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α_1-Adrenoceptors in testosterone-induced prostatic hypertrophy

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Abstract

Modifications of rat prostatic α_1-adrenoceptors were investigated in testosterone-induced prostatic hypertrophy. [^{3}H]prazosin bound to a single class of binding sites with a dissociation constant of 57.9 ± 5.02 pM. The greater part of the binding capacity (24.6 ± 1.02 fmol/mg protein) was made up of chloroethylclonidine-resistant binding sites that showed high-affinity for oxymetazoline and 5-methyl-urapidil, and was identified as α_{1A}-adrenoceptors. The remaining chloroethylclonidine-sensitive binding sites that showed low-affinity for oxymetazoline and 5-methyl-urapidil were preferentially identified as α_{1B}-adrenoceptors. mRNA for the three α_1-adrenoceptors (α_{1a}, α_{1b} and α_{1d}) was detected. Testosterone administration produced a 23% decrease of α_1-adrenoceptor density, likely by an increase of prostatic glandular epithelium and a decrease in the relative proportion of smooth muscle, thus of α_1-adrenoceptor density. The steady state level of mRNAs for α_1-adrenoceptors was not modified by testosterone treatment. These results indicate that prostate α_1-adrenoceptors are not affected in the prostatic hypertrophy induced by testosterone. © 1998 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor; Prostate; (Rat); Testosterone; Prostatic hypertrophy, benign

1. Introduction

Benign prostatic hypertrophy results in an age-related and androgen-dependent enlargement of the prostate. The consecutive constriction of the urethra may lead to reduction in urine flow rate, bladder outlet obstruction and irritation symptoms. The two components of benign prostatic hypertrophy could be determined: a static component due to compression induced by the increased prostate volume, and a dynamic component related to contraction of urethra and prostatic smooth muscle (Caine, 1990). It is generally accepted that α_1-adrenoceptors primarily mediate the contractile response of the prostate (Caine et al., 1975; Hieble et al., 1985) and are responsible for about 50% of the prostatic urethra pressure in patients with benign prostatic hypertrophy (Furaya et al., 1982). α_1-Adrenoceptor antagonists are widely used as therapeutic agents for benign prostatic hypertrophy (George et al., 1995).

Three distinct subtypes of α_1-adrenoceptor have been cloned and characterized in various species: α_{1A} (Schwinn et al., 1990; Hirasawa et al., 1993; Laz et al., 1994; Perez et al., 1994), α_{1B} (Cotecchia et al., 1988; Voigt et al., 1990; Ramarao et al., 1992) and α_{1D}-adrenoceptors (Bruno et al., 1991; Lomasney et al., 1991; Perez et al., 1991). Unlike prazosin, which binds to the three α_1-adrenoceptor subtypes with the same affinity, 5-methyl-urapidil, (+)-niguldipine and oxymetazoline show higher affinity for α_{1A}-adrenoceptors. Spiperone is relatively selective for α_{1B}-adrenoceptors whilst BMY 7378 and (−)-norepinephrine recognize preferentially α_{1D}-adrenoceptors (Hieble et al., 1995a; Graham et al., 1996). Another tool to discriminate between α_1-adrenoceptor subtypes is their sensitivity to the alkylating agent, chloroethylclonidine. Chloroethylclonidine is an irreversible antagonist that inactivates only 20% of the α_{1A}-adrenoceptors whereas 80% of the α_{1B} and α_{1D}-adrenoceptors are inactivated (Laz et al., 1994).

α_{1A}-Adrenoceptors are predominant in the prostate (Hieble et al., 1995b). Evidence for an additional subtype showing low-affinity for prazosin and mediating some of norepinephrine-induced prostatic contraction in human i.e. α_{1L}-adrenoceptor, has been presented (Muramatsu et al., 1994; Ford et al., 1996; Testa et al., 1996). Determination of α_{1L}-adrenoceptor rests only on functional studies. The

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cDNA clone encoding the α_{1L}-adrenoceptor has not yet been identified and specific ligands for this receptor subtype are lacking.

In the present study, we examined whether rat prostate α_1-adrenoceptors are affected by testosterone-induced prostatic hypertrophy. This model was chosen because although the density of smooth muscle in the rat prostate is low as compared to other species, a similar density of α_1-adrenoceptors was found in rat, rabbit, dog and human prostate (Testa et al., 1993; Lepor et al., 1994). Furthermore, testosterone administration to rats provokes a prazosin-sensitive simulation of the urodynamic alterations observed in patients with benign prostatic hypertrophy (Maggi et al., 1989). The binding characteristics of prostatic α_1-adrenoceptors were investigated using [^{3}H]prazosin, chloroethylclonidine and α_1-adrenoceptor agonists and antagonists and compared with those of recombinant rat α_1-adrenoceptors (Laz et al., 1994). The mRNA content for the cloned α_1-adrenoceptor subtypes (α_{1a}, α_{1b} and α_{1d}) was evaluated in the rat prostate using reverse transcription combined with polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Induction of prostatic hypertrophy by testosterone administration

Male Sprague Dawley rats weighing 240–250 g (Charles River, France) were randomly distributed into two groups of 8–12 animals. Testosterone propionate (3 mg/kg) was dissolved in olive oil and administered according to the method described by Chen et al. (1988) and Maggi et al. (1989). Subcutaneous injections of testosterone were given for 15 d (daily injection for 5 d/week) at independent sites. Control rats received on the same schedule the vehicle alone. The rats were killed 72 h after the final injection. The prostates were separated from bladder and seminal vesicles, weighed and immediately placed in 50 mM Tris–HCl, pH 7.7 at 4°C for membrane preparation, or frozen at −80°C for RNA extraction.

2.2. α_1-Adrenoceptor binding assay

The prostates were dissected into small pieces and homogenized with a Polytron (set at 8) for 30 s. Homogenates were filtered through four layers of gauze and centrifuged at 52,000 × *g* for 10 min at 4°C. The pellets were resuspended and incubated for 30 min at 37°C with or without 10 μM chloroethylclonidine in 50 mM Tris–HCl, pH 7.7. After the incubation period, the membrane preparation was diluted with ice-cold buffer, and centrifuged at 52,000 × *g* for 10 min at 4°C. The pellets were resuspended in the same buffer for another centrifugation at 52,000 × *g* for 10 min at 4°C. The resulting pellets were stored at −80°C. An aliquot was taken for the assessment of protein content by Bradford's method (Bradford, 1976) with bovine serum albumin as standard.

Prostate membranes (250 μg protein) were incubated for 30 min at 25°C in 50 mM Tris–HCl, pH 7.4 containing 1 mM EDTA and 0.01% (w/v) ascorbic acid. Saturation experiments were performed with [^{3}H]prazosin in concentrations ranging from 0.06 to 2 nM. Competitive inhibition of [^{3}H]prazosin (0.15 nM) binding was measured with unlabelled competitors at concentrations ranging from 10 pM to 100 μM. Non-specific binding was determined in the presence of 10 μM unlabelled prazosin. Bound [^{3}H]prazosin was separated from free [^{3}H]prazosin by immediate filtration through Whatman GF/B glass fiber filters presoaked with 0.05% (w/v) polyethylenimine for 24 h, using a Brandel cell harvester. The filters were washed three times with 50 mM Tris–HCl, pH 7.4 at 0–4°C and assayed for radioactivity by liquid scintillation spectrometry using a β-counter (Wallac) with 45% efficiency.

2.3. Determination of α_1-adrenoceptor mRNA by RT-PCR

Currently, the α_1-adrenoceptor classification recognizes three native subtypes, designated α_{1A}, α_{1B} and α_{1D}-adrenoceptors. The cloned counterparts are now termed α_{1a}, α_{1b} and α_{1d}-adrenoceptors, corresponding to the previously cloned subtypes α_{1c}, α_{1b} and $\alpha_{1a/d}$, respectively.

Total RNA was isolated from frozen prostate by a modification of the method of Chomczynski and Sacchi (1987) using a one-step guanidinium thiocyanate–phenol–chloroform extraction (RNA B, Bioprobe Systems, Montreuil sous Bois). RNA samples were quantified and their purity was assessed by spectrophotometry at 260 and 280 nm. Ethanol precipitates were stored at −80°C.

Synthesized specific oligonucleotide primers (Genset, Paris) were selected by the PCRBASE program from the BISANCE server (CITI2, Paris), based on the published rat sequence of α_{1a} (Laz et al., 1994), α_{1b} (Gao and Kunos, 1993) and α_{1d} (Lomasney et al., 1991) -adrenoceptors. α_{1a}-Primers were 'upstream' 5′-GAGGGACAGCACAGAGACAT-3′, starting at nucleotide 1137 and 'downstream' 5′-GACTTCCTCCCCGTTTTCAC-3′, starting at nucleotide 1414. α_{1b} Primers were 'upstream' 5′-CATCCTCTTTGCCATCGTG-3′, starting at nucleotide 179 and 'downstream' 5′-GGAGATGACCGTGGACAAGA-3′, starting at nucleotide 526. α_{1d}-Primers were 'upstream' 5′-CCTGGTGGTATCTGTGGGAC-3′, starting at nucleotide 1133 and 'downstream' 5′-CCTTGCTACTCTGTGTTCCG-3′, starting at nucleotide 1418. The lengths of resulting cDNA fragments to be amplified were predicted to be 297, 367 and 305 bp for α_{1a}, α_{1b} and α_{1d}-adrenoceptors, respectively. As previously described (Roubert et al., 1993), the positive control

was β-actin mRNA. The length of the resulting cDNA fragment to be amplified for β-actin was predicted to be 253 bp. Negative controls were determined in the absence of RNA and without RT.

RT-PCR (reverse transcription combined with polymerase chain reaction) of α_1-adrenoceptor mRNA was performed using recombinant *Thermus thermophilus* (rTth) DNA polymerase (Perkin Elmer Cetus, Saint Quentin–Yveline). Total prostatic RNA (100 ng) was reverse transcribed for 15 min at 65°C for α_{1A}-adrenoceptor and β-actin and 60°C for α_{1B} and α_{1D}-adrenoceptors, using a thermal cycler (TRIO-Thermoblock, Biometra). Template-specific cDNA was then amplified for 30, 36, 40 and 34 cycles for β-actin, α_{1A}, α_{1B} and α_{1D}-adrenoceptors, respectively: denaturation for 1 min at 94°C, annealing for 30 s at 57, 61, 55 and 53°C for β-actin, α_{1A}, α_{1B}, and α_{1D}-adrenoceptors, respectively, and polymerization at 72°C for 1 min. The PCR mixture consisted of 15 pmol of primers, all four deoxynucleoside triphosphates (each at 200 mM), 5 units of rTth DNA polymerase and 1.5, 0.6, 0.8 and 0.4 mM $MgCl_2$ for β-actin, α_{1A}, α_{1B}, and α_{1D}-adrenoceptors, respectively.

Aliquots of the PCR products were resolved by electrophoresis on 2.5% NuSieve agarose gel (FMC bioproducts, Rockland, ME) in Tris borate EDTA buffer containing ethidium bromide (0.5 μg/ml). The gel was placed under ultraviolet light, and a process image of the nucleic acid was taken with the Vilber Lourmat densitometer. Amplified cDNA sample length was evaluated using molecular weight standards (phi-X-174-RF DNA *hae* III digest and phi-X-174-RF DNA *hin*c II digest, Pharmacia Biotech, Orsay).

Nucleotide sequences of the RT-PCR products were determined by fluorescent dideoxynucleotide chain-termination method with RT-PCR primers and with AmpliTaq DNA polymerase (Perkin Elmer Cetus, Saint Quentin–Yveline) using an automated sequencer (Applied Biosystems, Foster city, CA).

2.4. Data analysis

Binding data were analyzed by computer-assisted nonlinear regression analysis using the LIGAND program (Biosoft, Cambridge). Statistical comparisons between single and multiple binding site models were made by determining the best fit of the affinity constant (dissociation constant, K_d, in saturation experiments, or inhibition constant, K_i, in competitive inhibition experiments) and binding capacity (B_{max}). The results were expressed as means $\pm$ S.E.M. Pearson correlation analyses between the negative logarithm of the K_i (pK_i) of various agonists and antagonists of prostatic α_1-adrenoceptors, and recombinant adrenoceptors were assessed by least-square linear regression.

2.5. Drugs

[^{3}H]prazosin (74.4 Ci/mmol), from New England Nuclear (Les Ulis), was stored at −20°C. EDTA, unlabelled prazosin, bovine serum albumin and testosterone propionate were purchased from Sigma (St. Louis, MO). Tris was from Solvabio (Arcueil). The following drugs were stored at room temperature and were purchased from RBI (Natrick, MA): BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) and oxymetazoline were dissolved at 10^{-1} M in water, (−)-norepinephrine and chloroethylclonidine, were dissolved at 10^{-2} M in water, prazosin, was dissolved at 10^{-2} M in methanol, spiperone and (+)-niguldipine, were dissolved at 10^{-1} M in DMSO (dimethyl sulfoxide), WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and 5-methyl-urapidil were dissolved at 10^{-2} M in acetic acid. Gel-loading buffer was purchased from Tebu (Le Perray en Yvelines), ethidium bromide from Interchim (Asnieres) and Tris borate EDTA from Bioprobe Systems (Montreuil sous Bois).

3. Results

3.1. Characteristics of [^{3}H]prazosin binding

The binding of [^{3}H]prazosin to rat prostate α_1-adrenoceptors was specific, saturable and of high-affinity (Fig. 1). It showed an apparent single class of binding sites with a

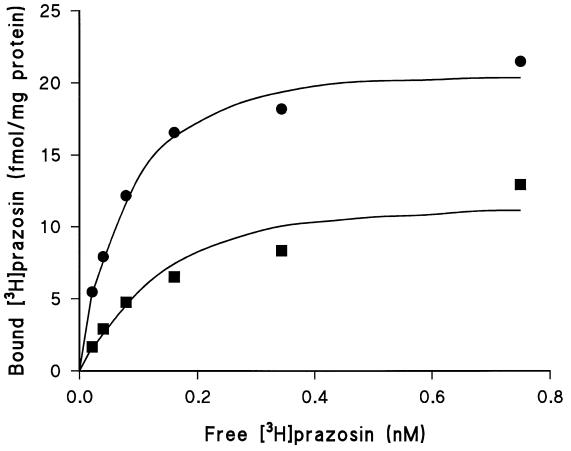

Fig. 1. Isotherm of [^{3}H]prazosin binding to rat prostate. Crude membranes from rat prostate were incubated in the absence (●) or presence (■) of 10 μM chloroethylclonidine for 30 min at 37°C and specific binding of [^{3}H]prazosin was measured as described in Section 2. Specific binding represented approximately 80% of total binding. Data are from a typical experiment which was replicated 6 times. Binding capacities (B_{max}) for control and chloroethylclonidine-treated membranes were 22.57 and 14.07 fmol/mg protein, respectively. K_d values were 67.9 and 158.5 pM for control and chloroethylclonidine-treated membranes, respectively.

Table 1
Characteristics of competitive inhibition of [^{3}H]prazosin binding to rat prostate by selected α_1-adrenoceptor ligands. Binding of [^{3}H]prazosin (0.15 nM) to chloroethylclonidine-treated and -untreated rat prostate membranes was measured in the presence of increasing concentrations of competing ligands. Binding data were analyzed using the LIGAND program and inhibition constants (K_i) were determined. Results are the means ± S.E.M. of three experiments. When fitting was significantly improved using a two-site model (oxymetazoline, $P < 0.05$ and 5-methyl-urapidil, $P < 0.05$), K_i and ratio are provided for the two affinity states

	Chloroethylclonidine-untreated			Chloroethylclonidine-treated
	$K_{i\,high}$ (nM)	$K_{i\,low}$ (nM)	ratio (high:low)	K_i (nM)
Agonists				
(−)-norepinephrine	1060 ± 182			559 ± 27.8
oxymetazoline	8.10 ± 2.687	799 ± 85.4	77:23	9.65 ± 1.741
Antagonists				
prazosin	0.243 ± 0.0388			0.619 ± 0.1876
WB-4101	1.45 ± 0.197			1.59 ± 0.301
5-methyl urapidil	0.789 ± 0.1617	78.5 ± 12.52	73:27	1.51 ± 0.193
(+)-niguldipine	30.7 ± 12.81			29.4 ± 13.47
spiperone	10.8 ± 2.40			15.0 ± 0.61
BMY 7378	106 ± 8.5			156 ± 67.0

dissociation constant (K_d) of 57.9 ± 5.02 pM and a binding capacity (B_{max}) of 24.6 ± 1.02 fmol/mg protein (n = 6). Pretreatment of prostate membranes with 10 μM chloroethylclonidine for 30 min at 37°C reduced the number of binding sites to 13.2 ± 1.20 fmol/mg protein with an increase of the K_d value (92.5 ± 15.68 pM), indicating that chloroethylclonidine-sensitive and -insensitive α_1-adrenoceptor subtypes co-exist in rat prostate. As chloroethylclonidine preferentially inactivates α_{1B} and α_{1D}-adrenoceptors (Laz et al., 1994), the results suggest that chloroethylclonidine-sensitive α_1-adrenoceptors (45%) are mainly α_{1B} and/or α_{1D} subtype and that chloroethylclonidine-insensitive α_1-adrenoceptors (55%) are mainly α_{1A} subtype.

In competition experiments, prazosin, WB-4101, (+)-niguldipine, spiperone, BMY 7378 and (−)-norepinephrine inhibited [^{3}H]prazosin binding according to a one-site model (Table 1). Oxymetazoline and 5-methyl-urapidil significantly improved the fit to a two-site model. The proportion of high-affinity sites for 5-methyl-urapidil and oxymetazoline, characterizing the α_{1A}-adrenoceptor subtype, was approximately 75% of total binding sites. The high-affinity binding observed with WB-4101 (1.45 nM) was consistent with the preponderance of α_{1A}-adrenoceptors in rat prostate whereas the apparently low-affinity binding observed with (+)-niguldipine (30.7 nM) was unexpected. The low-affinity sites for 5-methyl-urapidil and oxymetazoline may represent α_{1B} or α_{1D}-adrenoceptor subtypes. The low-affinity binding observed with spiperone (10.8 nM), a selective α_{1B}-adrenoceptor antagonist, and BMY 7378 (106 nM), a selective α_{1D}-adrenoceptor antagonist, did not allow differentiation of α_{1B} and α_{1D}-adrenoceptor subtypes.

The calculated pK_i of the ligands investigated in the rat prostate were compared to their binding affinity for rat recombinant α_1-adrenoceptor subtypes expressed in COS cells (Laz et al., 1994; Piascik et al., 1995, Table 2). The high-affinity site for 5-methyl-urapidil and oxymetazoline in the prostate was better correlated with cloned α_{1a}-adrenoceptors ($r = 0.93$, $P = 0.001$). The low-affinity site for 5-methyl-urapidil and oxymetazoline was correlated with α_{1b}-adrenoceptors ($r = 0.90$, $P = 0.006$). Chloroeth-

Table 2
Correlation between rat recombinant α_1-adrenoceptor subtypes and native α_1-adrenoceptors from the rat prostate. Data show correlation coefficients that were calculated by least-square linear regression by plotting the negative logarithm of the K_i (pK_i) presented in Table 1 at α_1-adrenoceptors of the rat prostate, against pK_i of the same compounds at rat recombinant α_1-adrenoceptors. The values of pK_i at cloned α_1-adrenoceptors were from Laz et al. (1994) and Piascik et al. (1995). Results from chloroethylclonidine-untreated prostate membranes were separated by observing high- and low-affinity binding sites for oxymetazoline and 5-methyl-urapidil.

Recombinant α_1-adrenoceptors	Native prostatic α_1-adrenoceptors		
	chloroethylclonidine-untreated		chloroethylclonidine-treated
	high affinity	low affinity	
α_{1a}	0.93 ($P = 0.001$)	0.71 ($P = 0.048$)	0.95 ($P < 0.001$)
α_{1b}	0.81 ($P = 0.028$)	0.90 ($P = 0.006$)	0.79 ($P = 0.034$)
α_{1d}	0.47 ($P = 0.238$)	0.81 ($P = 0.014$)	0.44 ($P = 0.271$)

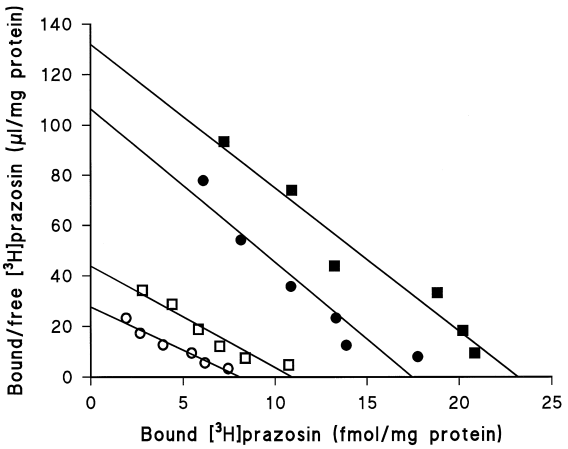

Fig. 2. Effect of testosterone administration on prostatic α_1-adrenoceptors. Scatchard plot of [^{3}H]prazosin binding to crude prostate membranes from control (■) and testosterone-treated (●) rats. The effect of 30 min incubation of the membranes with 10 μM chloroethylclonidine at 37°C was determined in control (□) and testosterone-treated (○) animals. B_{max} and K_d values for this typical experiment which was replicated 6 times were 23.47 fmol/mg protein and 55.8 pM, respectively, for chloroethylclonidine-untreated membranes from control rats, 10.75 fmol/mg protein and 77.3 pM, respectively, for chloroethylclonidine-treated membranes from control rats, 17.08 fmol/mg protein and 50.6 pM, respectively, for chloroethylclonidine-untreated membranes from testosterone-treated rats and 8.01 fmol/mg protein and 94.4 pM respectively, for chloroethylclonidine-treated membranes from testosterone-treated rats.

ylclonidine-pretreated prostate membranes showed a complete inactivation of the low-affinity site for 5-methylurapidil and oxymetazoline. The pK_i values of the ligands investigated measured with these membranes were correlated with pK_i obtained at recombinant α_{1a}-adrenoceptors ($r = 0.95$, $P < 0.001$).

Subcutaneous injection of testosterone for 15 d significantly decreased body weight from 421 ± 11.2 g for control rats to 386 ± 8.1 g for testosterone-treated rats ($P < 0.01$). Testosterone administration significantly increased the prostate weight from 225.0 ± 7.89 mg/100 g body weight in vehicle-treated controls to 393.3 ± 4.75 mg/100 g body weight in testosterone-treated rats. The binding capacity of [^{3}H]prazosin to crude prostate membrane preparations was slightly but significantly lower in treated rats than in the control group ($P < 0.01$). Scatchard analysis of the saturation curves gave a B_{max} of 19.44 ± 1.604 fmol/mg protein with a K_d of 61.3 ± 4.60 pM in testosterone-treated rats (Fig. 2). Pretreatment with chloroethylclonidine significantly reduced α_1-adrenoceptor density ($B_{max} = 10.68 \pm 1.329$ fmol/mg protein) and affinity ($K_d = 103.8 \pm 24.68$ pM). This effect of chloroethylclonidine was similar to that observed in control animals. Based on whole prostate, the total amount of α_1-adrenoceptors was 253 ± 9.6 fmol/prostate for control rats and 322 ± 27.2 fmol/prostate for testosterone-treated rats.

3.2. α_1-Adrenoceptor mRNA measurement

Total RNA from rat prostate was isolated, and the relative levels of α_1-adrenoceptor subtype mRNA were measured by RT-PCR (Fig. 3). RT-PCR using α_{1a} α_{1b} or α_{1d}-adrenoceptor primers resulted in a single band at the expected length: 297 bp, 367 bp or 305 bp, respectively. Each PCR product was extracted and digested with restriction enzymes, providing specifically sized fragments, and the nucleotide sequence of PCR products was established by DNA sequencing (data not shown), suggesting highly

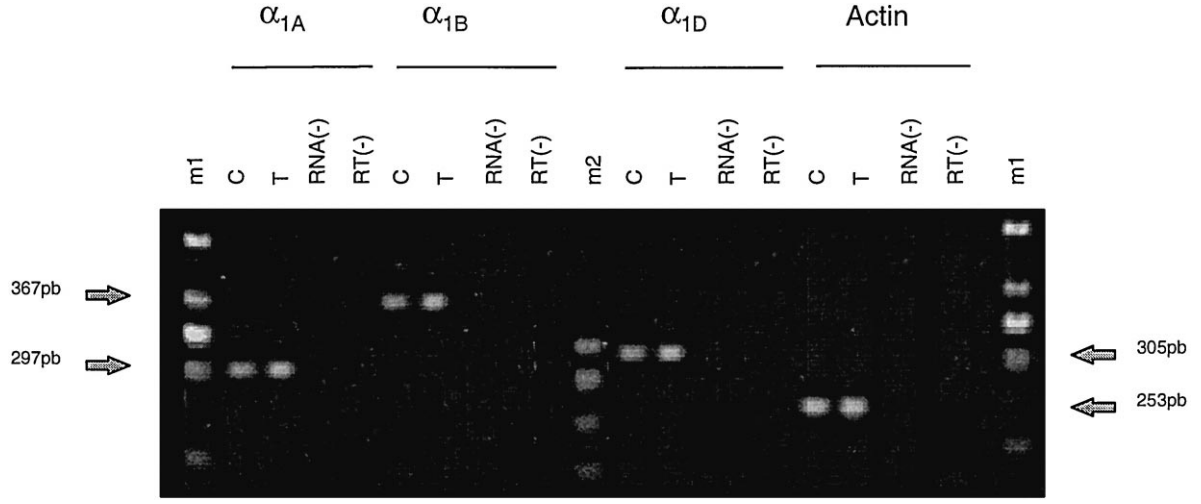

Fig. 3. Effect of testosterone administration on α_1-adrenoceptor mRNA expression in rat prostate. The expression of α_1-adrenoceptor mRNA was evaluated by RT-PCR in hypertrophic prostate from testosterone-treated rats (T) and compared with that in control animals (C). RT-PCR assay was performed on total RNA using specific primers for rat α_{1a}, α_{1b} and α_{1d}-adrenoceptors, as described in Section 2. The resulting products were resolved by electrophoresis on a 2.5% agarose gel in Tris borate EDTA buffer and visualized by ethidium bromide staining. Phi-X-174-RF DNA *hinc* II digest (*m1*) and phi-X-174-RF DNA *hae* III digest (*m2*) from Pharmacia Biotech were used as DNA size markers. Arrows indicate expected PCR product sizes. RT-PCR using specific primers for β-actin was used as positive control. Negative controls were performed in the absence of RNA [RNA(−)] and without RT [RT(−)]. Data shown are from a typical experiment which was replicated 3 times.

specific primers. The lack of signals in unmatched lanes (without RT) as well as without RNA demonstrated the absence of DNA contamination. For each sample, amplification with β-actin primers elicited an equal signal. These results indicate that the three α_1-adrenoceptor subtypes are expressed in the rat prostate.

The signal obtained from RT-PCR for α_{1a}, α_{1b} and α_{1d}-adrenoceptor subtypes was not modified when the animals were treated with 3 mg/kg testosterone for 15 d. This suggests that the expression of the three α_1-adrenoceptor mRNA is not affected by testosterone treatment (Fig. 3).

4. Discussion

The present results of ligand binding studies and mRNA identification, confirm that α_{1A}, α_{1B} and α_{1D}-adrenoceptors are present in rat prostate. A single band corresponding to the expected position for mRNA encoding each α_1-adrenoceptor subtype was found, using the RT-PCR technique. Although Price et al. (1994) only detected α_{1A} mRNA with an RNase protection assay, our results agree with previous reports based on either RNase protection assay (Roskosh et al., 1994) or RT-PCR (Scofield et al., 1995), demonstrating all three cloned adrenoceptor expression in rat prostate.

Although mRNA for the three α_1-adrenoceptor subtypes is detected, ligand binding demonstrates that most of the prostatic α_1-adrenoceptors are of the α_{1A} subtype. We found that the number of specific binding sites for [^{3}H]prazosin was affected by 45% by chloroethylclonidine. Since chloroethylclonidine preferentially alkylates α_{1B} and α_{1D}-adrenoceptor subtypes (Laz et al., 1994), this suggests that about 55% of the α_1-adrenoceptors are of the α_{1A} subtype, the remaining binding sites being α_{1B} and/or α_{1D}-adrenoceptors. The preponderance of chloroethylclonidine-insensitive α_1-adrenoceptors in rat prostate is consistent with results of previous radioligand binding (Testa et al., 1993; Yazawa and Honda, 1993; Lepor et al., 1994) and functional (Coudwell et al., 1993; Yazawa and Honda, 1993) studies.

Competitive inhibition of [^{3}H]prazosin binding shows that the α_{1A} subtype-selective ligands, 5-methyl-urapidil and oxymetazoline, discriminate between high- and low-affinity binding sites with a 75:25 ratio. These data confirm that the α_{1A}-adrenoceptor is the predominant α_1-adrenoceptor subtype in rat prostate. This is further supported by the good correlation between the affinity of selected compounds for the cloned rat α_{1a}-adrenoceptor (Laz et al., 1994; Piascik et al., 1995) and the potency of the same ligands observed in the present work on chloroethylclonidine-treated rat prostate membrane, as well as at the high-affinity site for 5-methyl-urapidil and oxymetazoline in chloroethylclonidine-untreated tissue. The remaining low-affinity site for 5-methyl-urapidil and oxymetazoline is preferentially correlated with the cloned rat α_{1b}-adrenoceptor. However, in contrast with published K_i values on recombinant α_{1b}- and α_{1d}-adrenoceptors (Laz et al., 1994; Piascik et al., 1995), neither spiperone, that preferentially recognizes α_{1b}-adrenoceptors, nor BMY 7378, that preferentially recognizes α_{1d}-adrenoceptors, show binding affinity in the low nanomolar range in rat prostate. This is presumably due to a lack of selectivity of these drugs in the rat and to the weak proportion for the low-affinity site for 5-methyl-urapidil and oxymetazoline (25%), i.e. α_{1B} and/or α_{1D}-adrenoceptors, we now found. The predominance of α_{1A}-adrenoceptors in rat prostate as shown by both binding affinity of subtype-specific ligands and the effect of chloroethylclonidine pretreatment on prazosin binding capacity are consistent with a recent report from Scofield et al. (1995), who used competitive RT-PCR, that describes mRNA steady state expression levels in rat prostate of about 680, 8 and 0.4 molecules/ng total RNA for α_{1a}, α_{1b} and α_{1d}-adrenoceptors, respectively.

Testosterone administration to rats represents an experimental model of prostatic hypertrophy that induces urodynamic symptoms of outflow obstruction (Maggi et al., 1989). As a result of the hyperthermia, reduction in food intake, increase in lean tissue and decrease of fat that are induced by testosterone (Hervey and Hutchinson, 1973; Nunez, 1982; Abelenda et al., 1992), the rat body weight decreased by 8.5% in the present study. Subcutaneous injection of testosterone for 15 d produced a 75% increase in prostate weight. Binding experiments showed that the density of α_1-adrenoceptors in the prostate was slightly but significantly decreased in testosterone-treated animals, whereas the total amount of α_1-adrenoceptors per prostate was increased by 27%, probably because of the increase in prostate weight. The relative proportion of receptor subtypes was unchanged. Preincubation of the preparations with chloroethylclonidine reduced the number of [^{3}H]prazosin binding sites to the same extent in the testosterone-treated group and the control group. Concurrently, our RT-PCR data showed that the steady state level of mRNA for α_{1a}, α_{1b} and α_{1d}-adrenoceptors was not modified by testosterone treatment.

In summary, our findings suggest that the prostatic α_1-adrenoceptors are not affected by testosterone-induced prostatic hypertrophy in rats. Since α_1-adrenoceptors predominate in the fibromuscular stroma (Lacey et al., 1996) and testosterone induces hypertrophy of the glandular epithelial component of the prostate (Flickinger, 1976; Limanowski et al., 1995), the observed decrease of α_1-adrenoceptor density likely results from a decrease in the relative proportion of smooth muscular tissue in the entire prostate, reducing the density of α_1-adrenoceptors. This hypothesis is consistent with a recent report from Lacey et al. (1996) of an apparent increase in prostatic α_1-adrenoceptor density in androgen deprivation by castration in rats, resulting from a relative increase in the ratio of smooth

muscle to epithelium, rather than from direct up-regulation of α_1-adrenoceptors. Since benign prostatic hypertrophy in man is age- and androgen-dependent, the preservation of α_1-adrenoceptors in an experimental model of prostatic hypertrophy supports the clinical use of α_1-adrenoceptor antagonists in benign prostatic hypertrophy.

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