Regulation of Estrogen Receptor α and β Expression by Testosterone in the Rat Prostate Gland

KOSUKE ASANO*, SATOSHI MARUYAMA*, TSUGURO USUI* AND NARIKI FUJIMOTO

Department of Developmental Biology, Research Institute for Radiation Biology and Medicine (RIRBM), Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
*Department of Urology, School of Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Abstract. Although ERβ is known to be expressed at high levels in the rat prostate gland, its regulation is not well understood. Here we examined ER mRNA expression and the effects of testosterone administration in male rats at 1, 4 and 9 weeks of age who were castrated and/or treated with testosterone for a week, and then sacrificed. ERα was the major type of ER expressed in 2 week-old animals while dominant expression of ERβ mRNA was apparent in older age groups. Interestingly while ERβ expression was diminished and ERα mRNA increased in the castrated group, testosterone administration reversed this effect. A time-course study indicated that induction of ERβ mRNA increased within 9 hr and ERα decreased in 2 days after an injection (i.p.) of testosterone. Our results suggested that 1) testosterone up-regulates ER β mRNA expression while ER α is down-regulated; and that 2) great changes in ERα and β expression in the prostate gland during development from the newborn to adult may be due to the influence of testosterone.

Key words: Estrogen receptor α and β, Testosterone, Rat prostate, Competitive RT-PCR


IT has been demonstrated that appreciable numbers of estrogen binding sites are present in rat prostate tissue, although their significance is not clearly understood [1–3]. In the human prostate tissue, despite conflicting early results, immunohistochemical studies as well as radioligand-binding assays have also demonstrated the presence of ER, albeit at low concentrations [4–6]. While some involvement in tumorigenesis might be expected, studies of ER levels in prostate cancer have shown no correlation with pathologic features [7–9].

Interest in ER and its roles in the prostate gland was recently revived by the cloning and characterization of a second ER, ERβ [10, 11]. Subsequent studies revealed that ERα is localized in the stromal tissue and ERβ is present in epithelial cells in both primates and rodents [12–14]. Furthermore, in the rat prostate, expression of ER β mRNA is developmentally regulated and a significant increase takes place during the initial stages of epithelial cytodifferentiation. It is assumed that androgen plays the major role in this developmental change since castration has been known to decrease ER expression in the gland. However, the effects of testosterone on ERα and β expression have not been quantitatively addressed in the prostate gland [15]. In the present study, ERα and ERβ mRNA levels were therefore measured with a competitive RT-PCR technique in tissue of rats castrated and treated with testosterone (T).

Materials and Methods

Hormones

17β-estradiol and diethylstilbestrol (DES) were purchased from Sigma Chemicals, St. Louis, MO, U.S.A., and testosterone (T) was from Wako Junyaku KK,
Osaka, Japan.

Animal experimentation

Male F344 rats were purchased from Charles River Co., Kanagawa, Japan, and maintained under constant condition with free access to basal diet and tap water. All experiments were conducted under the guidelines of the Guide for the Care and Use of Laboratory Animals of Hiroshima University. Animals were sacrificed under anesthesia and ventral prostate tissues were dissected and frozen. Blood samples were collected from the abdominal artery and separated sera were stored at −20°C until assayed.

Experiment 1. One, 2, 4 and 9 week-old rats were maintained for a week and sacrificed to examine the prostate gland.

Experiment 2A. Four and 9 week-old animals were castrated and/or treated with 5 mg of T in a silicone tube subcutaneously for a week. For 2 week-old rats, 0.5 mg of T in silicone tubes was given instead of 5 mg for a week and 0.5 mg of DES was administered instead of surgical castration.

Experiment 2B. For the cytosolic ER binding assay of the prostate tissue, 9 week-old animals were castrated and treated with 5 mg of T for a week.

Experiment 3. Four week-old rats were castrated. One week later, 10 mg of T was administered i.p. They were sacrificed at hours 0, 3, 9, 24 and 48 after the injection.

PCR primers

RER-a1u and RER-a1d with the sequences 5'-TCTGACA ATCGAGCGCA G (473–493) and 5'-GT GCTTCAAC ATTCCTCCCT CTC (794–816) were employed for RERα detection (344 bp), and RER-b1u and RER-b1d with the sequences 5'-TTCTTTGGCAG CACCAGTAAC C (38–58) and 5'-TCCCTCTTTG CGTTTGGACT A (279–299) for RERβ (262 bp). For construction of an ER α competitor, RER-a2u, RER-a2d, RER-a1u (RER-a1u linked to RER-a2u) and RER-a12d (RER-a1d linked to RER-a2d) with the sequences 5'-GAGACTCTCC AGCAGACGCC AG (515–536), 5'-AAAGCCTTGC AGCCTTCAAC GG (605–626), 5'-AATCTGACA ATCGAGCGCA GGAGACTCTCC AGCAGACGCC GAG and 5'-GT GCTTCAAC ATTCCTCCCT CTCAAGGCT TG CAGCTTCC ACAGG were used. For the ERβ competitor, RER-b2d and RER-b12d with the sequences 5'-GAGTTCTGC ATAGAGAGGC G (152–172) and 5'-TCCCTCTTTG CGTTTGGACT AGAG GTTCTG CATAGAGAGC CG were applied.

Construction of competitor DNAs

A RER α plasmid was mixed with 20 pmol each of primers RER-a2u and RER-a2d in a total volume of 50 μL containing 1.25 U of Ex-Taq DNase polymerase (Takara Shuzo Co., Otsu, Japan), 0.2 mM dNTP, 10 mM Tris-HCl (pH 8.3), 2 mM MgCl2, and 50 mM KCl. A 112-bp DNA fragment was isolated with a GFX Gel Band Purification Kit (Amersham pharma biotech, Uppsala, Sweden). Using this fragment as a template, a further PCR amplification with primers RER-a12u and RER-a12d and gel purification were performed to obtain a 126-bp competitor DNA for ERα. The same procedures with RER-b1u and RER-b2d followed by amplification of RER-b1u and RER-b12d was performed to obtain the ERβ competitor (156 bp). The sequences of the resulting DNA fragments were confirmed with a capillary sequencer, ABI PRISM 310 (Applied Biosystems, Foster City, CA, U.S.A.).

Reverse transcription (RT) and competitive PCR

These assays were performed as described previously [16]. Briefly, total RNAs from tissues were prepared by a modified acid guanidium thiocyanate-phenol-chloroform extraction method and treated with RQ1 DNase (Promega, Madison, WI, USA). One μg of total RNA was reverse-transcribed with 100 U of MMLV-RT (Invitrogen Corp., Carlsbad, CA, USA) and 1.25 pmol of oligo-dT primers.

Sample cDNA (equivalent of 0.2 μg of total RNA) and competitor DNA at various concentrations (0–20 fg) were co-amplified by PCR with Ex-Taq using the RER-a1u and RER-a1d primer set. The amplification conditions were as described above in a final volume of 20 μL. Each PCR product was electrophoretically separated on a 1.5% agarose gel containing ethidium bromide at 0.2 μg/mL. The image was digitized with a video capturing device, PrintGraph (Atto Co., Tokyo, Japan) and intensities of the blots were quantified using Scion Image software (Scion Corp., Frederick, MD, U.S.A.). The log ratio of the blot intensities of sample cDNA over the competitor in each lane was plotted against amounts of the competi-
tor. Quantity of rER mRNAs was determined where the ratio was equal to 1.

**ER assay**

Cytosol fractions of prostate tissues were prepared in TEDMG buffer (10 mM Tris-HCL, 1 mM disodium EDTA, 1 mM dithiothreitol (DTT), 10 mM sodium molybdate and 10% (v/v) glycerol, pH 7.4) and incubated in 0.05–50 nM of [17β-2, 4, 6, 7-3H] - estradiol (NEN Life Science Products, Boston, MA, U.S.A.), with or without a 100-fold molar excess of unlabelled estradiol (10 nM), at 30°C for 40 min. Bound [3H]-estradiol was separated by the hydroxylapatite method and binding data were analyzed by Scatchard plotting.

**Serum T levels**

Serum T was measured with a RIA kit for rat serum E2, purchased from Immunotech Inc. (Marseille Cedex, France).

**Statistical analysis**

Statistical comparisons were made using the Student’s t-test.

**Results**

**Age dependent changes in ER α and ER β mRNA levels in rat prostate**

ERα mRNA was the major type of ER expressed in the 2 week-old rat prostate. Its expression was decreased at later time points and ERβ mRNA expression became apparent after 3 weeks, coming to predominant in 5 and 10 week-old rats (Fig. 1).

**Effects of DES and T administration on ER mRNA levels in the prostate gland in 3 week-old rats**

ERα mRNA in the prostate of 3 week-old rats, which was relatively high, was increased by DES and decreased by T (Fig. 2). ERβ, on the other hand, was decreased by DES treatment. There were no significant differences in ventral prostate weight among treatments.

**Effects of castration and T administration on ER mRNA levels in the rat prostate gland at 5 and 10 weeks old**

At ages of 5 and 10 weeks, the ERβ mRNA levels were decreased by castration and recovered on T administration (Fig. 2). Although the control ERα mRNA level was low, it was increased by castration at 5 weeks old. At 10 weeks old, however, castration did not alter the ERα mRNA level and T administration decreased it. The weight of the ventral prostate was decreased by castration and increased by T administration.

**Time dependent changes in ER mRNA levels in the prostate gland after T injection in 5 week old rats**

Changes in ER α and β mRNA levels in the ventral prostate in castrated rats after 10 mg T injection are summarized in Fig. 3. At time 0, higher ERα and lower ERβ mRNA levels were noted in the prostate gland as the result of castration. The level of the ERβ mes-
Fig. 2. Effects of castration and testosterone on ER mRNA levels (A, B), ventral prostate weight (C) and serum testosterone levels (D) in rats at different ages. F344 male rats were castrated (Cast) and treated with 5 mg of testosterone in a silicone tube subcutaneously (Cast+T) for a week. For 3 week-old rats, 0.5 mg of DES was administered (DES) and 0.5 mg of testosterone in a silicone tube was given (T). Intact animals were also examined (C). Bars indicate mean ± SEM (n = 6, each point). * and ** indicate significant difference from the control at p<0.05 and p<0.01, respectively.

Fig. 3. Time dependent changes in ER mRNA levels (A, B), ventral prostate weight (C) and serum T levels (D) in 5-week-old rats after a T injection. 10 mg of T was administered i.p. to castrated F344 rats. The levels in intact animals are also noted (C). Bars indicate mean ± SEM (n = 6, each point). * and ** indicate significant differences from the values at 0 hr at p<0.05 and p<0.01, respectively.
Table 1. Cytosolic ER levels in the prostate determined by competitive binding assay

<table>
<thead>
<tr>
<th>Prostate lobe</th>
<th>Treatment</th>
<th>Specific binding (fmol/mg prl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral</td>
<td>Control</td>
<td>15.9, 8.6</td>
</tr>
<tr>
<td></td>
<td>Castration</td>
<td>0.0</td>
</tr>
<tr>
<td>Dorsolateral</td>
<td>Control</td>
<td>24.6, 13.7</td>
</tr>
<tr>
<td></td>
<td>Castration</td>
<td>0.0</td>
</tr>
</tbody>
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The number of $^3$H-17β estradiol specific binding sites in prostate cytosol in 10 week-old F344 rats were determined. Each assay was conducted with combined tissues from two animals.

Sage began to increase 3 hr after the injection and reached a maximum at 24 hours. The level of ERα mRNA decreased gradually. The weight of the ventral prostate remained unchanged over 48 hours. The serum T level reached a peak at 3 hr after the injection.

*Number of specific estradiol binding sites in the prostate cytosol in normal and castrated rats*

Table 1 shows ER levels in the prostate gland determined by competitive binding assay. No specific estradiol binding sites were detected in the prostate cytosol in castrated animals, while significant numbers were apparent in the controls.

**Discussion**

Several studies have indicated that ERβ in the rat prostate is developmentally regulated, probably by androgen [12, 15, 17, 18]. The present results confirmed that testosterone controls the expression of ERα mRNA as well as ERβ in the rat prostate gland. More importantly, we showed that a significant amount of ERα mRNA to be expressed in younger rats when ERβ expression was still low.

It has been reported that ERβ is localized in prostatic epithelial cells, in contrast to the stromal localization noted for ERα. At birth, low levels of ERβ message are observed in the epithelial and mesenchymal cells followed by decreases as mesenchymal cells differentiate. Subsequent increase in ERβ begins around day 15 probably due to elevation in testosterone with maximal expression at day 90 when adult levels of the hormone are at their peak [17]. Our quantitative results for ERβ mRNA measurement confirmed this to be the case while ERα was found to be regulated in the opposite way. These development changes in ER expression might be considered indirect effects of morphological change in the glands, since epithelial differentiation occurs in a proximal to distal fashion during this period. However, our finding of relatively rapid induction of ERβ after an injection of testosterone suggested that the regulation of ER is closely linked to the androgen. In fact, the androgen receptor (AR) colocalizes with ERβ in the same tissue, i.e. strong expression is apparent in the epithelium and low levels in the stroma [19]. On the other hand, AR could be detected in the prostate of long-term castrated animals. Promoter regions of the ERα gene have been reported and well characterized [20, 21]. The human ERα gene is transcribed from three different promoters, while only two promoters are involved in the rat case. Although there seem to be no androgen responsive transcriptional motifs in the known promoter structures, investigations of ERβ promoters are still ongoing [22, 23].

It is interesting to note that competitive binding studies, measuring the number of binding sites for both ERα and ERβ, have clearly demonstrated androgen down-regulation of ER in humans and monkeys, whereas the ER level increases by androgen treatment in rats [2, 24, 25]. This might be explained by the opposite regulation of ERα and β expression rather than interspecies variation, since relative expression of ERα is high in the human prostate gland while the ERβ level is higher in the case of rat [16].

The roles of ERβ in the prostate gland are not clear, although the differential distribution patterns of both receptors in the tissue and the distinctive regulation of the transcripts, as we demonstrated here, indicate physiological roles. ERβ may function to restrain epithelial growth since the tissue in ERβ knockout mice contains multiple hyperplastic foci [26]. In a human cancer study, loss of ERβ expression was reported to be associated with progression from normal prostate epithelium to cancer [27].

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