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Effect of Sex Hormones on the Tissue Localization of Nuclear Estrogen Receptor Positively Stained Cells in the Seminal Vesicle of Immature Castrated Rats

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Yuasa, H., Ono, Y., Fukabori, Y., Ohma, C., Suzuki, K. and Yamanaka, H. *Effect of Sex Hormones on the Tissue Localization of Nuclear Estrogen Receptor Positivey Stained Cells in the Seminal Vesicle of Immature Castrated Rats.* Tohoku J. Exp. Med., 1997, **181** (2), 297-309 —— We studied the changes in the tissue localization of nuclear estrogen receptor (ER) positively stained cells in the seminal vesicle of immature castrated rats under various environmental conditions of sex hormones by immunohistochemical methods. In castrated rats of 6 weeks of age, the percentage of nuclear ER positively stained cells showed remarkable increase in the periglandular stroma region, but not in the epithelium and the peripheral stroma region. Estrogen administration to castrated rats dramatically increased the percentage of nuclear ER positively stained cells in both the epithelium and the peripheral stroma region, whereas cessation of estrogen treatment caused a significant percentile decrease. These results suggest that the nuclear ER expression in both the epithelial cells and the peripheral stromal cells seems to respond to estrogen. The concomitant treatment of estradiol-17β (E2) with 5α-dihydrotestosterone (DHT) completely inhibited these E2 mediated ER expression in the epithelium and the stroma. This result suggests that ER works only when E2 is given in the absence of DHT in the seminal vesicle of immature castrated rats.——— seminal vesicle; estrogen receptor; estradiol; dihydrotestosterone

Androgen and estrogen are known to modulate the seminal vesicle proliferation and differentiation. The seminal vesicle consists of the epithelium and the stroma. The epithelium is sustained with androgen and the stroma is a potential target for both androgen and estrogen (Mariotti et al. 1981; Thornton et al. 1984; Neubauer et al. 1989). We have already reported that estrogen evoked the increase of collagen and smooth muscle in the stroma of the seminal vesicle of immature castrated rats (Yuasa et al. 1993). Recently, we have shown that the

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nuclear ER protein levels in the seminal vesicle increased after estrogen administration to male immature castrated rats (Yuasa et al. 1996). The stroma of rat seminal vesicle consists of two regions, that is, the periglandular stroma region and the peripheral stroma region. The former is collagen rich and the latter is smooth muscle rich (Ono et al. 1995). In the present study we examined the changes in the tissue localization of nuclear ER positively stained cells in the seminal vesicle of immature castrated rats under various environmental conditions of sex hormones by immunohistochemical methods.

Materials and Methods

Animals and chemicals

Male Wistar rats were obtained from Imai Animal Co. (Kodama, Saitama) and were housed in cages with ad libitum food and water access in a temperature (22±3°C) and light (12 hr of light, 12 hr of darkness)-controlled room. Castration was performed under ether anesthesia at 3 weeks of age. Estradiol-17β (E2) and 5α-dihydrotestosterone (DHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Monoclonal mouse anti-human estrogen receptor antibody (DAKO-ER, 1D5) was purchased from DAKO Japan Co., Tokyo. LSAB 2 Kit, HRP, Rat was purchased from DAKO Co. (Carpinteria, CA, USA).

Immunohistochemical staining of estrogen receptor

Tissue was fixed for 6 hr in buffered 20% formalin. After the tissue sections were deparaffinized, they were autoclaved at 121°C for 5 min under 2 atmospheric pressure in a polymethylpentene staining jar (Kartell, Milano, Italy) filled with 0.01 M citrate buffer, pH 6.0 to completely immerse the sections. The sections were then placed in methanol containing 0.3% hydrogen peroxide for 15 min. After rinsing with 0.01 M phosphate buffered saline, they were incubated overnight at 4°C with ×50 anti-ER antibody. The primary antibody was reacted with the biotinylated anti-mouse immunoglobulin for 10 min at room temperature and biotin was detected with peroxidase-conjugated streptavidin molecules, using diaminobenzidine tetrachloride as a chromogen. The sections were counterstained with hematoxylin for 20 sec. As a final step, the sections were dehydrated gradually with ethanol, cleared with xylene and covered with Marinol (Muto Chemical, Tokyo). For negative control staining, the sections were incubated with mouse IgG, negative control reagent (DAKO), in stead of with the primary antiboby, and for positive control staining, rat uterus was incubated with ×50 anti-ER antibody. The number of nuclear ER positively stained cells per 100 cells in the epithelium, the periglandular stroma region and the peripheral stroma region, were counted in a blind fashion, using a light microscope of 1,000 original magnification. Statistical analysis was carried out by using the Statview 4.5 program (Abacus Concept, Berkeley, CA, USA). Differences among treatment groups were determined by using the Mann-Whitney U-test.

Results

Effect of castration and E2 administration on the tissue localization of nuclear ER positively stained cells in the seminal vesicle

Immature rats of 3 weeks of age were castrated and left untreated for 3 weeks. From 6 weeks of age, a daily dose of 5 μg of E2 was administered for 3 weeks and then observed afterwards for 2 weeks without E2 administration. The effects of castration and E2 on the tissue localization of nuclear ER positively stained cells in the seminal vesicle of immature castrated rats are summarized in Table 1. In intact rats of 3 weeks of age, no ER positively stained nuclei were seen in the epithelium, the periglandular stroma region or the peripheral stroma region. In intact rats of 6 weeks of age, no ER positively stained nuclei were seen in the epithelium or the peripheral stroma region, and a few nuclei in the periglandular stroma region were positively stained (Fig. 1-a, b). In castrated rats of 6 weeks of age, the percentage of ER positively stained nuclei in the periglandular stroma region showed a remarkable increase to 52.89%, compared with that of intact rats of 6 weeks of age ($p<0.001$), whereas no ER positively stained nuclei were seen

Table 1. *The changes in percentage of nuclear ER positively stained cells in the epithelium, the periglandular stroma region and the peripheral stroma region*

	Age at autopsy (weeks)	Epithelium (%)	Stroma (%)	
			Periglandular region	Peripheral region
Intact	3	0 (10)	0 (10)	0 (10)
Intact	6	0 (10)	0.15±0.34 (10)	0 (10)
Castrated, 3W	6	0 (10)	52.89±6.38 (18)	0 (7)
Castrated, 6W	9	0 (10)	50.20±4.21 (10)	0 (5)
Castrated, 3W + E2, 1W	7	29.61±19.29 (41)	36.41±9.37 (22)	24.29±6.76 (17)
Castrated, 3W + E2, 3W	9	71.71±10.43 (35)	37.50±9.21 (40)	68.00±7.60 (14)
Castrated, 3W + E2, 3W and OFF, 2W	11	2.0±4.0 (29)	43.91±10.13 (23)	12.8±12.64 (12)
Castrated, 3W + DHT, 3W	9	0 (10)	0 (10)	0 (10)
Castrated, 3W + DHT+E2, 3W	9	0 (10)	0 (10)	0 (10)

All values are expressed as mean±S.D., and the numbers in parentheses indicate the number of individual observations. The percentage of nuclear ER positively stained cells was evaluated in a blind fashion, using a light microscope (×1,000).

in the epithelium (Fig. 2-a) or the peripheral stroma region (Fig. 2-b). At one week after E2 administration in castrated rats, 29.61% of nuclei in the epithelium became positively stained (Fig. 3-a), as well as a significant increase in the peripheral stroma region to 24.29% ($p<0.001$) (Fig. 3-b), whereas the percentage of ER positively stained nuclei in the periglandular stroma region significantly decreased from 52.89% to 36.41% ($p<0.001$). At three weeks after E2 administration, the percentage of ER positively stained nuclei in both the epithelium and the peripheral stroma region further increased to 71.71% and 68.00%, respectively (Fig. 4-a, 4-b), whereas the percentage of ER positively stained nuclei in the periglandular stroma region did not change, compared with that at one week after E2 administration (36.41% vs. 37.50%). At two weeks after the cessation of E2 administration, the percentage of ER positively stained nuclei both in the epithelium and the peripheral stroma region dramatically decreased from 71.71% to 2.00% and from 68.00% to 12.08% ($p<0.001$), respectively, whereas the percentage of ER positively stained nuclei in the periglandular stroma region slightly increased (37.50% vs. 43.51%, $p<0.05$).

Effect of DHT or DHT plus E2 administration on the tissue localization of nuclear ER positively stained cells in the seminal vesicle

In order to invesigate the effect of DHT or DHT plus E2 administration on the tissue localization of nuclear ER positively stained cells in the seminal vesicle, a daily dose of 500 μg DHT or 500 μg DHT plus 5 μg E2 were subcutaneously administered to castrated rats of 6 weeks of age for 3 weeks. The effect of DHT or DHT plus E2 on the tissue localization of nuclear ER positively stained cells in the seminal vesicle are summarized in Table 1. At three weeks after DHT administration, no ER positively stained nuclei were seen at all in the epithelium, the periglandular stroma region or the peripheral stroma region. At three weeks after DHT plus E2 administration, no ER positively stained nuclei were detected at all in the epithelium,the periglandular stroma region or the peripheral stroma region (Fig. 5-a, 5-b).

Discussion

The occurrence of estrogenic effects in the female estrogen-target organs have been thought to be mediated by intracellular estrogen receptor (King and Mainwaring 1974; Gorski and Gannon 1976). However, the physiological role of ER in the male reproductive organs has not yet been clarified precisely. Recently, the immunohistochemical methods employing a monoclonal antibody to ER have provided a technique superior to steroid autoradiography (Korach et al. 1988; Yamashita and Korach 1989). Using this technique, the existence of ER in the male reproductive organs such as epididymis, prostate and seminal vesicle have been reported (Iguchi et al. 1991; Sato et al. 1994). In our previous study, the immunohistochemical analysis demonstrated that estrogen treatment increased

ER in the seminal vesicle of immature castrated rats (Yuasa et al. 1996). In this study, we investigated the changes in the tissue localization of nuclear ER positively stained cells in the seminal vesicle of immature castrated rats under various environmental conditions of sex hormones and showed that castration increased the tissue concentration of nuclear ER positively stained cells only in the periglandular stroma region and DHT treatment erased completely ER staining. On the other hand, estrogen treatment dramatically increased the tissue concentration of nuclear ER positively stained cells in both the epithelium and the peripheral stroma region and the cessation of estrogen treatment caused a significant decrease. These results suggested that the physiological role of nuclear ER expression in the periglandular stromal cells might be different from that in the epithelial cells and the peripheral stromal cells. In the previous report, we demonstrated that in the seminal vesicle of immature castrated rats the long-term treatment with estrogen evoked partial metaplastic changes in the glandular epithelial cells (Yuasa et al. 1993). In this study, we had the impression that the epithelial cells with metaplastic changes appear to have a higher positive rate of nuclear ER. In this regard, it is of interest to recall the earlier findings that perinatal estrogen exposure causes hyperplastic and metaplasic changes in the epithelium of seminal vesicle, epididymis and ventral prostate in rats and mice (Arai 1968; McLachlan et al. 1975; Arai et al. 1983). In the previous studies, we reported that estrogen treatment evoked the proliferation of both the periglandular stroma and the peripheral stroma in the seminal vesicle of immature castrated rats (Yuasa et al. 1993; Ono et al. 1995). The periglandular stroma region is collagen rich and the peripheral stroma region is smooth muscle rich. ER expression in the stromal cells might be connected with the proliferation of collagen and smooth muscle. In this study, we showed that the concomitant treatment of E2 with DHT completely inhibited the estrogen mediated ER expression in both the epithelium and the stroma. This result suggests that ER works only when E2 is given in the absence of DHT in the seminal vesicle of immature castrated rats. Recently, Kesteren and colleagues (1996) studied the effects of estrogen only on prostate tissue in aging male transsexuals who had undergone orchiectomy and found that few androgen receptors were detectable but there were ample estrogen receptors in the epithelium and the stroma. It is very interesting that ER expression in the seminal vesicle of estrogen-treated immature castrated rats resemble that in the prostate of human castrated transsexuals.

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References

1) Arai, Y. (1968) Metaplasia in male rat reproductive accessory glands induced by neonatal estrogen treatment. *Experimentia*, **24**, 180-181.
2) Arai, Y., Mori, T., Suzuki, Y. & Bern, H.A. (1983) Long-term effects of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. Int. *Rev. Cytol.*, **84**, 235-268.
3) Gorski, J. & Gannon, F. (1976) Current models of steroid hormone action: A critique. *Annu. Rev. Physiol.*, **38**, 425-450.
4) Iguchi, T., Uesugi, Y., Sato, T., Ohta, Y. & Takasugi, N. (1991) Developmental pattern of estrogen receptor expression in male mouse genitalorgans. *Mol. Androl.*, **3**, 109-120.
5) Kesteren, P., Meinhardt, W., Valk, P., Geldof, A., Megens, J. & Gooren, L. (1996) Effects of estrogens only on the prostates of aging men. *J. Urol.*, **156**, 1349-1353.
6) King, R.J.B. & Mainwaring, W.I.P. (1974) Oestrogens. In: *Steroid-cell Interactions*, Butterworths, London, pp. 190-255.
7) Korach, K.S., Horigome, T., Tomooka, Y., Yamashita, S., Newbold, R.R. & McLachlan, J.A. (1988) Immunodetection of estrogen receptor in epithelial and stromal tissues of neonatal mouse tissue. *Proc. Natl. Acad. Sci. USA*, **85**, 3334-3337.
8) Mariotti, A., Thornton, M. & Mawhinney, M. (1981) Actions of androgen and estrogen on collagen levels in male accessory sex organs. *Eedocrinology*, **109**, 837-845.
9) McLachlan, J.A., Newbold, R.R. & Bullock, B. (1975) Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. *Science*, **190**, 991-992.
10) Neubauer, B.L., Biser, P., Jones, C.D., Mariotti, A., Hoover, D.M., Thornton, T., Thornton, M.O. & Goode, R.L. (1989) Antagonism of androgen and estrogen effects in guinea pig seminal vesicle epithelium and fibromuscular stroma by keoxifene. *Prostate*, **15**, 273-286.
11) Ono, Y., Yuasa, H., Ohma, C., Mashimo, T., Suzuki, K. & Yamanaka, H. (1995) Eosinophils infiltration in the rat seminal vesicle associated with estradiol-17β-related stromal proliferation. *Tohoku J. Exp. Med.*, **175**, 163-169.
12) Sato, T., Chiba, A., Hayashi, S., Okamura, H., Ohta, Y., Takasugi, N. & Iguchi, T. (1994) Induction of estrogen receptor and cell division in genital tracts of male mice by neonatal exposure to diethylstilbestrol. *Reprod. Toxicol.*, **8**, 145-153.
13) Thornton, M.O., Frederickson, R., Mata, L. & Mawhinney, M. (1984) Preliminary studies on the relationship between collagen and the growth of male accessory sex organ epithelial cells. In: *New Approaches to Study of Benign Prostatic Hyperplasia*, edited by F.A. Kimball, A.E. Buhl & D.B. Carter, Alan R. Liss. Inc., New York, pp. 143-158.
14) Yamashita, S. & Korach, K.S. (1989) A modified immunohistochemical procedure for the detection of estrogen receptor in mouse tissues. *Histochemistry*, **90**, 325-330.
15) Yuasa, H., Fukabori, Y., Mashimo, T., Ohma, C., Suzuki, T., Yamanaka, H. & Suzuki, K. (1993) The characteristics of hormone responsiveness of glandular epithelium and stroma in male accessory sex organs. *Acta Urol. Jpn.*, **39**, 631-637.
16) Yuasa, H., Ono, Y., Ohma, C., Shimizu, N., Yamanaka, H., Uchida, T., Mizunuma, H., Aoki, K. & Suzuki, K. (1996) The estrogen-induced changes of estrogen receptor in seminal vesicle of immature castrated rat. *Acta Urol. Jpn.*, **42**, 33-37.

Illustrations follow

Fig. 1. ER immunostaining in the epithelium, the periglandular stroma region and the peripheral stroma region in intact rats.
a: In intact rats of 6 weeks of age, no ER positively stained nuclei were seen in the epithelium (EP) and a few nuclei in the periglandular stroma region (PGS) (arrow) were positively stained. ABC stain, ×1,000.
b: In intact rats of 6 weeks of age, no ER positively stained nuclei were seen in the peripheral stroma region (PPS). ABC stain, ×1,000.

Fig. 2. ER immunostaining in the epithelium, the periglandular stroma region and the peripheral stroma region in immature castrated rats.
a: In immature castrated rats of 6 weeks of age, the percentage of ER positively stained nuclei in PGS (arrow) showed a remarkable increase to 52.89%. ABC stain, ×1,000.
b: In immature castrated rats of 6 weeks of age, no ER positively stained nuclei were seen in PPS. ABC stain, ×1,000.

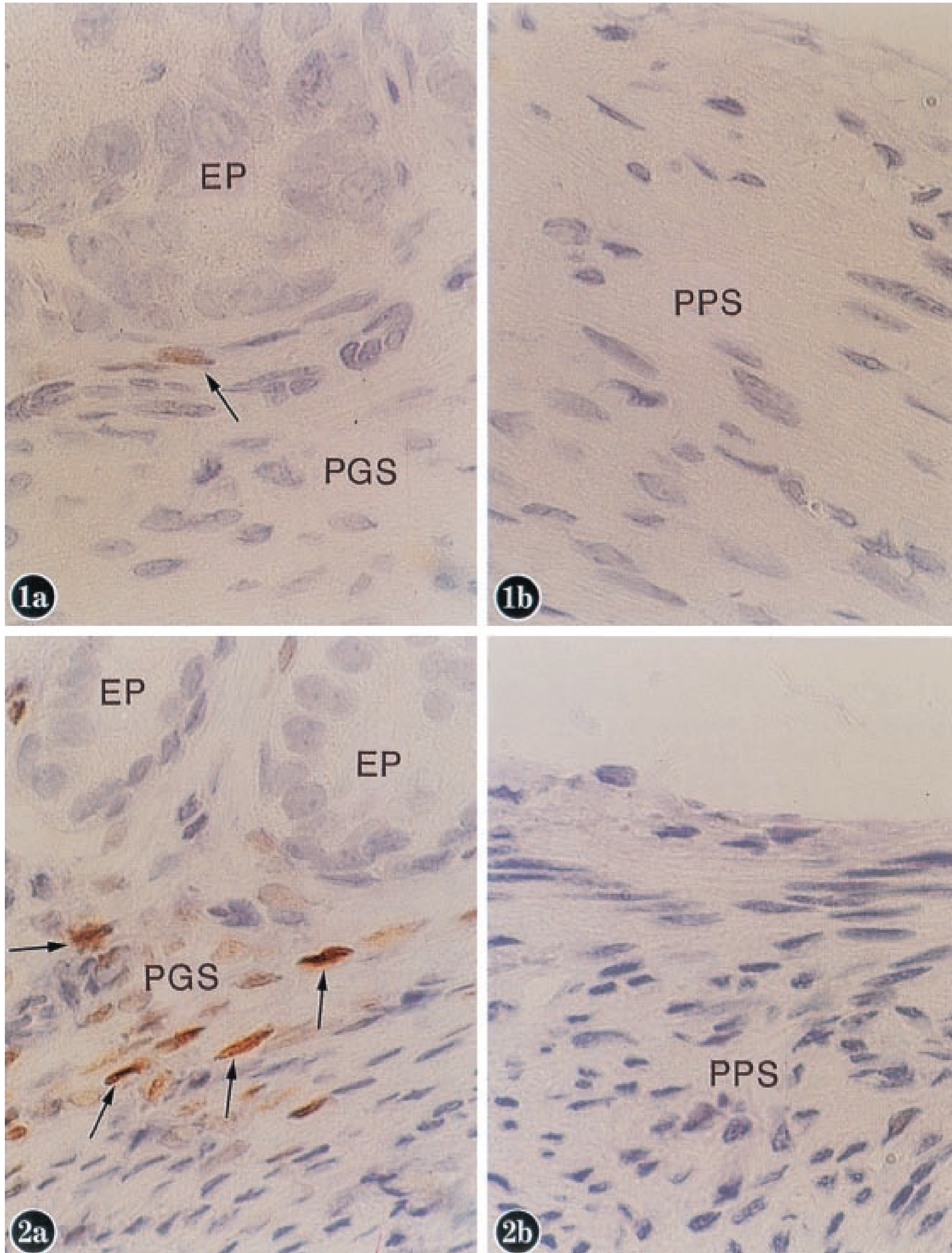

EP
PGS
1a
PPS
1b
EP
EP
PGS
2a
PPS
2b

Fig. 3. ER immunostaining in the epithelium, the periglandular stroma region and the peripheral stroma region at one week after E2 administration to immature castrated rats.
a: At one week after E2 administration to castrated rats, 29.61% of nuclei in EP (arrow) became positively stained. ABC stain, ×1,000.
b: At one week after E2 administration to castrated rats, the percentage of ER positively stained nuclei in PPS (arrow) significantly increased, compared with that of castrated rats. ABC stain, ×1,000.

Fig. 4. ER immunostaining in the epithelium, the periglandular stroma region and the peripheral stroma region at three weeks after E2 administration to immature castrated rats.
a: At three weeks after E2 administration to immature castrated rats, the percentage of ER positively stained nuclei in EP (arrow) further increased to 71.71%. ABC stain, ×1,000.
b: At three weeks after E2 administration to immature castrated rats, the percentage of ER positively stained nuclei in PPS (arrow) further increased to 68.00%. ABC stain, ×1,000.

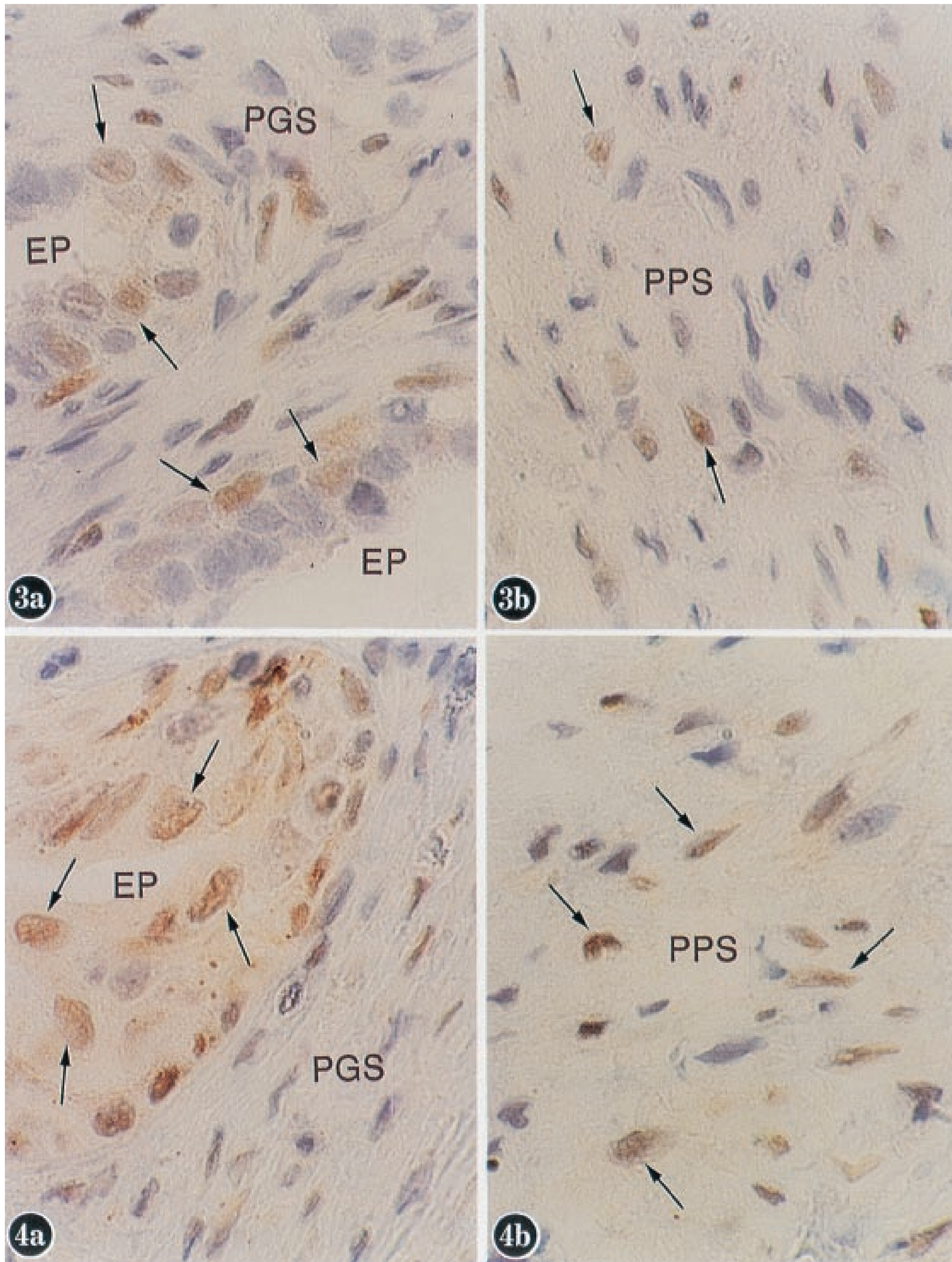
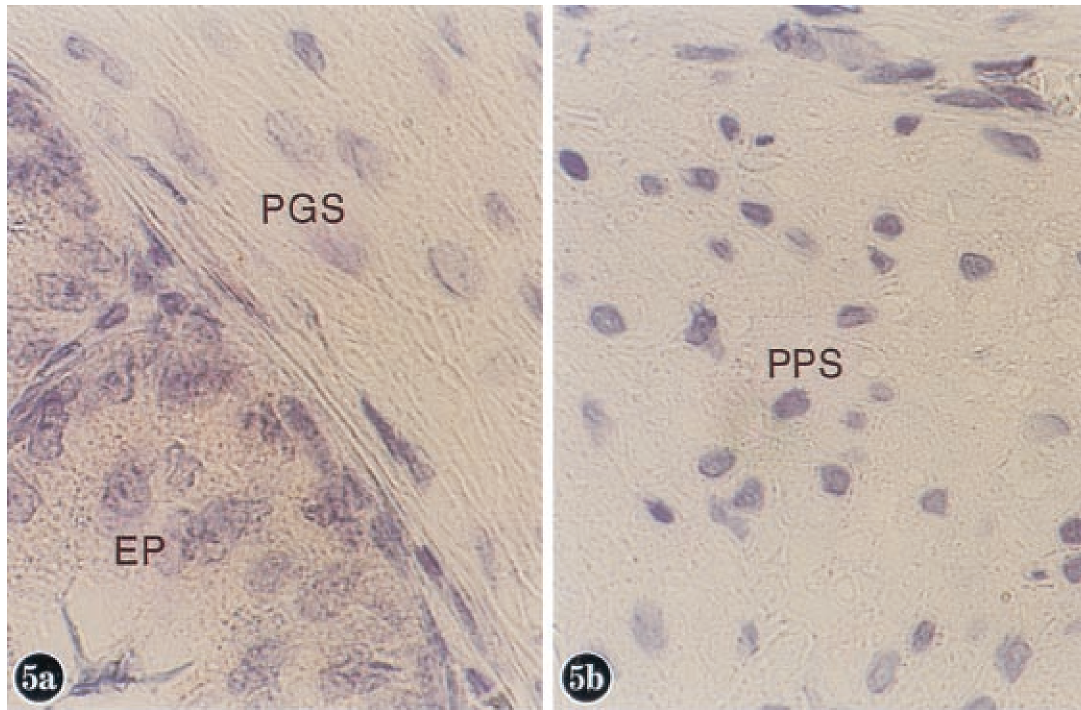

PGS
EP
EP
3a
PPS
3b
EP
PGS
4a
PPS
4b

Fig. 5. ER immunostaining in the epithelium, the periglandular stroma region and the peripheral stroma region at three weeks after DHT plus E2 administration to immature castrated rats.
a: At three weeks after DHT plus E2 administration to immature castrated rats, no ER positively stained nuclei were seen at all in EP and PGS.
ABC stain, $\times 1,000$.
b: At three weeks after DHT plus E2 administration to immature castrated rats, no ER positively stained nuclei were seen in PPS.
ABC stain, $\times 1,000$.


PGS
EP
5a
PPS
5b