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LOCALIZATION AND REGULATION OF ESTROGEN, PROGESTIN AND ANDROGEN RECEPTORS IN THE SEMINAL VESICLE OF THE RHESUS MONKEY*

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Summary—We have used monoclonal antibodies against the estrogen (E), progestin (P) and androgen (A) receptors (R) to study receptor localization and regulation in the seminal vesicles of rhesus monkeys under different hormonal conditions. The antibodies caused substantial shifts of the appropriately labeled receptors on sucrose gradients. ER levels were lower in intact males than in immature, castrate, and estrogen-treated castrates. With immunocytochemistry, ER were detectable only in stromal and smooth muscle cells, not the epithelium. The number of ER-positive stromal cells was significantly lower in intact males than in immature, castrate, and estrogen-treated castrates, and low in a DHT-treated castrate animal. Androgen receptors were localized in epithelial as well as stromal and smooth muscle cells, and the number of AR-positive stromal cells was highest in intact adults and lowest in castrated and immature animals. Estrogen treatment at the time of castration induced PR in the ER-positive stromal cells, prevented a decline in the number of AR-positive stromal cells, and caused stromal hypertrophy. In summary, in the seminal vesicle, as in the prostate, ER is restricted to the fibromuscular stroma, is suppressed by androgens, and can mediate induction of PR on estrogen treatment. Androgen receptors are present in epithelial as well as stromal and smooth muscle cells, but variations in hormonal state appear to affect regulation of AR more in the stroma than the epithelium.

INTRODUCTION

The presence of steroid hormone receptors in the male reproductive tract implies a direct role for steroids in the regulation of tissue responses. Although androgens, acting through androgen receptors (AR), are considered the most important effectors of hormonal control in the male, there is increasing evidence that estrogens and even progestins may act directly on the epididymis, prostate, and seminal vesicle [1–3]. In some cases, there are also interactions between steroids, probably mediated through the respective steroid receptors, and our investigative approach is to determine how the quantities of receptors are regulated, since fluctuations in receptor availability is a crucial limiting factor in hormonal action.

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We reported recently [2] that the prostate glands of intact adult rhesus monkeys had low levels of estrogen receptor (ER), that castration led to a significant increase in these levels and that androgen treatment at the time of castration prevented the increase in prostatic ER. We also found with immunocytochemistry (ICC) that ER was restricted to the fibromuscular stromal cells of the prostate and that estrogen treatment at the time of castration elevated progesterone receptor (PR) in the ER-positive stromal cells. We concluded that in the prostate, stromal ER was physiologically significant because it was able to mediate estrogen-dependent PR synthesis, but that in normal adults, stromal ER levels were low due to suppression by androgens.

The seminal vesicle is an accessory sex organ which is highly dependent on androgens for maintenance of its functions. Castration results in a greatly diminished weight, lowered rates of transcription and protein synthesis, and decreased secretory activity [4–7]. Treatment of

castrates or immature animals with androgens alone can increase these functions, but, depending on the species, estrogens may also have effects [5–10]. For instance, Van Wagenen [11] showed that treatment of immature rhesus monkeys with estrogen resulted in a marked increase in the size of the seminal vesicles, due primarily to growth of the fibromuscular stroma. In a subsequent study, Zuckerman and Parkes [12] found that when androgen and estrogen were injected simultaneously, the androgen suppressed the effects of estrogen on seminal vesicle.

We now report on some new work on steroid receptor regulation and localization in the macaque seminal vesicle. Specific monoclonal antibodies against ER, PR and the androgen receptor (AR) were used in sucrose gradient analyses and immunocytochemical procedures to evaluate these receptors in tissues from immature, mature, castrate and estrogen-treated castrate rhesus monkeys.

METHODS

Animals

Rhesus monkeys were used under the supervision of the veterinary staff of the Oregon Regional Primate Research Center according to the guidelines established by the National Institutes of Health and based on the current Animal Welfare Act. Orchidectomies and seminal vesicle biopsies were performed under Fluothane/nitrous oxide anaesthesia. Autopsy specimens were obtained from animals which had been chemically restrained with ketamine, overdosed with pentobarbitol, and exsanguinated. Serum estradiol (E_2) and testosterone (T) were determined by RIA as previously described [13, 14].

We examined seminal vesicle tissues from four different groups of rhesus monkeys as follows: immature animals (1–2 yr old, $N = 3$), intact adults (over 4 yr old, $N = 5$), untreated castrate adults (castrated for either 5 yr, $N = 3$ or 6 weeks, $N = 2$) and E_2-treated castrated adults (one or two 3 cm long E_2 capsules implanted at time of castration and removed 6 weeks later). In addition, to validate that dihydrotestosterone (DHT) could restore the seminal vesicle of a castrate adult rhesus male to normal, we treated an animal for 6 weeks with four 4 cm long DHT capsules and biopsied the seminal vesicle. The cross-sectional dimensions of all the Silastic capsules used were i.d., 0.132 in.; o.d., 0.183 in.

We also removed the spleen from some of these and other animals at the time of autopsy to serve as negative controls for ICC of AR.

Monoclonal antibodies

Five monoclonal antibodies (IgGs) were used. The anti-ER was H222, courtesy of Dr Chris Nolan, Abbott Laboratories [15], the anti-PR was JZB39, courtesy of Dr Geoffrey Greene, University of Chicago [16] and anti-ARs were AN1-15 and AN1-7 [17] and the control, irrelevant antibody was against antigen B of Timothy pollen (AT) courtesy of Dr Arthur Malley, Oregon Regional Primate Research Center.

Estrogen receptor assay

Chunks of seminal vesicle (5–10 mm diameter) were sliced into 0.5 mm sections with a Stadie–Riggs tissue slicer (Arthur H. Thomas Co., Philadelphia, Penn). The tissues were rinsed with Hepes buffered Trowell's medium (pH 7) and incubated for 1 h at 37°C in the same medium containing 10 nM [^{3}H]E_2 ([2,4,6,7,16,17-^{3}H (*N*)]estradiol [150 Ci/mmol]; New England Nuclear, Boston, Mass). The assay for total, activated nuclear ER was carried out by the identical procedure previously used on macaque prostates [2]. Briefly, the tissues were homogenized and the crude nuclear pellets obtained by centrifugation were washed repeatedly in low salt buffer. Then a nuclear extract was prepared with high salt (0.5 M KCl) buffer and mixed with 2.5 μg of either a specific monoclonal antibody (H222) prepared against ER or 10 μg of an irrelevant monoclonal antibody (AT) of the same IgG subclass. Finally, ultracentrifugation on sucrose gradients containing high salt was used to physically separate radiolabeled ER complexed to antibody from nonspecifically bound and excess free [^{3}H]E_2. The amount of radioactivity in the heavier, shifted ER peak is taken as the amount of specific ER and is expressed as fmol/mg DNA in the extracted nuclear pellet.

Antibody–PR and –AR complexes on sucrose gradients

Slices of seminal vesicle were prepared as described for ER except that for progestin receptor (PR) they were incubated in [^{3}H]R5020 (Promegestone[17α-methyl-^{3}H]R5020; 86.9 Ci/mmol; New England Nuclear) and for AR they were incubated in [^{3}H]5αDHT ([1,2,4,5,6,7-^{3}H (*N*)]5α-androstan-17β-ol-3-one; 148 Ci/mmol;

New England Nuclear). After homogenization and washing of nuclei, aliquots of the nuclear high salt nuclear extracts were mixed with either 10 μg AT (control antibody) or the appropriate antireceptor antibody (7.5 μg B39 for PR; 6.4 μg AN1-15 or 12.8 μg AN1-7 for AR). All other procedures and conditions were identical to those used for ER.

Immunocytochemistry

Receptors were localized with the immunocytochemical method previously described [18] and subsequently modified [19]. Briefly, 2–3 mm pieces of tissue were placed in a drop of embedding medium (Tissue-Tek O.C.T. Compound, Miles Laboratories, Naperville, Ill.) and frozen in liquefied propane. Frozen tissue was sectioned (5 μm) on a cryostat, sections were mounted on gelatin-coated glass slides, lightly fixed, washed, and incubated overnight at 4°C with either H222 (10 μg/ml), B39 (1 μg/ml), or AN1-15 (1–2 μg/ml) for ER, PR and AR respectively or with AT (10 μg/ml) as a control for nonspecific staining. The primary antibodies were reacted with an anti-rat IgG biotinylated second antibody and the biotin was detected with an avidin–biotin peroxidase kit from Vector Laboratories. The percentage of stromal cells that contained detectable staining for each receptor was determined at a magnification of 400 × by counting cells in 10 nonoverlapping fields, approximately 200 cells/field on at least 2 sections from each seminal vesicle for a total of approximately 4000 cells/seminal vesicle.

In selected instances, serial frozen sections, 1.5 μm thick were prepared on a Hacker–Bright cryostat with freshly sharpened steel knives. Consecutive sections were mounted on slides, one section per slide, and one section was stained for ER and the other for PR. Slides were examined to determine whether the nucleus of the same cell in the two adjacent sections stained for both ER and PR. Similarly, members of other pairs of serial sections were stained for ER and AR.

Histology

After overnight fixation in 3% paraformaldehyde and 1% glutaraldehyde, tissues were embedded in glycol methacrylate and stained with Gill's hematoxylin and Lee's stains [20].

RESULTS

Serum steroid hormone levels

The concentrations of estradiol (E_2) and testosterone (T) in serum prepared from peripheral blood are shown in Table 1. The E_2-treated castrate adults had significantly higher ($P < 0.05$) serum E_2 levels than all other groups (Table 1). Testosterone levels were significantly higher ($P < 0.05$) in the intact adults than in all other groups (Table 1). Treatment with four DHT capsules produced a DHT level of 23.4 ng/ml in serum.

Sucrose gradient profiles

Sucrose gradient analysis showed that the anti-ER, anti-PR and anti-AR monoclonal antibodies reacted respectively with the ER, PR and AR extracted from the seminal vesicle of the rhesus monkey (Fig. 1). In all cases, the addition of 10 μg of an irrelevant monoclonal antibody, AT, resulted in radiolabeled gradient profiles with a peak at 4–5S, a position on these high salt gradients consistent with a peak of receptor uncomplexed with any antibody. With H222, a shifted peak of ER–antibody complex was observed at approximately 8S (Fig. 1A). With B39 a peak of antibody–PR complex appeared at approximately 6S (Fig. 1B). The addition of either AN1-15 or AN1-7 gave a shifted peak of AR–antibody at approximately 7S (Fig. 1C).

Estrogen receptor quantitation

The quantities of activated ER prepared by extraction of nuclei with 0.5 M KCl are shown

Table 1. Effects of hormone treatment on ER, PR and AR in the seminal vesicle of rhesus monkeys

Treatment	n	Serum steroid concentration: E_2 (pg/ml)	Serum steroid concentration: T (ng/ml)	ER (fmol/mg DNA)	Immunopositive cells in stroma (percentage stained): ER	PR	AR
Immature	3	7 ± 7^{a}	0.14 ± 0.03^{a}	427 ± 121^{a}	54 ± 3^{a}	0^{a}	38 ± 6
Intact	5	17 ± 7^{b}	3.3 ± 0.6^{abc}	55 ± 17^{abc}	8 ± 4^{abc}	15 ± 6^{b}	$59 \pm 3^{a*}$
Castrate	5	5 ± 2^{c}	0.23 ± 0.08^{b}	382 ± 114^{b}	45 ± 3^{b}	7 ± 2^{c}	28 ± 6^{ab}
E_2-treated castrate	5	89 ± 35^{abc}	0.21 ± 0.09^{c}	206 ± 54^{c}	38 ± 5^{c}	49 ± 3^{abc}	$54 \pm 2^{b*}$
DHT-treated castrate	1	19	—	55	3	6	44

Data are presented as the mean ± SEM. They were analyzed by a one-way analysis of variance, log transformed whenever appropriate, and tested for significance by Duncan's multiple range test. Treatment groups with the same letter superscript differ significantly ($P < 0.05$). Treatment groups with an asterisk superscript differ significantly ($P < 0.02$) from the immature group only with an unpaired t-test. n, Number of animals per treatment group.

in Table 1. Estrogen receptor is expressed as fmol/mg DNA in the extracted nuclear pellet. Levels of ER in the adult, intact seminal vesicle are significantly lower ($P < 0.05$) than those measured in the immature, castrate or E_2-treated castrate treatment groups (Table 1). In the castrate male treated with 4 capsules of DHT, the ER level was similar to the mean level in the intact adults (55 fmol/mg DNA).

Histology

Light micrographs prepared from GMA sections from the different treatment groups are shown in Figs 2–5. In intact adult animals, the secretory epithelium is the major component of the gland and the stroma represents a much smaller fraction of the tissue volume. The epithelial cells are tall, columnar and filled with secretory material (Fig. 2). In the untreated castrate animals, the epithelium atrophied dramatically and the stromal component made up a larger portion of the gland (Fig. 3). In the E_2-treated castrate group there was a substantial hypertrophy of the stromal cells but no evidence of any stimulation of either the size or secretory activity of the epithelial cells (Fig. 4). In immature animals, the seminal vesicles were much smaller than those from intact or castrate adults, their epithelial cells were small and had no signs of secretion, and their stroma made up a larger fraction of the gland just as in the untreated castrate animals (Fig. 5). In the castrate animal treated with DHT the relationship between the epithelium and stroma and the histological structure of the gland was identical to the glands of intact adult males as shown in Fig. 2.

Immunocytochemistry

ER, when present, was found only in the nuclei of stromal and smooth muscle cells, not in epithelial cells. The intact adult males (and the DHT-treated castrate male) had the lowest number of ER-positive stromal cells (Fig. 6, Table 1), and the untreated or E_2-treated castrates (Figs 7 and 8 respectively, Table 1) had a significantly higher number. There was no difference between the immature males (Fig. 9) and the castrates in the number of ER-positive stromal cells, and both had significantly more ER-positive cells than the intact adults (Table 1). These findings correlate very well with the levels of ER measured with the biochemical assay (Table 1).

PR was also present only in the nuclei of stromal and smooth muscle cells. Intact (Fig. 10) and castrate (Fig. 11) animals had low numbers of PR-positive cells (Table 1). In the E_2-treated castrate males (Fig. 12), the number of PR-positive cells was much higher, similar to the number of ER-positive cells in the same animals, and significantly more than in all other groups (Table 1). In immature males (Fig. 13) the number of PR-positive cells was very low.

In contrast to ER and PR, AR was present in the nuclei of epithelial as well as stromal and smooth muscle cells (Figs 14–17). The AN1-15 anti-AR antibody stained the cytoplasm and pericellular background to a slight degree, but similar background staining was evident in the spleen (Fig. 18) which has been reported to lack AR [21, 22]. This suggests that the majority of the cytoplasmic staining seen by light microscopy with the anti-AR antibody AN1-15 is nonspecific. Regardless of hormonal state, there was always nuclear staining for AR (compare Figs 14–17). This suggests that nuclear occupancy of AR is ligand independent.

In the stroma, but not the epithelium, the number of AR-positive cells varied significantly with hormonal state. The intact adults (Fig. 14) had a significantly higher percent of AR-positive stromal cells than the untreated castrates (Fig. 15) or the immature males (Fig. 17; $P < 0.05$, Duncan's, for castrate; $P < 0.01$, t-test, for immature; Table 1).

Fig. 1(A–C). Sucrose density gradients showing that estrogen receptors (A), progestin receptors (B) and androgen receptors (C) extracted from seminal vesicles can be recognized and shifted in the gradient by specific monoclonal antibodies. Receptors were specifically radiolabeled by incubating slices of seminal vesicle with either [^{3}H]estradiol, [^{3}H]R5020 or [^{3}H]5αDHT for estrogen, progestin and androgen receptors respectively. Nuclear extracts from incubated slices were mixed with the appropriate monoclonal antibodies (2.5 μg H-222 for ER, 7.5 μg B-39 for PR and 6.4 μg AN1-15 or 12.8 μg AN1-7 for AR) and analyzed on 5–20% sucrose gradients containing 0.5 M KCl. In each case aliquots were also mixed with 10 μg of AT, a nonspecific monoclonal antibody of the same IgG subclass. In Fig. 1A, extracts radiolabeled for ER gave a 5S peak with AT and an 8S peak with H222. In Fig. 1B, extracts radiolabeled for PR gave no receptor peak with AT, due to high nonspecific binding and free radiolabel, and a peak at approximately 6S with B39. In Fig. 1C, extract radiolabeled for AR gave a 4S peak with AT and an approxiately 7S peak with either AN1-15 or AN1-7. The positions of aldolase (8S) and bovine serum albumin (BSA; 4.5S) marker proteins are indicated by arrows.

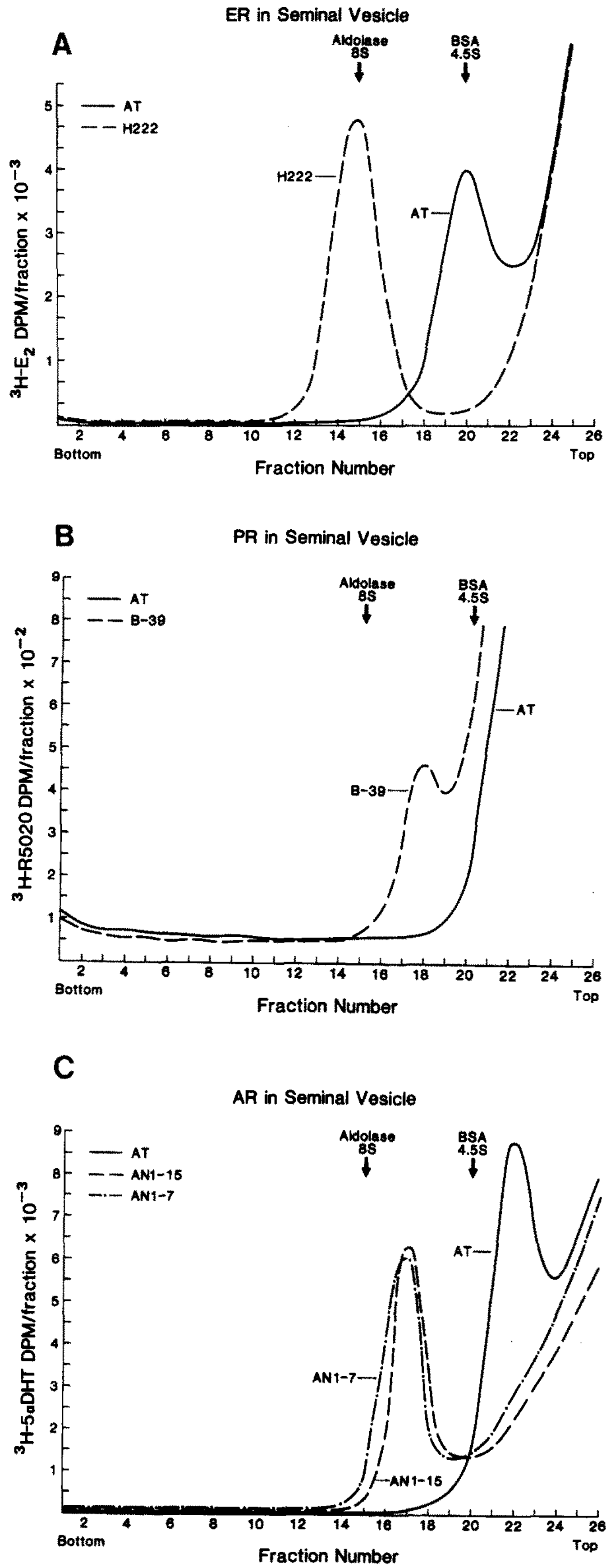


Fig. 1

Figs 2–17 are presented as a grid with four columns and four rows. The columns represent the four hormonal states and are headed as follows: intact (for adult male), castrate, E_2-treated castrate, and immature. The rows represent the different histological and immunocytochemical techniques indicated as follows: GMA (glycol methacrylate embedded, hematoxylin and Lee's stained), ER (stained for estrogen receptor), PR (stained for progestin receptor), and AR (stained for androgen receptor). Figure 18, which is a spleen stained for AR as a negative tissue control, is from an adult male. All magnifications are at 400 ×.

Fig. 2. Intact adult male. In intact adults the epithelium (E) consists of tall columnar cells filled with fine vacuoles typical of highly active secretory cells. The lamina propria (stroma) is relatively scanty and the stromal cells (S) appear compressed by the hypertrophied epithelium.

Fig. 3. Castrate adult male. 6 weeks after castration, the epithelium has become severely atrophied and all histological evidence of secretion has disappeared. Shrinkage of the epithelium has led to a loosening of the stroma so that the stroma occupies relatively more volume than in the intact adult.

Fig. 4. E_2-treated castrate. When E_2 is present during the 6-week period after castration, it has little or no ability to prevent the regression of the epithelium but it causes a definite hypertrophy of the stroma. In this micrograph, the increased distance between the glandular elements due to the expansion of the stroma is apparent.

Fig. 5. Immature male. Before puberty, the epithelium is low cuboidal and shows no signs of secretory activity. The stromal cells are relatively uncrowded, and the spatial relationships between the epithelium and the stroma resemble that seen in the castrates.

Fig. 6. ER in intact adult. In intact adult males, there are very few ER-positive stromal cells. None are evident in this micrograph.

Fig. 7. ER in castrate adult. By 6 weeks after castration, the number of ER-positive stromal cells has increased significantly but the epithelium remains ER-negative.

Fig. 8. ER in E_2-treated castrate adult. When E_2 is present during the six week period after castration, there is an increase in the number of ER-positive stromal cells over the number in intact adults, but the increase is no greater than occurs in untreated castrates (see Table 1). No ER-positive cells are induced in the epithelium.

Fig. 9. ER in immature male. Before puberty, a substantial number of stromal cells are ER-positive but the epithelium is entirely ER-negative.

Fig. 10. PR in intact adults. The number of PR positive cells in intact cells in intact adults is quite low. None are evident in this micrograph.

Fig. 11. PR in castrates. The number of PR-positive cells remains very low after castration. None are evident in this micrograph.

Fig. 12. PR in E_2-treated castrates. When E_2 is present for 6 weeks after castration, a large number of PR-positive cells are induced in the stroma, but not in the epithelium.

Fig. 13. PR in immature male. Before puberty, the number of PR-positive cells is extremely low. None are evident in this micrograph.

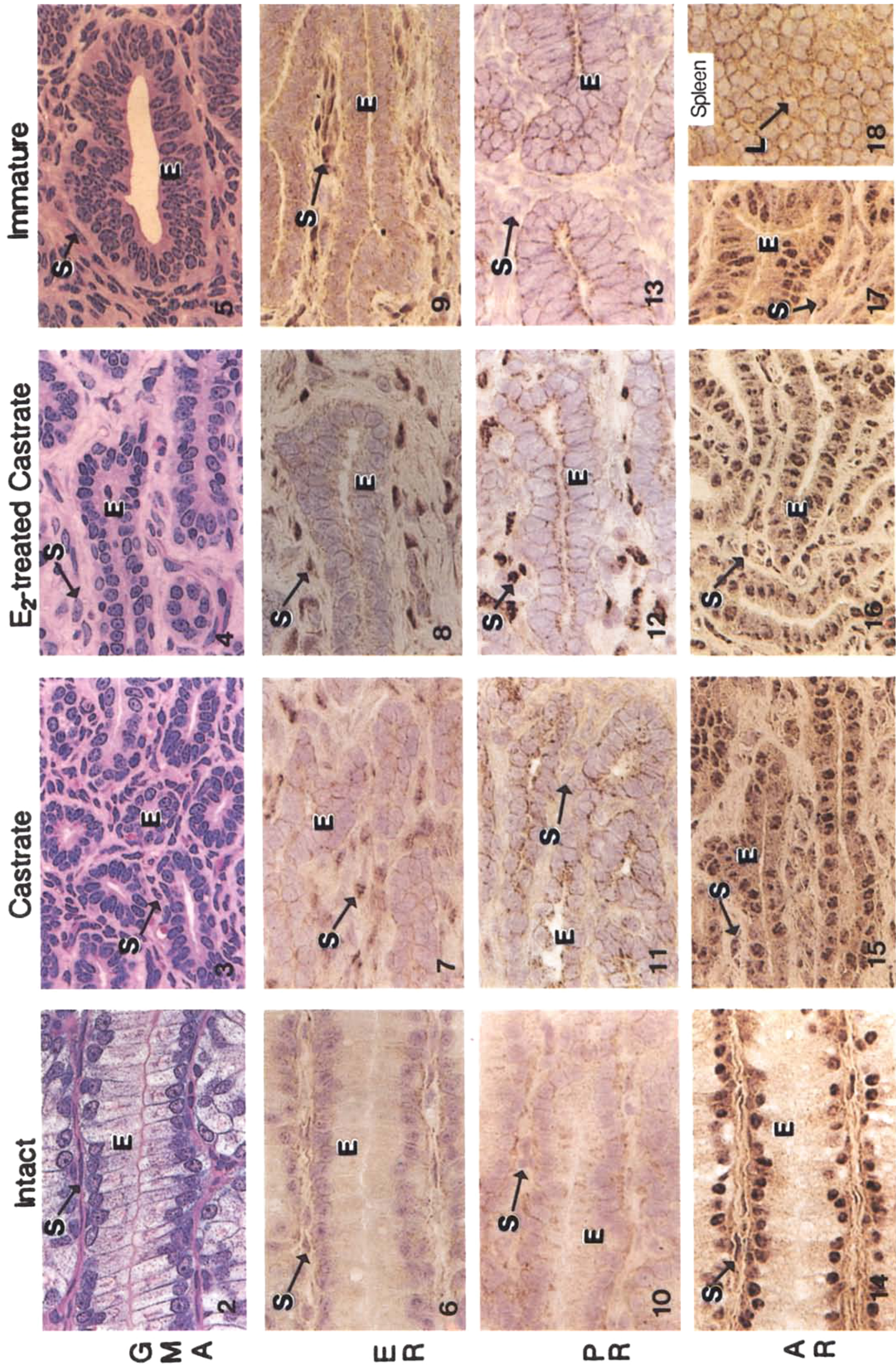
Fig. 14. AR in intact adults. AR is present in essentially all the epithelial cells and in a large number (59%, Table 1) of the stromal cells.

Fig. 15. AR in castrates. After castration, the number of AR-positive cells in the stroma was reduced to 28% (Table 1) but there was no evident change in the number of AR-positive cells in the epithelium.

Fig. 16. AR in E_2-treated castrates. When E_2 was present for 6 weeks after castration, the number of AR-positive stromal cells (54%, Table 1) was maintained at the level present before castration. There was no apparent effect on AR in the epithelium.

Fig. 17. AR in immature animals. Before puberty, the entire population of epithelial cells were AR-positive, as in adult males. However, in the stroma, the number of AR-positive cells (38%, Table 1) was significantly less than in adults, and was essentially identical to the number found 6 weeks after castration.

Fig. 18. Spleen of adult male stained with AN1-15. There was no evidence of nuclear staining in any of the splenic lymphocytes (L). The light brown staining evident in the cytoplasm, pericellular connective tissue and extracellular matrix background appears to be nonspecific.


Intact
Castrate
E2-treated Castrate
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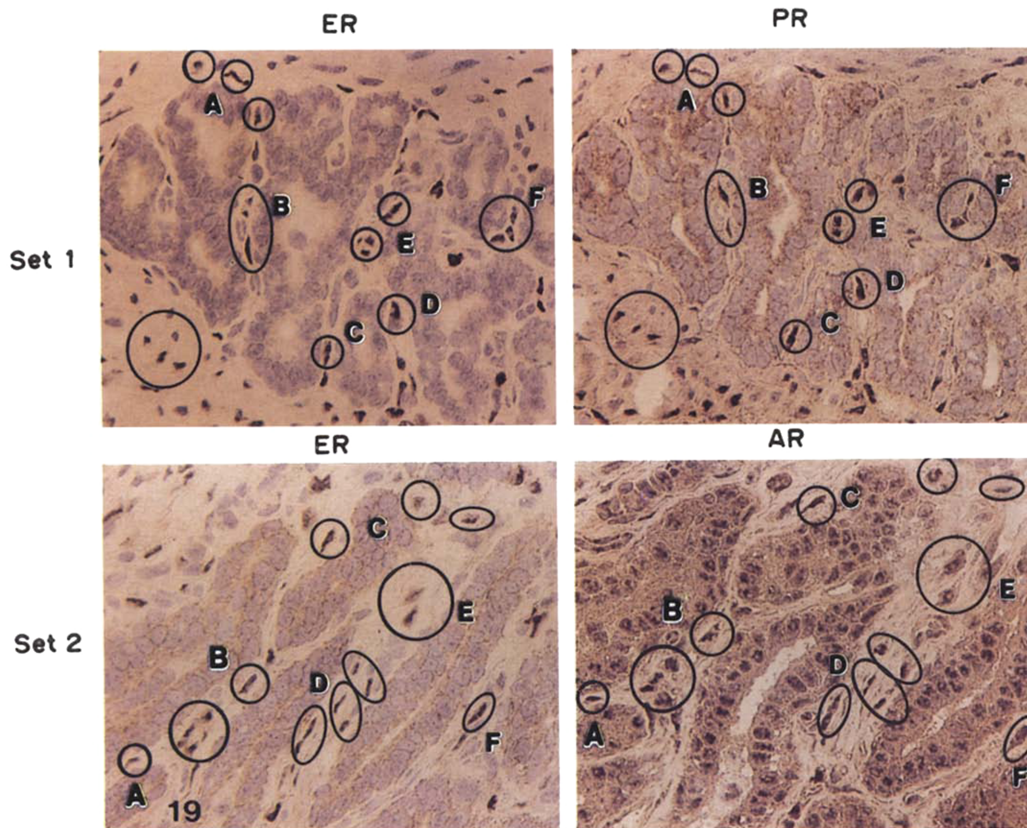

Fig. 19. Serial frozen sections, 1.5 μm thick, of seminal vesicles from E_2-treated castrates. Pairs of sections were stained for either ER and PR or ER and AR. In set 1, one section was stained for ER and the other for PR. In Set 2, one section was stained for ER and the other for AR. In each set, landmarks identifiable in both sections are labeled A–F. Matching sections of individual nuclei that contain both receptors are circled. Other matched sections of nuclei that contain both receptors can be observed. In set 1, it is apparent by inspection that many stromal cells contain ER and PR. In set 2, it is apparent that many stromal cells contain both ER and AR. In the epithelium, none of the AR-positive cells contain ER.

E_2-treatment of castrates not only caused stromal cell hypertrophy (Fig. 4), but also elevated the number of AR-positive stromal cells (Fig. 16, Table 1) up to the level seen in intact adult males, and significantly above the number in either the untreated castrates ($P < 0.05$) or the immature males (t-test, $P < 0.02$, Table 1). DHT treatment of a castrate also elevated the percentage of AR-positive stromal cells to the level characteristic of intact adult males (Table 1).

In pairs of very thin (1.5 μm) serial sections from E_2-treated castrate animals, where one section was stained for ER and the other for PR, it was evident that the majority of stromal nuclei contained both ER and PR (Fig. 19, Set 1). Similarly, where one member of a pair of sections from the same animal was stained for ER and the other for AR, large numbers of stromal nuclei contained both ER and AR while none of the AR-positive epithelial cells contained ER (Fig. 19, Set 2).

DISCUSSION

We have used monoclonal antibodies prepared against ER, PR and AR to document the presence in macaque seminal vesicle of these 3 classes of steroid hormone receptors. Immunocytochemical analysis revealed that specific receptor staining appeared to be exclusively nuclear. Estrogen receptors and PR were present only in the fibroblasts and smooth muscle cells of the stroma, while AR was present in the stroma, smooth muscle and epithelium. Colocalization studies on frozen serial sections indicated that ER, PR and AR could all occur in the same stromal cells.

Before puberty, when serum testosterone was low, ER levels in the macaque seminal vesicle were high, and the number of ER-positive stromal cells was elevated. In adult males, when serum testosterone was high, the ER levels and the number of ER-positive stromal cells were significantly reduced. Castration of adults resulted in an increase in ER levels and an increase in the number of ER-positive stromal cells to values that did not differ from the levels that had been present before puberty. Also, DHT-treatment of a castrate male decreased the percentage of ER-positive stromal cells and lowered the quantity of ER to values similar to those found in intact adult males. These findings are consistent with the hypothesis that in the intact macaque seminal vesicle, androgens suppress ER, a finding which we have previously reported for the prostate [2]. Our detection of ER only in stroma confirms autoradiographic studies on castrated baboons [23] rhesus macaques [24] and mice [25] which showed that $[^3H]E_2$ accumulated more in the interstitial cells than in the epithelial cells of the seminal vesicle. Biochemical studies on the mouse [26] and guinea-pig [27] seminal vesicle also found ER primarily in the fibromuscular stroma, while AR were concentrated in the glandular epithelium.

Progesterone receptor induction is often a specific indicator of estrogen action. Estrogen treatment induced PR in about the same percentage of cells as contained ER, and the serial sections indicated that ER and PR occurred in the same cells. Consequently we have concluded that E_2-treatment induced PR in those cells that contained ER. We drew a similar conclusion from similar findings in the prostate [2]. Whether treatment with exogenous progestin could activate the PR and evoke any cellular response may depend on dose and differential tissue sensitivities. In older work, Zuckerman and Sandys [10] found that simultaneous treatment with progesterone inhibited the estrone-induced changes in prostates of immature rhesus macaques, but the seminal vesicles of the same animals did not exhibit any P effects. We have not yet treated any animals with progestins.

In the seminal vesicles of intact adult males, the stroma occupies a much smaller volume of the tissue than the epithelium. Whether peripheral estrogens or local products of aromatization could interact with ER to modulate the structure and function of the stroma in intact, adult macaques is unknown. What is known is that when immature rhesus monkeys are injected with either androgens alone, estrogens alone, or a combination of the two, the combination produces effects intermediate between those seen with either steroid alone [10]. It seems quite likely that in cases of idiopathic hormonal imbalance, excess estrogens would result in stromal hypertrophy in the seminal vesicle. The effect such hypertrophy would have on seminal vesicle function deserves further study.

Our ICC studies suggest that AR in stromal cells were more heavily androgen dependent than AR in epithelial cells. For example, AR were detectable in the epithelium in all hormonal states whereas there were fewer

AR-positive stromal cells in immature and castrate animals than in adult, intact males, and DHT-treatment of a castrated male prevented the decline in stromal AR. Interestingly, E_2-treatment of castrated animals prevented the decline in the number of AR-positive stromal cells that normally followed castration. Serial sections showed that ER and AR could be present in the same stromal cells, and this suggests that the upregulatory effects of estrogen on stromal AR, the suppressive effects of androgens on stromal ER and the upregulatory effects of androgen on stromal AR are mediated directly at the receptor level within stromal cells. Although E_2-treatment of castrated animals had clear effects on stromal cells, little effect was observed in the epithelium, presumably because epithelial cells lack ER.

In summary, our findings on receptor regulation and localization in the rhesus monkey seminal vesicle suggest that ER and PR only occur in the stroma and smooth muscle and that ER is normally suppressed by androgens, just as in the prostate. Any direct, receptor-mediated effects of estrogens and progestins on the seminal vesicle would therefore be restricted to the stroma. The most dramatic histological effect of androgens is the maintenance of a hypertrophied, secretory epithelium, presumably mediated by the AR in the epithelial cells, but the presence of AR in stromal and smooth muscle cells indicates that androgens can have direct effects on these cells as well. One of the novel findings of this work, which grew out of the availability of new monoclonal anti-AR antibodies for immunocytochemical research, was that the stromal cell population was more dependent on androgens than the epithelial cell population for maintenance of normal numbers of AR-positive cells. Further studies of the significance of these differences in AR regulation in epithelial versus stromal cells are warranted.

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