Regulation of the β₂-adrenergic receptor and its mRNA in the rat ventral prostate by testosterone

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To investigate the regulation of β_2AR expression in rat ventral prostate the effects of castration and testosterone replacement on the β_2AR were studied by ligand binding and Northern blot analysis. Orchidectomy depressed β_2AR number by 50% within 4 days and testosterone administration to 4-day castrates produced a rapid and complete recovery of β_2AR number within 24 h. In contrast to receptor number, β_2AR mRNA levels did not change relative to total RNA following castration. However, during the testosterone replacement period β_2AR mRNA levels rose transiently, reaching a maximum (3.5-fold) between 8 and 12 h, and this increase in mRNA preceded the recovery in β_2AR number in the membrane. Regulation of β_2AR gene expression by testosterone in the ventral prostate is thus complex and probably involves both transcriptional and post-transcriptional components.

β_2-Adrenergic receptor; Androgen; Ventral prostate; mRNA

1. INTRODUCTION

Hormonal responsiveness of tissues can be regulated by several mechanisms, one of which is the dynamic regulation of the number of specific receptor sites on the cell surface. In the case of the β-adrenergic receptor (βAR), a prototypic G-protein coupled receptor, both homologous and heterologous hormones can affect receptor number [1].

The rat ventral prostate contains one of the highest densities of β_2AR compared to other tissues [2,3], and recent evidence implicates both neurotransmitters and androgens in the development and function of the prostate and other ac-

cessory sex organs [4,5]. In addition, prostatic adenylate cyclase and β_2AR number are known to be significantly altered by circulating androgens [2,6,7]; however, the mechanisms involved in this regulation of β_2AR by testosterone are unknown. The cloning of the genes and cDNAs for several adrenergic receptors [8–11] now permits an examination of the mechanisms involved in heterologous hormonal regulation of the members of this receptor family.

2. MATERIALS AND METHODS

Adult, male, Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were maintained on a 12 h light:dark cycle. Daily subcutaneous injections of 1.0 mg testosterone propionate (4 mg/kg body wt in 0.1 ml vegetable oil) were as previously described [12]. At the time points indicated in the figures and legends, ventral prostate tissue was quickly removed from animals killed by decapitation, trimmed free of excess fat, and either flash-frozen in liquid nitrogen, or minced finely on ice and used immediately. No differences in binding characteristics were observed between frozen and fresh tissue. Tissue was suspended in buffer (75 mM Tris, pH 7.5, 12.5 mM MgCl₂, 0.25 M sucrose containing 5 μg/ml soybean

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Abbreviations: βAR, β-adrenergic receptor; β_2AR, β_2-adrenergic receptor subtype; CYP, cyanopindolol; SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0

trypsin inhibitor, 5 μg/ml leupeptin and 15 μg/ml benzamidine) and homogenized in a motor-driven, glass-teflon homogenizer maintained on ice. The homogenate was filtered through two layers of cheesecloth and centrifuged at 40000 × g for 20 min. The crude membrane pellet was washed twice with buffer and resuspended.

Binding of the β-adrenergic ligand [^{125}I]cyanopindolol (CYP) was performed in homogenization buffer in the absence or presence of 1 μM alprenolol at 25°C for 90 min as previously described [13]. Competition binding assays to determine βAR subtype were performed with betaxolol (β_1-selective) and ICI 118,551 (β_2-selective). Binding parameters were estimated by nonlinear least squares curve fitting [14,15]. Protein concentrations were determined by the method of Bradford [16].

Total cellular RNA was isolated by the cesium chloride gradient method of Chirgwin [17]. RNA was denatured and electrophoresed through 1.2% agarose gels using the glyoxal procedure [18] and transferred to Biodyne (Pall Corp., Glen Cove, NY) membranes. Hybridizations with either the hamster β_2-adrenergic receptor [8] or chicken β-actin [19] cDNA probes were conducted as previously described [20]. These probes were labeled by nick translation with [α-^{32}P]dCTP (3–8 × 10^8 dpm/μg DNA). Following hybridization the filters were washed successively in 2 × SSC, 0.1% SDS at room temperature and 0.1 × SSC, 0.1% SDS at 55°C.

3. RESULTS AND DISCUSSION

Saturation binding studies with [^{125}I]CYP in ventral prostate membranes from intact animals indicated a βAR density of 436 ± 29 fmol/mg membrane protein and a K_d for CYP binding of 15 ± 4 pM which did not change following castration. Competition binding studies with the subtype selective antagonists betaxolol (β_1-selective) and ICI 118,551 (β_2-selective) classified the receptors in the ventral prostate as being of the β_2 subtype (not shown).

Following castration, there was a steady decline in the number of β_2AR in ventral prostate membranes to 50% of control (~200 fmol/mg protein) by 4 days (fig.1). Testosterone replacement promoted a rapid and complete recovery of prostatic β_2AR (fig.1). Within 24 h of androgen administration, the receptor density returned to the level of the intact animal. During the first 24 h period, when there were dramatic changes in receptor number, there was no discernable tissue growth based on tissue weight (not shown).

The decrease in prostatic β_2AR observed following castration was not reflected in the steady-state mRNA levels analyzed by Northern blot hybridization (fig.2). Four days after castration β_2AR

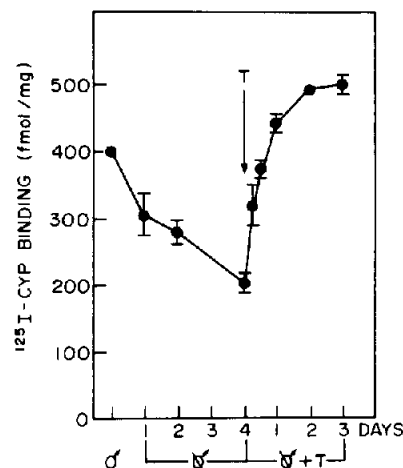Fig.1. Change in β-adrenergic receptor number in ventral prostate membranes following castration (♂̶) and testosterone replacement (♂̶ + T). Male CD rats (250–300 g) were castrated as described in section 2 and killed at the indicated times. On day 4 of ♂̶, daily injections of testosterone propionate (1.0 mg in 0.1 ml vegetable oil) were begun and animals were killed at the indicated time. 6–8 animals were used per point. Membrane preparations and [^{125}I]CYP binding were performed as described in section 2.

mRNA relative to total RNA was unchanged from levels in intact animals. In other experiments equivalent amounts of β_2AR mRNA were still detected on days 1, 2, 4 and 7 post-castration (not shown). Testosterone replacement in 4-day castrates produced a rapid but transient increase in β_2AR mRNA levels in the ventral prostate (fig.3A). Peak induction in β_2AR mRNA was found between 8 and 12 h. By comparison, actin mRNA levels appeared slightly elevated following castration, but they remained relatively constant following testosterone (fig.3B). Relative to actin expression maximum increases in β_2AR mRNA were estimated by densitometry to be 3.5-fold.

These data demonstrate that β_2AR levels in the rat ventral prostate are regulated by testosterone and that they display an immediate response to changes in androgen status. However, it was unexpected that the number of β_2AR in the ventral prostate was not a direct function of the level of β_2AR transcripts. Instead, when β_2ARs declined following castration, mRNA levels remained virtually unchanged. The process of receptor turnover may be sensitive to testosterone, such that following castration the rate of degradation of the
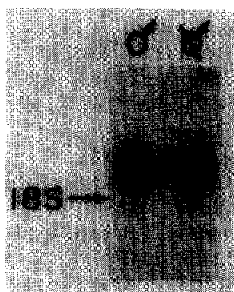
Fig.2. Northern blot analysis of β_2-adrenergic receptor mRNA levels in the ventral prostate of intact (σ) or 4-day castrated (σ) rats. 20 μg of total cellular RNA were electrophoresed and blotted as described in section 2 and hybridized to a hamster β_2-adrenergic receptor cDNA probe labeled with $[\alpha-^{32}P]dCTP$ by nick-translation. The filter was exposed to Kodak XRP film for 4 days at $-80°C$.

receptor was elevated. Alternatively, efficiency of translation of the β_2AR message could have been influenced by hormonal status, as shown for a number of other proteins [21–23]. At present we are unable to distinguish between these various possibilities due to the limitations of this animal model.

During the hormone replacement phase of these experiments, testosterone promoted a rapid and steady rise in prostatic β_2AR. Within 12 h, levels of β_2AR in testosterone-treated animals reached 80% of that in intact animals. At the same time there was an increase in β_2AR mRNA levels which peaked 8 to 12 h after initiation of hormone treatment. However, this enhancement was not maintained and it soon returned to the level of the untreated control. Similar findings of temporary transcriptional enhancement and mRNA accumulation have been reported for other hormonally-regulated genes in vivo [24–26]. In some of these cases, the transient nature of the rise in mRNA levels has been ascribed to increased translational efficiency of the newly synthesized message. In the case of the β_2AR in the ventral prostate, several events may be occurring. First, new mRNA may be synthesized in response to the androgen stimulus, and this might allow the cell to quickly recover the lost complement of receptors. Second, as a new steady-state level of β_2AR (which has been reported to have a relatively long half-life [27]) is reached, mRNA synthesis declines.

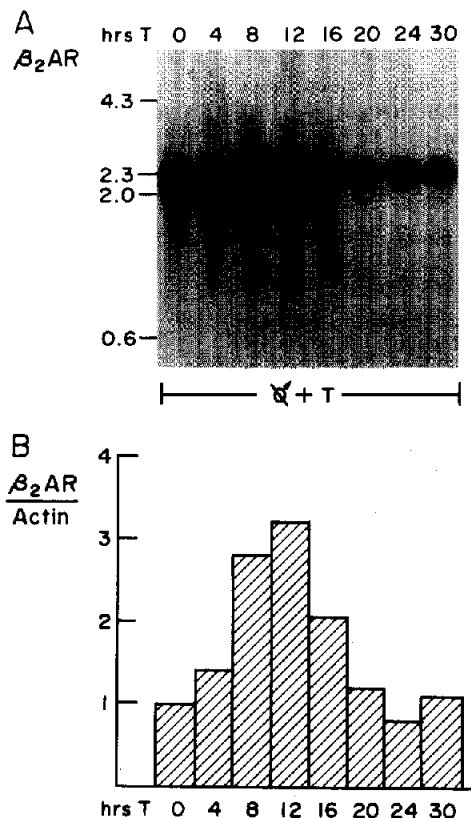Our results indicate that androgenic regulation

Fig.3. Northern blot analysis of mRNA levels for β_2-adrenergic receptor in rat ventral prostate after testosterone administration to 4-day castrates. Total cellular RNA (20 μg) was electrophoresed and blotted as described in section 2, and hybridized to a hamster β_2-adrenergic receptor cDNA probe labeled with $[\alpha-^{32}P]dCTP$ by nick-translation. Following exposure to Kodak XRP film, blots were stripped and reprobed with a ^{32}P-labeled actin cDNA probe. The fold increase in β_2-adrenergic receptor mRNA relative to actin mRNA was computed from densitometric tracings of the autoradiograms.

of β_2AR is complex, and that both transcriptional and post-transcriptional components are probably involved. The availability of gene probes for several adrenergic receptor subtypes and model systems to study their expression should further our understanding of the genetic regulation of this receptor family.

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