment. Levels of nuclear AR protein in specific cell types were quantified by immunohistochemistry in conjunction with computer-assisted image analysis. Levels of AR in testicular cells declined sharply after treatment with the LHRH-antagonist. In Sertoli cells, nuclear AR levels decreased to 8% of control (P < 0.01) after 4 wk treatment; and to 12% and 17% of control (P < 0.01) in Leydig and myoid cells, respectively. Androgen replacement resulted in complete recovery of nuclear AR levels in Sertoli cells (93%, P > 0.05) but in only partial recovery in myoid (69%, P < 0.01) and Leydig cells (56%, P < 0.01). In the epididymis, tubular epithelial cells and stromal cells differed in their responses to the LHRH-antagonist. After 1 wk, nuclear AR levels in caput stromal cells decreased dramatically to 34% of control (P < 0.01) and in cauda stromal cells to 43% (P < 0.01). In contrast, the decline of AR levels in epididymal epithelial cells was not as dramatic as that in stromal cells. After 1 wk, the decline in the caput and cauda was to 87% and 76% of control, respectively. After 8 wk, nuclear AR levels in stromal cells further declined to 1.1% in caput and 1.4% in cauda, whereas in the epithelial cells, a smaller decline in nuclear AR was noted (to 30% in the caput and 45% in the cauda). After androgen replacement with MENT, nuclear AR levels recovered to more than 90% of control in both epididymal cell types. These results indicate that AR levels in the nuclei of adult Sertoli cells depend mainly on the level of androgen, whereas in the adult Leydig and myoid cells, the androgen dependency is more limited. The results also indicate that in the epididymis, stromal cells are more sensitive than epithelial cells to the regulation of AR levels by androgen.

INTRODUCTION

The androgen receptor (AR) plays a key role in androgen action. This nuclear transcription factor, on binding to androgen, becomes competent to bind DNA and to stimulate androgen-dependent gene transcription [1, 2]. Expression of AR itself is hormonally regulated by androgen, but the mechanisms of this regulation are not completely understood. In the various tissues or cells that contain AR, response to androgen can result in differences in AR expression. Thus, androgen up-regulates AR mRNA levels in avian kidney [3], rat penile smooth muscle [4], and prostate cancer cell lines PC3 [5] but down-regulates AR mRNA in avian liver [3] and rat ventral prostate [6]. At the protein level, androgen up-regulates AR in LNCaP cells, which are a human prostate cancer cell line [7], but down-regulates AR in rat prostate and seminal vesicles [6]. These studies reveal that androgen regulation of AR expression occurs in a tissue- and cell-type-specific fashion. In the male reproductive system, the testis and epididymis are major targets of androgen action, and androgen is critical for maintenance of spermatogenesis and secretory function in epididymal epithelial cells [8, 9]. In fact, AR has been detected in the nuclei of Sertoli, Leydig, and peritubular myoid cells in the testis and in the epithelial and stromal cells of the epididymis [10–12]. Whether differences occur in AR expression among these cell-types in response to androgen in adult animals, however, is not known. Therefore, we sought to determine whether AR expression in the testis and epididymis is regulated by androgen in a cell-type-specific manner. Animals were first treated with an LHRH-antagonist for as long as 8 wk to suppress circulating levels of LH, FSH, and testosterone. Exogenous androgen was coadministered with the antagonist during the last 4 wk of treatment in one of the experimental groups. The AR protein in nuclei of specific testicular and epididymal cell types was visualized by immunohistochemistry and quantified by computer-assisted image analysis. Androgen-dependent decreases in nuclear AR occurred in rat testis and epididymis, with the magnitude of the decrease depending on testicular and epididymal cell type. To our knowledge, this study is the first to quantify nuclear AR levels in different cell types.
EFFECTS OF ANDROGEN ON AR

FIG. 1. Serum hormone profiles during LHRH antagonism (A–C) and androgen replacement (D–F). Serum levels of testosterone (A), LH (B), and FSH (C) in male rats after continuous administration of LHRH antagonist, Azaline B, by mini-osmotic pump (●, 100 µg/day) were all significantly suppressed compared with control (○) and remained suppressed throughout treatment. Androgen replacement with MENT by osmotic pump during the last 4 wk of the 8-wk treatment (Ant + MENT) did not result in any significant change in these serum hormone levels compared with Azaline B alone (D–F). Results for intact control (Con), animals treated with MENT for 4 wk (MENT), and those treated with LHRH antagonist for 8 wk (Ant) are also shown. Data are given as the mean ± SEM.

within the adult rat testis and epididymis after gonadotrophic suppression and androgen replacement. Our results indicate that androgen regulation of AR in the testis and epididymis occurs in a cell-type-specific manner.

MATERIALS AND METHODS

Animals and Hormonal Treatment

Sixty adult male Sprague-Dawley rats (age, 90 days) were obtained from Charles River Laboratory (Kingston, MA) and housed at the Rockefeller University Laboratory Animal Research Center in a controlled environment (12L:12D) with free access to rat chow and water. To suppress circulating levels of gonadotropins and testosterone and determine the time sequence in which this could be achieved, animals were randomly assigned to 12 groups, each consisting of five animals. Animals in the experimental groups were treated with the LHRH-antagonist Azaline B (100 µg/day by Alza mini-osmotic pump) for 1, 2, 3, 4, and 8 consecutive wk, respectively. The dose of Azaline B was based on the results of previous dose-finding studies in rats conducted at our laboratory. Azaline B was provided by the Salk Institute (La Jolla, CA). Appropriate amounts of Azaline B were dissolved in 5% (w/v) mannitol in water and loaded into the mini-osmotic pumps to give the desired release rate of 100 µg/day. Concentrations of Azaline B used in models 2mL2 and 2mL4 pumps were 0.835 µg/ml and 1.66 µg/ml, respectively. Purity of the Azaline B was 98%. Control groups consisted of untreated, intact animals. Two groups received androgen replacement. One of these groups received Azaline B for 8 wk plus an androgen, 7-methyl-19-nortestosterone (MENT), at 30 µg/day by osmotic pump for the last 4 wk, and the second group received MENT alone for the last 4 wk. The vehicle for MENT was 45% (w/v) aqueous solution of hydroxypropyl β-cyclodextrin (Trappsol) purchased from Cyclodextrin Technologies Development, Inc. (Gainesville, FL). A synthetic androgen, MENT is more potent than either testosterone or dehydrotestosterone [13]. The MENT used was made by a privately contracted manufacturer for the Population Council and was 98.3% pure as assessed by HPLC. The MENT dose of 30 µg/day was chosen because it restored muscle, seminal vesicles, and prostate weights when administered daily for 2 wk in castrated rats [14]. Animal procedures were approved by Rockefeller University’s Institutional Animal Care and Use Committee.

Serum Hormones

Blood samples were collected when the animals were sacrificed. Serum testosterone and FSH levels were measured by radioimmunoassay. Serum LH was measured by an immunofluorometric assay [15].

Antibody

Polyclonal antiandrogen receptor antibody was purchased from Affinity Bioreagents, Inc. (Golden, CO) (catalog number PA1-111). The antibody was made by immunizing New Zealand White rabbits with the synthetic peptide MEVQGLGLGRVYPRPSKTYRGC, corresponding to the N-terminal 21 amino acids of rat and human AR. It does not cross-react with estrogen, progesterone, or glucocorticoid receptors [16].
FIG. 2. Distribution of AR immunohistochemical staining in various rat tissues. Immunostainable AR can be detected in the nuclei of cells in testis (A), epididymis (B), and prostate (C), but not in spleen (D). When the primary antibody (rabbit anti-AR immunoglobulin [Ig] G) was replaced with nonimmune rabbit IgG at the same working dilution, no staining was observed in any of these tissues (a-d). Bar = 100 μm.

Immunohistochemistry

Six groups of animals with five rats in each were used in the immunohistochemical study. This included the animals treated with Azaline B for 1, 2, 4, and 8 wk; treated with Azaline B for 8 wk and with MENT for the last 4 wk; and intact controls.

Animals were anesthetized with CO₂ and killed by cervical dislocation. Testes, epididymis, ventral prostate, and spleen were immediately removed. The epididymides were trimmed of fat and divided into three segments: caput, corpus, and cauda. All tissues were immediately embedded in O.C.T. compound (Miles Scientific, Elkhart, IN), frozen in liquid nitrogen, and then stored at −70°C until use. Sections (thickness, ~6 to 7 μm) were cut with a cryostat microtome (Hacker, Fairfield, NJ) at −20°C and mounted on glass slides coated with poly-L-lysine (molecular weight, >150,000). Twelve sections were cut from each piece of tissue, eight of which were used for AR immunostaining and four of which were used as controls. Sections were air-dried at room temperature and then fixed in 4% paraformaldehyde in PBS (pH 7.4) for 10 min and washed thoroughly with PBS. Streptavidin-biotin-peroxidase immunostaining was performed using the Histostain-SP kit (Zymed Laboratories, Burlingame, CA). Endogenous peroxidase activity was blocked with a 10-min incubation in 3% H₂O₂ in methanol, and nonspecific antibody binding was minimized by a 20-min incubation in 10% nonimmune goat serum. Sections were then incubated with the rabbit anti-AR antibody with 5 μg/ml PBS in a moist chamber at 4°C overnight. Thereafter, sections were washed thoroughly with PBS containing 0.2% Triton X-100 and treated with biotinylated goat antirabbit IgG for 30 min and with streptavidin-peroxidase for 30 min. A colored reaction product was developed using an aminoethyl carbazole (AEC) mixture for 6 min. To determine the incubation time...
FIG. 3. Immunohistochemical staining of AR in rat testes during LHRH antagonism and with androgen replacement. Immunostainable AR in rat testes of control animals (A) and of animals treated with Azaline B for 1 wk (B), 4 wk (C), 8 wk (D), and 8 wk with MENT during the last 4 wk (E). In the testis, AR is localized in the nuclei of Sertoli cells (s), peritubular myoid cells (m), and Leydig cells (l). Bar = 50 μm.

for the color development, sections of prostate, testis, epididymis, and spleen were collected on the same slide and monitored under the microscope during incubation with AEC. A nuclear signal was apparent in prostate, epididymis, and testis after 2 min, but this signal did not increase further after 10 min, even in epithelial cells of the prostate, which contain the highest nuclear AR concentration. Hence, the incubation time was set at 6 min.

Control procedures consisted of the following: 1) substituting the primary antibody with nonimmune rabbit IgG (Zymed, catalog number 02-6102) at the same working dilution, 2) substituting the primary antibody with anti-AR antibody preabsorbed with synthetic peptide (Affinity Bioreagents, catalog number PEP-006) at the same working dilution, 3) substituting the primary antibody with PBS, and 4) substituting the secondary antibody with PBS.

**Image Analysis**

The AR immunohistochemical staining resulted in the nuclear areas of some testicular and epididymal cells staining dark-red in color, which was distinguishable from the nonstained background. Stained testicular and epididymal areas were first recorded using a Nikon Optiphot-2 microscope (Nikon Corp., Melville, NY) equipped with ×40 objective lens and a Dage MTI video camera (CCD 72, Michigan City, IN). The video images were then digitized using a frame grabber (Quick Capture; Data Translation, Inc., Marlboro, MA) and displayed on a Sun IPC workstation (Mountain View, CA). The stained nuclear areas of labeled cells (i.e., Sertoli, Leydig, and myoid cells in testis; epithelial and interstitial stromal cells in caput and cauda epididymis) were traced. Different testicular and epididymal cells could be identified by the shapes and location of their nuclear areas. For testicular cells, Sertoli cells were characterized by their large, irregularly shaped nuclei on the basal layer of the seminiferous epithelium; myoid cells by their elongate nuclei around the periphery of the tubule; and Leydig cells by their round nuclei in the interstitial tissue. For epididymal cells in both caput and cauda, the epithelial cells were characterized by the oval or round nuclei that were regularly arranged on the surface of lumen, whereas the stromal cells had irregularly shaped nuclei in
FIG. 4. Relative signal intensity of nuclear AR (RSI-AR) in rat Sertoli cells (A), Leydig cells (B), and peritubular myoid cells (C) during LHRH antagonism and with androgen replacement. The RSI-AR in these three types of cells decreased markedly (P < 0.01) after LHRH-antagonist treatment. Androgen replacement resulted in RSI-AR recovery to normal levels in Sertoli cells, but in Leydig and myoid cells, levels remained lower than control (P < 0.01). Data are given as the mean ± SEM.

Statistics

Sections of one testis and one epididymis from each animal were used in the immunohistochemical study; hence, each group included five testes and epididymides. In the image analysis, Sertoli cells were measured from stage VII seminiferous epithelium, and all other cell types were measured in a randomly observed field. For each cell type, 10 cells on the same section were measured from each animal. Only one (usually the fourth or fifth) of eight serial sections was selected for image analysis. The reference areas within the observed fields were randomly chosen on each section, and the start site within the reference area was also randomly chosen, being a cell that met the criteria for cell-type identification. Neighboring cells that also met the identification criteria were scored as well, until 10 cells were sampled. If 10 cells were not counted in a reference area, the field was shifted randomly by one position, and the sampling procedure was then renewed. Hence, each average was based on 50 observations from five animals. Data were analyzed by ANOVA, and significant differences between groups were identified by multiple comparisons (Fisher’s LSD). Differences were regarded as being significant at P < 0.05.

RESULTS

Serum Hormone Levels

Changes in serum levels of testosterone, LH, and FSH after Azaline B treatment are shown in Figure 1, A–C. Within 1 wk of Azaline B treatment, serum testosterone and LH levels decreased dramatically to 4.2% and 3.5% of control, respectively. Both hormones remained at low levels during the 8 wk of antagonist treatment. In contrast to LH and testosterone, the decrease in serum FSH was not as dramatic, reaching 70% of control after 1 wk of treatment and remaining at 50%–55% of control during subsequent weeks. Figure 1, D–F, compares the serum testosterone, LH, and FSH levels between the antagonist treatment and the androgen replacement. Androgen replacement with MENT did not affect any serum hormone levels compared with Azaline B alone (P > 0.05).

Distribution of AR Immunohistochemical Staining in Various Rat Tissues

Immunostainable AR could be detected in rat testis (Fig. 2A), epididymis (Fig. 2B), and prostate (Fig. 2C) but not in spleen (Fig. 2D). In testis, positive staining of AR was localized to the nuclei of Sertoli, Leydig, and peritubular myoid cells. By the method used in this study, no signal for AR was found in germ cells (Figs. 2A and 3A). In caput and cauda epididymis, immunostainable AR was localized in the nuclei of both tubular epithelial cells and interstitial stromal cells (Figs. 2B and 5, A and a). In addition, AR was observed in the nuclei of prostate epithelial and stromal cells (Fig. 2C), which have high AR concentrations. In the present study, prostate was used as a positive tissue control. No positive staining for AR could be detected in spleen (Fig. 2D), which is known to be AR negative. Immunore-
EFFECTS OF ANDROGEN ON AR

FIG. 5. Immunolocalization of AR in rat epididymis during LHRH antagonism and with androgen replacement, showing immunostainable AR in rat epididymis from controls (A, a) and in animals treated with Azaline B for 1 wk (B, b), 4 wk (C, c), 8 wk (D, d), and 8 wk with MENT during the last 4 wk (E, e). Epididymal AR was present in the nuclei of luminal epithelial cells (e) and interstitial stromal cells (s) in both caput (A–E) and cauda (a–e). The LHRH antagonism reduced AR staining in stromal cells to near-undetectable levels after 4 and 8 wk of treatment. However, the epithelial cells in both caput and cauda epididymis remained labeled. Bar = 50 μm.

active product was not visible in sections of prostate, testis, and epididymis when the primary antibody was substituted with nonimmune rabbit IgG (Fig. 2, a–d), preabsorbed antibody or PBS, or when the secondary antibody was substituted with PBS.

AR Immunostaining in Rat Testis During Treatment with LHRH Antagonist and After Androgen Replacement

In normal rat seminiferous epithelium, immunostainable AR was detected in the nuclei of Sertoli cells, which were recognized by their irregular contours and localization on the basal layer of the epithelium (Fig. 3A). The intensity of AR immunostaining in the Sertoli cells varied according to the cycle of the seminiferous epithelium, but it appeared to be strongest at stage VII. Immunostainable AR could also be detected in the nuclei of Leydig cells and peritubular myoid cells (Fig. 3A). One week after Azaline B treatment, the intensity of AR immunostaining decreased markedly in these three cell types (Fig. 3B). Stages of the seminiferous epithelium cycle could still be identified, however, and the strongest staining for AR in Sertoli cells still appeared to be at stage VII. After 4 wk of Azaline B treatment, the testes shrank, and the seminiferous epithelium lost all the elongate and most of the round spermatids. Stages of the cycle could not be identified. Similar intensities of AR immunostaining in the nuclei of Sertoli cells could be found in every tubule. Although weak, the signal for AR in Sertoli, Leydig, and myoid cells was still detectable (Fig. 3C). However, immunostainable AR was undetectable in the testicular cells when the animals were continuously treated with Azaline B for 8 wk (Fig. 3D). Androgen replacement with MENT elicited recovery of spermatogenesis. The regular arrangement of various grades of spermatogenic cells in the epithelium and the changes of epi-
thelial structure characteristic of the seminiferous cycle reappeared as well. Mature elongate spermatids were observed adjacent to luminal regions of stage VII and VIII seminiferous tubules. Immunostainable AR reappeared at different levels in Sertoli, Leydig, and myoid cells. In Sertoli cells, immunostainable AR was equivalent to control, whereas AR levels were lower in Leydig and myoid cells (Fig. 3E).

Image analysis indicated that the relative signal intensity of immunostainable nuclear AR (RSI-AR) in Sertoli cells (Fig. 4A) decreased markedly, to 67%, 81%, and 1.1% of control \(P < 0.01\), after 1, 4, and 8 wk of Azaline B treatment, respectively. Androgen replacement resulted in recovery to 93% of control, which was not significantly different from untreated controls at the start \(P > 0.05\). In Leydig cells (Fig. 4B), RSI-AR was also significantly suppressed, to 75%, 12%, and 0.5% of control \(P < 0.01\), after 1, 4, and 8 wk of LHRH-antagonist treatment, respectively. However, in contrast to Sertoli cells, recovery after androgen replacement was only to 56% of control, which was significantly different from untreated normal rats \(P < 0.01\). Similar results were also noted for the peritubular myoid cell (Fig. 4C), in which RSI-AR decreased to 44%, 17%, and 1.4% of control after 1, 4, and 8 wk of Azaline B treatment, respectively. Androgen replacement resulted in recovery to 69% of control, which was significantly lower than untreated normal rats \(P < 0.01\).

**AR Immunostaining in Rat Epididymis During Treatment with LHRH Antagonist and After Androgen Replacement**

In normal rat epididymis, immunostainable AR was located within nuclei of luminal epithelial cells and interstitial stromal cells in both caput and cauda. The AR staining in the epithelial cells appeared to be stronger than in the stromal cells (Fig. 5, A and a). In the epithelium, principal cells displayed stronger staining than basal cells. Clear and halo cells could not be identified with the methods used in this study. After 1 wk of Azaline B treatment, immunostainable AR decreased dramatically in stromal cells but showed little change in epithelial cells (Fig. 5, B and b). After 4 and 8 wk of treatment, nuclear AR was undetectable in stromal cells, whereas epithelial cells in both caput and cauda epididymis remained labeled. The staining was weaker than in controls, but it was still detectable (Fig. 5, C, c, D, and d). Furthermore, weak cytoplasmic staining of AR in epithelial cells could also be observed after 4 and 8 wk of treatment but was not seen in stromal cells. Androgen replacement resulted in complete recovery of immunostainable AR to normal levels in both epithelial and stromal cells (Fig. 5, E and e).

Image analysis indicated that after 1 wk of Azaline B treatment, RSI-AR in stromal cells decreased to 34% of control \(P < 0.01\) in caput and to 43% of control \(P < 0.01\) in cauda epididymis. After 4 and 8 wk of treatment, this reached 12% and 1.1%, respectively, in caput (Fig. 6A) and 2.6% and 1.4%, respectively, in cauda epididymis (Fig. 6C). Androgen replacement resulted in recovery of RSI-AR to 94% in caput \(P > 0.05\) and to 86% in cauda epididymis, which were not significantly different from untreated control rats \(P > 0.05\). In contrast, the decrease of RSI-AR in epithelial cells was not as dramatic compared with stromal cells during Azaline B treatment. After 1 wk of treatment, RSI-AR were still at 87% of the control level in caput and 76% of control level in cauda. After 4 and 8 wk of treatment, RSI-ARs were 39% and 30% of control, respectively, in caput epididymis (Fig. 6B) and 46% and 45% of control, respectively, in cauda epididymis (Fig. 6D). In the epithelial cells, androgen replacement led to 94% recovery RSI-AR in caput \(P > 0.05\) and to 86% in cauda epididymis.

**DISCUSSION**

In the present study, immunohistochemistry was used to investigate the effects of androgen on concentrations of AR protein in testicular and epididymal cells of adult rats. Immunostainable AR was observed in the nuclear areas of Sertoli cells, peritubular myoid cells, and Leydig cells in the testis, and in both the epithelial and interstitial cells of the epididymis. Localization in these cell types is consistent with the results of previous reports [10, 11, 17]. The nuclear AR levels were estimated by measuring the RSI of AR-positive cells according to procedures described in our pre-

**FIG. 6.** Relative signal intensity of nuclear AR (RSI-AR) in the epididymis during LHRH antagonism and with androgen replacement. In both caput (A, B) and cauda (C, D) epididymis, RSI-AR in the interstitial stromal cells (A, C) decreased dramatically \(P < 0.01\) after the LHRH-antagonist treatment. However, in the luminal epithelial cells (B, D), the decrease of RSI-AR was not as obvious. Androgen replacement resulted in near-complete recovery of RSI-AR in both epithelial and stromal cells to control levels. Data are given as the mean ± SEM.
vious studies of AR mRNA and protein levels among developing and adult rat Sertoli and Leydig cells [18, 19].

To see the changes of AR levels during gonadotropin withdrawal and androgen replacement, animals were first treated with an LHRH antagonist, Azaline-B, to suppress endogenous secretion of both LH and testosterone. Then, a synthetic androgen, MENT, was administered exogenously. The results showed that after Azaline-B treatment, both endogenous LH and testosterone decreased to undetectable levels, and this was followed by shrinkage of the testes and epididymis, cessation of spermatogenesis, and death of germ cells [20]. Androgen replacement with MENT resulted in increased testis weight and recovery of spermatogenesis, suggesting that both androgen withdrawal, induced by Azaline B, and androgen replacement were highly efficient.

Levels of nuclear AR protein in rat Sertoli cells can be modulated by hormonal treatment. In vitro studies have shown FSH to be a major regulator that can stimulate AR mRNA expression and protein synthesis in cultured Sertoli cells [21–23], but the effects of androgen on AR regulation were not as pronounced. Some studies have shown that in cultured Sertoli cells, androgen increases AR protein but has no effect on, or even decreases, AR mRNA expression [21–23]. This led to the hypothesis that androgen plays a role only in stabilization of the receptor protein structure [21, 24]. However, these findings were based on studies of immature animals. To our knowledge, few studies have addressed the hormonal regulation of AR in adult Sertoli cells.

In the present study, our data demonstrated that the serum FSH level was lower, and that the serum LH and testosterone levels were markedly suppressed to the lower limits of detection for the assay methods, by LHRH-antagonist treatment. The AR levels in Sertoli cell nuclei of adult rats decreased to less than 10% of control. Exogenous androgen replacement alone brought nuclear AR levels back to normal. Other studies have shown that when endogenous testosterone is suppressed by ethane dimethane sulfonate (EDS), which induces higher serum FSH and LH concentrations [25], immunostainable AR in stage VII Sertoli cells decreased to less than 10% of control. Androgen replacement in EDS-treated animals leads to the return of AR immunostaining, back to approximately 75% of the normal level [17]. Considered together, our results and those of previous reports indicate that nuclear AR levels in adult rat Sertoli cells mainly depend on the circulating level of androgen. Hence, androgen can be considered as the major regulator of AR levels in adult rat Sertoli cells. The AR levels in adult rat Sertoli cells are more sensitive to stimulation by androgen compared with immature Sertoli cells [18]. In addition, as the rat testis matures, the stimulatory effects of FSH on the Sertoli cell are thought to be diminished by an FSH-stimulated, cyclic AMP phosphodiesterase [24, 26]. Hence, the differences between results of the present in vivo study and the previous in vitro studies may be caused by differences in the age of the animals.

Differences were observed between various testicular cells in the extent of AR regulation by androgen. After androgen withdrawal, exogenous androgen replacement restored nuclear AR in Sertoli cells to normal levels, whereas AR levels in the nuclei of Leydig and myoid cells were not completely restored. These findings demonstrate that hormonal regulation of nuclear AR is specific to each testicular cell type. One explanation for this specificity may be residual circulating FSH after LHRH-antagonist treatment. Because the Sertoli cell is the only cell in the male with detectable numbers of FSH receptors [27, 28], we assume that residual FSH allows Sertoli cells to maintain their reactivity to stimulation by androgen, and that this does not apply to Leydig and myoid cells. The concentration of MENT used in the present experiments provided replacement levels of androgen action in peripheral tissues [14], but it probably did not do so in the different types of testicular cells. This is attributed to untreated animals with steroidogenically active Leydig cells maintaining testicular levels of testosterone that are 10-fold higher compared with blood levels [29]. However, insufficient androgen stimulation in the MENT-treated rats caused the lowered AR levels seen in the Leydig cells of these animals is unlikely, because the same dose of MENT maintained the Sertoli cell AR level. Therefore, other factors (e.g., LH) might be needed for complete recovery of AR levels in Leydig cells [30].

The epididymis is also a target organ for androgen action. In fact, AR is localized in both epithelial and stromal cells [10, 11], and results of androgen-binding experiments indicate that AR levels in rat epididymis are controlled by the circulating level of androgen [31]. However, previous studies have not addressed possible differences in the androgen regulation of AR between epididymal cell types. The present study showed that nuclear AR levels in rat epididymal stromal cells are more sensitive than that in epithelial cells to circulating androgen. Similarly, compared with other tissues or organs in the male reproductive system, epididymal epithelial cells have a greater ability to maintain AR levels during androgen deprivation [32]. After treatment with an LHRH antagonist, the reduction in AR immunostaining in rat epididymal epithelial cells was not as obvious as the declines that occurred in epithelial cells of ventral prostate and seminal vesicle in one report [32]. Differences in the extent of androgen regulation of nuclear AR between rat epididymal epithelial cells and stromal cells suggests separate regulatory mechanisms. Considering the distinct structure and function of the epididymis, testicular factors such as testosterone and androgen-binding protein (ABP) in the seminiferous fluid that enters the epididymis may contribute to the observed differences. Secretd by Sertoli cells, ABP works as a transporter of testosterone from the testis to the epididymis and as a carrier to maintain high concentrations of androgen [33, 34]. Secretion of ABP is regulated by FSH and androgen [34]. After LHRH antagonism, the transport of ABP is decreased but still detectable in epididymal tissue [35, 36]. In addition, after LHRH-antagonist treatment, serum testosterone decreases to undetectable levels, whereas testicular testosterone remains detectable [37]. Based on these findings, residual testicular testosterone might still bind to the residual ABP and then be transported to the epididymis, allowing the epididymal epithelial cells to receive more androgen support than the stromal cells.

Another factor in mediating differences among maintenance of AR expression by androgen could be 5α-reductase in the rat epididymal epithelium. Androgenic control of epididymal functions is modulated by the 5α-reductase metabolism of testosterone, such as 5α-dihydrotestosterone (DHT), which has a biological activity two- to threefold greater than that of testosterone [2, 38]. Northern blot analysis has shown that 5α-reductase, particularly the type 2 isozyme, is highly expressed in the rat epididymis [39, 40]. Tissue distribution studies have shown that both type 1 and 2 5α-reductase mRNA and protein in rat epididymis are predominantly located in the epithelial cells but not in the stromal cells [41, 42]. Therefore, we propose that expres-
sion of the genes encoding 5α-reductase in the epithelial cells, and low expression of these genes in the stromal cells, causes a difference in full use of the residual testosterone, with preferential action of the more potent DHT on epithelial cells. This, and the increased bioavailability of androgen to epididymal epithelial cells afforded by ABP, may explain their relative insensitivity to suppression of androgen during treatment with the LHHR antagonist.

ACKNOWLEDGMENT

We thank Dr. Barry Zirkin for providing comments on the manuscript.

REFERENCES

12. Huhtaniemi I. A supersensitive immuno¯uorometric assay for rat lu-
19. Viger RS, Robaire B. Immunocytochemical localization of 4-ene ste-
roid 5α-reductase type 1 along the rat epididymis during postnatal development. Endocrinology 1994; 134:2298±2306.