



Effects of castration on contraction and α_1 -adrenoceptor expression in rat prostate

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1 The prostate function is regulated by androgens and α -adrenergic activity. Clinically, antiandrogens and/or α_1 -adrenergic antagonists are commonly used to treat symptomatic prostatic hypertrophy. To elucidate the effects of androgen deprivation on prostate contractility via α_1 -adrenoceptor, the characteristics and expression of α_1 -adrenoceptors were examined in castrated rats.

2 Isolated prostate strips from intact and castrated rats were subjected to a phenylephrine stimulated contraction. Prazosin (10 nM), [³H]-prazosin and phenoxybenzamine (3–300 nM) were used for inhibition assay, receptor characterization and partial alkylation of α -adrenoceptor, respectively. The mRNA content of three subtypes of α -adrenoceptors was determined by reverse transcription combined with polymerase chain reaction (RT–PCR).

3 Contractile response to phenylephrine increased in castrated rats, which could be explained by a relative increase of the stromal component. A lowered contraction potency was also noted in castrated rats. Receptor binding assay indicated minimal changes in the affinity or density of α_1 -adrenoceptor. Escalating alkylation of the α_1 -adrenoceptor population resulted in a rightward shift in the contraction-response curves before depressing maximal contractile force, and the suppression was detected at lower doses in castrated rats. RT–PCR study confirmed the expression of three types of α_1 -adrenoceptor, α_{1a} , α_{1b} and α_{1d} -adrenoceptors, in intact rat prostate, and revealed that α_{1a} -adrenoceptor, but not α_{1b} or α_{1d} -adrenoceptors, was down-regulated in castrates.

4 The results show that androgen deprivation suppressed α_1 -adrenergic contractility of rat prostate strips, and the suppression was associated with down-regulation of receptor reserve for the α_{1a} -adrenoceptor population expressed in intact rat prostate.

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Abbreviations: BPH, benign prostatic hyperplasia; RT–PCR, reverse transcription combined with polymerase chain reaction

Introduction

The sex hormone environment is known to influence the contraction of smooth muscle in response to adrenergic stimulation. For example, in the female rabbit the sensitivity of urethral smooth muscle to α -adrenergic stimulation is increased after bilateral oophorectomy (Andersson *et al.*, 1984). Estradiol treatment of immature rabbits enhances sensitivity of the uterus to norepinephrine-induced contraction (Roberts *et al.*, 1981).

The prostate is a contractile organ that responds to α -adrenergic stimulation (Furuya *et al.*, 1982). Although there are substantial differences in prostate stromal contents among species (Lepor *et al.*, 1994), stromal smooth muscle cells that express α_1 -adrenoceptors (Lepor *et al.*, 1987) are responsible for contractility with α -adrenergic agonists. Three distinct subtypes of α_1 -adrenoceptor have been cloned and characterized: α_{1a} (Schwinn *et al.*, 1990), α_{1b} (Cotecchia *et al.*, 1988), and α_{1d} -adrenoceptors (Lomasney *et al.*, 1991). Although all three α_1 -adrenoceptor subtypes have been detected in the prostate, the α_{1a} -adrenoceptor subtype predominates in prostatic stromal tissue (Hiraoka *et al.*, 1999; Walden *et al.*, 1999). This was evidenced at RNA level (Hirasawa *et al.*, 1993; Nasu *et al.*, 1996; Price *et al.*, 1993) as well as at the protein level and in smooth muscle contraction assays (Faure *et al.*, 1994; Lepor *et al.*, 1994; Marshall *et al.*, 1995).

Another pharmacologically distinct α_1 -adrenoceptor that mediates prostatic contraction has been suggested as a receptor with low affinity for prazosin (α_{1L} -adrenoceptor) (Muramatsu *et al.*, 1994); it may represent a functional phenotype of α_{1a} -adrenoceptor (Daniels *et al.*, 1999).

Contraction of the prostate increases intraurethral pressure and bladder outlet resistance (Furuya *et al.*, 1982; Caine, 1990) and, in turn, relaxation of muscle tone by α -adrenergic antagonists has been shown to be effective in treating symptomatic benign prostatic hyperplasia (BPH) (Chapple, 1995; Kawabe & Nijima, 1987; Matyus & Horvath, 1997). On the other hand, the prostate is an androgen-dependent organ. Biological functions such as prostate fluid secretion and morphological characteristics are affected considerably by androgen deprivation (Cheng *et al.*, 1993). This androgen dependency is a theoretical basis for clinical utility of antiandrogens or five α -reductase inhibitors in benign and malignant diseases of the prostate (Gormley *et al.*, 1992).

It could, therefore, be hypothesized that the contractile properties of prostate smooth muscle cells under α -adrenergic stimulation might be affected by changes in androgen levels. This is clinically relevant in that an anti-androgen and an α -adrenergic antagonist may be simultaneously prescribed for BPH patients, which resulted in no synergy (Lepor *et al.*, 1996) or modest synergy (Okada *et al.*, 1996). However, the interaction between androgen deprivation and adrenergic contractile response of the prostate has been investigated in

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only a few studies (Akiyama *et al.*, 1999; Lacey *et al.*, 1996; Lin *et al.*, 1995). The present study explored the effects of castration on the contractility and/or pharmacological characteristics of α -adrenoceptors in rat prostate. Receptor expression was evaluated using reverse transcription combined with polymerase chain reaction.

Methods

Animals

Male Sprague-Dawley rats weighing 300–500 g (Japan SLC Co., Shizuoka, Japan) were used throughout. Rats were housed in groups of four in a room maintained at 21–25°C and 45–65% humidity, controlled at an alternating 12 h light/dark cycle with lights on at 0700 h. Food and water were given *ad libitum*. Two weeks after bilateral orchidectomy under anaesthesia, the following studies were conducted.

Contraction study

Animals were killed and the ventral and dorsal lobes of the prostate removed. Two strips of about 2 mm in width and 5–10 mm in length were prepared from each lobe. Each strip was weighed and mounted in an organ bath containing 10 ml Krebs solution (mM: NaCl 111, KCl 5.9, MgCl₂ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, NaH₂PO₄ 1.2, glucose 11.5), gassed with 95% O₂ and 5% CO₂, maintained at 37°C. The strip was stretched to a resting tension of 1 g and equilibrated for 60 min. Changes in tension were isometrically detected with a force-displacement transducer connected to a polygraph recorder (T7-30-240, Toyo Baldwin, Tokyo, Japan) *via* a strain-gauge pressure amplifier (AP-620G, Nihon Kohden, Tokyo, Japan) and recorded on a pen recorder (RJG-4024, Nihon Kohden). K⁺ (100 mM) induced contraction was obtained by exchange of Krebs solution to high K⁺ Krebs solution (mM: NaCl 17, KCl 100, MgCl₂ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, NaH₂PO₄ 1.2, glucose 11.5). When maximal contraction was obtained, the strip was washed with normal Krebs solution until the tone returned to the basal level. The concentration-response curves were generated by cumulative addition of phenylephrine (0.01–1000 μ M) and repeated for the same strip at 60 min intervals. The first curve was taken as the control response. For the inhibition study, prazosin (10 nM) was added to the bath 15 min before the initiation of phenylephrine application, and reproducibility of the curve was confirmed in the absence of antagonist. The maximal contraction was obtained from the concentration-response curve and the pEC₅₀ value for phenylephrine was calculated from a regression line between logarithmic concentrations and logit-converted response rate based on the Hill equation by the least square method (Jenkinson *et al.*, 1995). The pK_B values for antagonists were determined according to the method of van Rossum (1963).

Alkylation of α -adrenoceptors

After a control concentration-response curve for phenylephrine had been determined, the strip was equilibrated with phenoxybenzamine at 3–300 nM for 10 min and then washed for 2 h in Krebs solution. During the first hour of washing, sodium thiosulphate (100 μ M) was added to prevent further alkylation of α -adrenoceptors. After washing for 2 h, phenylephrine was applied cumulatively. Reproducibility of the response to phenylephrine without phenoxybenzamine

treatment was confirmed in other strips. Maximal contraction and pEC₅₀ values were determined as described above.

Receptor preparation and binding assay

Prostate tissue was minced into small pieces and homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) with a Polytron homogenizer (Kinematica, Littau, Switzerland) for 30 s. The homogenate was centrifuged at 39,000 \times g for 20 min at 4°C. The pellet was suspended in 20 volumes of ice-cold buffer, and the suspension was centrifuged at 39,000 \times g for 20 min at 4°C. The pellet was then resuspended in 10 to 40 volumes of ice-cold buffer and the suspension was filtered through gauze. The filtrate was used for the binding assay. Protein concentration was determined by the method of Bradford (1976). The binding assay was performed at six different concentrations (in the range 0.02–3 nM) of [³H]-prazosin (2671.4 GBq mmol⁻¹). The membrane preparation (0.4 ml) was incubated with [³H]-prazosin in the presence and absence of 10 μ M phentolamine at 25°C for 30 min in a total volume of 0.5 ml. The reaction was terminated by the addition of 3 ml of ice-cold buffer and the reaction mixture was rapidly filtered under a vacuum through a glass-fibre filter (Whatman GF/B, Maidstone, U.K.) presoaked with 0.1% polyethyleneimine. Filters were washed three times with 3 ml ice-cold buffer and then placed in vials with 10 ml scintillation fluid. Radioactivity was determined in a liquid scintillation counter (Tri-Carb4640, Packard, Downers Grove, IL, U.S.A.). The dissociation constant (K_d) and the binding capacity (B_{max}) were obtained by non-linear regression analysis of logistic equation (De Lean *et al.*, 1978). Inhibitory effects of phenylephrine on [³H]-prazosin (0.2 nM) binding to membrane preparations were examined at nine concentrations (in range 0.1–100 μ M) of phenylephrine. IC₅₀ values for phenylephrine were determined by regression analysis of logarithmic concentration versus logit-converted inhibition rate. Inhibition constant (K_i) was calculated by the method of Cheng & Prusoff (1973).

Determination of α_1 adrenoceptor mRNAs by RT-PCR

Total cytoplasmic RNA was extracted from rat prostate tissues with ISOGEN and subjected to reverse transcription and quantitative competitive PCR. Briefly, 5.0 μ g total RNA was converted to cDNA using SuperScript II reverse transcriptase in the presence of d(N) 6 random primers. To confirm that all samples contained equivalent amounts of RNA, total RNA from samples was separately co-amplified in the presence of serial dilutions of an RNA internal standard (mimic) prepared for β -actin. The β -actin RNA mimic shares the same primer template sequence but contains a smaller intervening sequence. Samples containing 5.0 μ g of prostate total RNA were co-reverse-transcribed with 2 fold serial dilutions of β -actin mRNA. The β -actin DNA fragments that share the same primer template sequence with the target cDNA but contain a completely different, smaller intervening sequence were prepared and used as DNA internal standards. Aliquots of sample cDNA mixed with serial dilutions of DNA mimics were co-amplified as templates in the presence of 10.0 μ mol μ l⁻¹ downstream and upstream primers for β -actin (downstream primers: 5'-CAG GAG ATG GCC ACT GCC GCA-3', upstream primers: 5'-TCC TTC TGC ATC CTG TCA GCA-3'). The PCR mixture consisted of 2.5 mM of each deoxyribonucleotide, 10 \times ExTaq buffer and 0.015 units of ExTaq DNA polymerase. The DNA was denatured by heating at 95°C for

5 min, followed by 28 cycles of 30 s at 95°C, annealing at 56°C for 30 s and extension at 72°C for 1 min (Perkin-Elmer thermocycler). The amplified products were analysed by electrophoresis on 3.0% agarose gels followed by staining with ethidium bromide. The stained products were scanned and quantified, and all samples were prepared to contain 3 ng β -actin cDNA. To determine the amounts of α_1 adrenoceptor mRNAs, samples were amplified as templates in the presence of 10.0 $\mu\text{mol } \mu\text{l}^{-1}$ downstream and upstream primers for α_{1a} , α_{1b} , α_{1d} (downstream primers; α_{1a} : 5'-AGG CTG CTC AAG TTT TCTCG-3', α_{1b} : 5'-ATC CTG AGC CTA TGT GCC AT-3'-3', α_{1d} : 5'-CGC TGT GGT GGG AAC CGG CAG CGG A-3', upstream primers; α_{1a} : 5'-ACT TCC TCC CCG TTT TCACC-3', α_{1b} : 5'-TTG GTA CTG CTG AGG GTG TC-3', α_{1d} : 5'-ACA GCT GCA CTC AGT AGC AGG TCA-3') in a PCR mixture. The DNA was denatured by heating at 95°C for 5 min, followed by 28 cycles of 30 s at 95°C, annealing at 56°C (α_{1d} : 58°C) for 30 s and extension at 72°C for 1 min. This reaction was followed by a final elongation step for 7 min at 72°C. The amplified products were analysed by electrophoresis on 3.0% agarose gels, stained with ethidium bromide and quantified.

Drugs

Phenoxybenzamine (Nacalai Tesque Inc., Kyoto, Japan); [^3H]-prazosin (New England Nuclear, Boston, MA, U.S.A.); ISOGEN (Nippon Gene, Toyama, Japan); SuperScript II reverse transcriptase (Gibco BRL); ExTaq DNA polymerase (TaKaRa, Shiga, Japan). All other compounds were purchased from Sigma, St Louis, MO, U.S.A.

Statistics

Results are expressed as mean \pm s.e.mean. Unpaired *t*-tests were used to assess the difference between intact and castrated rats. Multiple comparison of contraction variable values at various doses of phenoxybenzamine with control values was done with Dunnett's test. *P* values less than 0.05 by two-tailed analysis were considered significant.

Results

Castration caused a dramatic decrease in prostate weight from 525 \pm 37 mg ($n=4$) to 74 \pm 3 mg in ventral lobes, and 350 \pm 15 mg ($n=4$) to 115 \pm 10 mg in dorsal lobes, respectively. On the other hand, the maximal contraction force of prostatic strips provoked by 100 mM K^+ , which was adjusted for strip weight, was greater in castrated rats: 271% of control for ventral lobes and 157% for dorsal lobes (Table 1). Similarly, the phenylephrine-induced contraction force per mg tissue was higher in castrates than in controls: 341% of control for ventral lobes, and 200% for dorsal lobes, whereas pEC_{50} values for phenylephrine were slightly but significantly lowered in castrates (Table 2). The antagonist study of phenylephrine-induced contraction by prazosin revealed comparable pK_B values for both prostatic lobes of castrated rats (Table 3). The representative contraction-response curves for phenylephrine with or without prazosin in ventral lobe strips are shown in Figure 1.

The characteristics of [^3H]-prazosin binding to membrane preparations obtained from ventral lobes are shown in Table 4. Saturation curves of [^3H]-prazosin binding appeared monophasic in tissue from both control and castrated rats (data not shown). The K_d value was significantly increased in

Table 1 K^+ -induced contractions of rat prostatic strips

	Maximal contractile force (mg force mg tissue $^{-1}$)	
	Ventral lobes	Dorsal lobes
Intact	3.36 \pm 0.53	3.26 \pm 0.84
Castrated	9.09 \pm 1.52**	5.13 \pm 0.69*

Maximal contractile force was measured in the presence of K^+ at 100 mM using tissue strips from ventral lobes and dorsal lobes of intact and castrated rats. Results are expressed as the mean \pm s.e.mean of four experiments. Increased contractility was apparent in castrates, especially for ventral lobes. **P* < 0.05, ***P* < 0.01.

Table 2 Phenylephrine-induced contractions of rat prostatic strips

	Maximal contractile force (mg force mg tissue $^{-1}$)		Potency pEC_{50} (M)	
		%KCl		
<i>Ventral lobes</i>				
Intact	3.29 \pm 0.61	96.7 \pm 3.6	6.25 \pm 0.13	
Castrated	11.22 \pm 2.23*	122.9 \pm 9.5	5.42 \pm 0.10*	
<i>Dorsal lobes</i>				
Intact	2.33 \pm 0.43	77.0 \pm 8.6	5.88 \pm 0.09	
Castrated	4.67 \pm 0.55*	91.8 \pm 2.3	5.01 \pm 0.08**	

Contraction-response curves were generated for cumulative doses of phenylephrine (0.01–1000 μM) using the tissue strips from ventral lobes and dorsal lobes of intact and castrated rats. Results are expressed as the mean \pm s.e.mean of four experiments. Androgen deprivation increased contractility to phenylephrine in both lobes, especially for ventral lobes. This apparent increase was normalized when the proportion due to KCl-induced contraction was taken into account. Contraction potency for phenylephrine decreased significantly in castrates. **P* < 0.05, ***P* < 0.001.

Table 3 Antagonistic effects of prazosin on phenylephrine-induced contractions of rat prostate strips

	pK_B value (M)	
	Ventral lobes	Dorsal lobes
Intact	8.78 \pm 0.12	8.76 \pm 0.08
Castrated	8.61 \pm 0.11	8.64 \pm 0.03

Tissue strips were obtained from ventral lobes and dorsal lobes of intact and castrated rats. Prazosin (10 nM) was added to the bath before contraction-response curves were generated for phenylephrine (0.01–1000 μM). pK_B values were calculated according to the method of van Rossum (1993). Results are expressed as the mean \pm s.e.mean of four experiments.

castrated rats, although it would not be physiologically significant. An increase in B_{max} values was also apparent when expressed per mg protein, but not on a tissue weight basis. The study of competitive inhibition by phenylephrine of [^3H]-prazosin (0.2 nM) binding to membrane preparations gave pK_1 values (M) for phenylephrine of 5.66 \pm 0.06 ($n=4$) and 5.67 \pm 0.09 ($n=3$) in intact and castrated rats, respectively.

The effect of various doses of phenoxybenzamine pre-treatment on phenylephrine contraction-response curves are shown in Figure 2, and the related parameter values are summarized in Table 5. In intact rats, extending alkylation by phenoxybenzamine at 3–30 nM produced a decrease in pEC_{50} without a change in the maximal response size, and further extended alkylation at 100 nM and 300 nM suppressed the maximal response. Furthermore, the suppression of

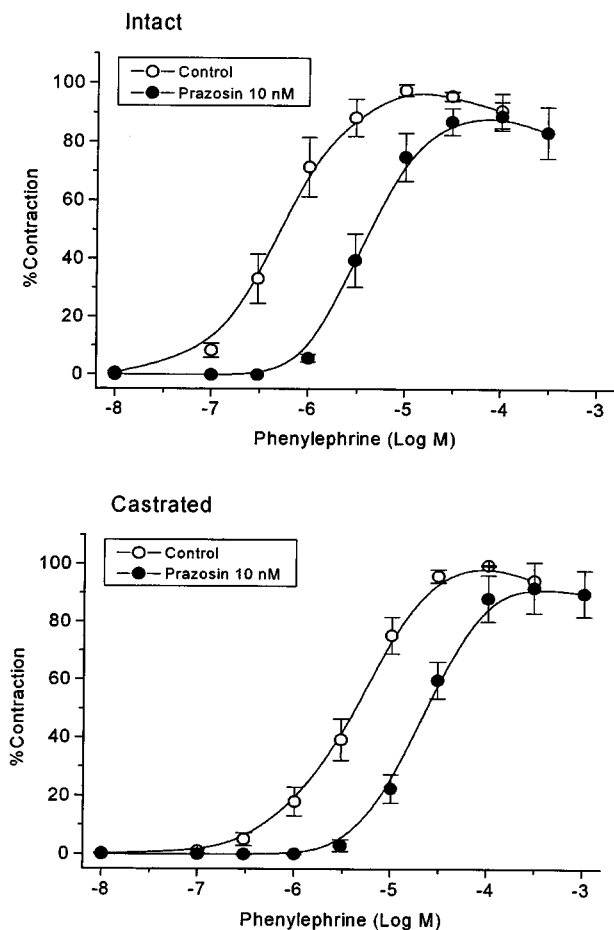


Figure 1 Contraction-response curves for phenylephrine with or without prazosin (10 nM) in isolated strips from prostate ventral lobes of intact and castrated rats. Each point and bar represents mean \pm s.e. mean of four experiments.

Table 4 Binding assay of [3 H]-prazosin to membrane preparations from rat prostate ventral lobes

	K_d (nM)	(fmol mg tissue $^{-1}$)	B_{max} (fmol mg protein $^{-1}$)
Intact	0.17 \pm 0.01	0.49 \pm 0.01	27.7 \pm 1.3
Castrated	0.29 \pm 0.03*	0.51 \pm 0.07	46.8 \pm 3.7**

Membrane fraction was obtained from prostate ventral lobes of intact and castrated rats. Binding of [3 H]-prazosin was measured at increasing concentrations of the ligand (0.02–3 nM). Results are expressed as mean \pm s.e. mean of four experiments. Increase in K_d value in castrates was detected, but this may not be of biological significance. B_{max} was elevated in castrated when calculated per protein unit. * $P < 0.05$, ** $P < 0.01$.

maximal response was more readily detected at the lower doses of phenoxybenzamine (30 nM) in castrated rats. In both intact and castrated rats, pEC $_{50}$ values were relatively unchanged at lower doses of phenoxybenzamine and decreased when the suppression of maximal contraction started, but again stabilized despite further suppression by increasing doses of phenoxybenzamine.

The expression of mRNAs for α_1 adrenoceptor subtypes in the prostate was measured by RT-PCR (Figure 3). RT-PCR using three different subtypes of α_1 adrenoceptor primers resulted in a single band at the expected length. Three fold dilutions of DNA internal standards were used to

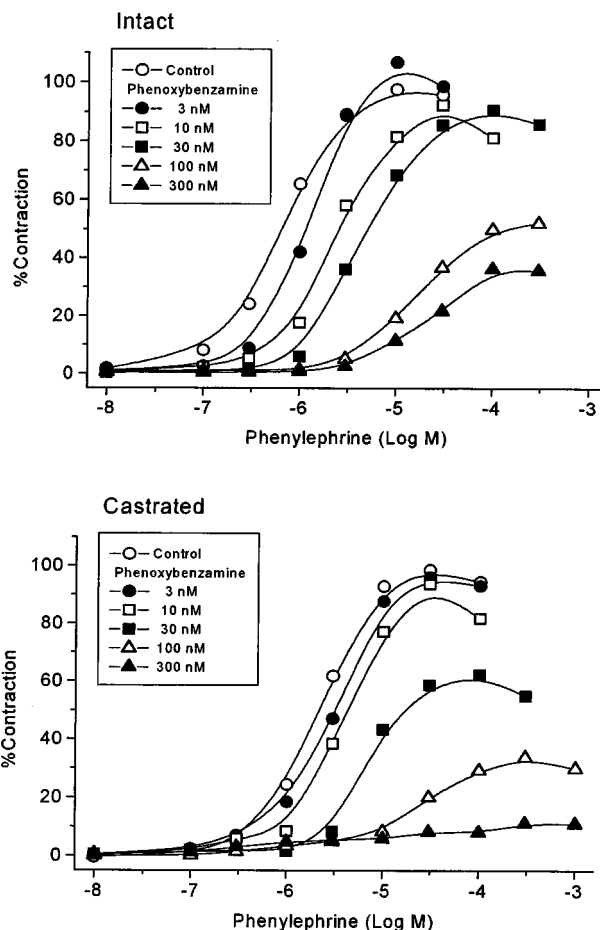


Figure 2 The effects of partial alkylation of α -adrenoceptor population on phenylephrine-induced contractions of isolated rat prostatic strips. Tissue strips from ventral lobes of intact and castrated rats were equilibrated with phenoxybenzamine at 3–300 nM for 10 min. After washing for 2 h, with sodium thiosulphate (100 μ M) added during the first hour, contraction-response curves for phenylephrine were produced. Each point represents the mean of four to 20 experiments. Error bars are not shown for simplicity. Parameter values calculated from the curves are given in Table 5. A rightward shift of curves was observed before maximal contraction size was suppressed. The suppression started at lower doses of phenoxybenzamine in castrates.

determine the concentration range necessary to compete for the PCR amplification of reverse-transcribed sample cDNAs. Cycle number was also varied to determine the linear amplification range for sample cDNA and internal standard DNA. Each cDNA sample was prepared at a concentration of 3 ng μ l $^{-1}$ β -actin cDNA. The expression of adrenoceptor mRNA was reduced in castrated rats for α_{1a} adrenoceptor, but not for α_{1b} or α_{1d} adrenoceptors. The reduction of α_{1a} adrenoceptor expression was more pronounced in ventral lobes than in dorsal lobes.

Discussion

Both androgen receptors and α -adrenoceptors are present in the prostate, and their physiological, pathological and therapeutic implications have been extensively documented (Cheng *et al.*, 1993; Furuya *et al.*, 1982; Gormley *et al.*, 1992; Lepor *et al.*, 1987; Matyus & Horvath, 1997). In principle, secretory and proliferative activities of the prostate are regulated by androgens, whilst the contractile properties of stromal cells are mediated *via* α -adrenoceptors, with α_{1a} -

Table 5 Effect of phenoxybenzamine pre-treatment on phenylephrine-induced contractions in strips of rat prostate ventral lobes

Treatment	No.	pEC_{50} (M)	E_{max} (%)
<i>Intact</i>			
Control	20	6.17 ± 0.04	100
Phenoxybenzamine 3 nM	4	$5.90 \pm 0.03^*$	107.0 ± 5.9
Phenoxybenzamine 10 nM	4	$5.59 \pm 0.16^{**}$	95.0 ± 11.2
Phenoxybenzamine 30 nM	4	$5.30 \pm 0.17^{**}$	93.5 ± 10.2
Phenoxybenzamine 100 nM	4	$4.79 \pm 0.06^{**}$	$53.0 \pm 5.7^{**}$
Phenoxybenzamine 300 nM	4	$4.66 \pm 0.15^{**}$	$37.3 \pm 5.5^{**}$
<i>Castrated</i>			
Control	6	5.69 ± 0.03	100
Phenoxybenzamine 3 nM	5	5.55 ± 0.09	97.6 ± 1.4
Phenoxybenzamine 10 nM	5	5.45 ± 0.05	93.9 ± 11.9
Phenoxybenzamine 30 nM	5	$5.12 \pm 0.04^{**}$	$62.8 \pm 7.9^{**}$
Phenoxybenzamine 100 nM	5	$4.58 \pm 0.12^{**}$	$33.9 \pm 4.2^{**}$
Phenoxybenzamine 300 nM	5	not calculated	$12.1 \pm 3.3^{**}$

After a control concentration-response curve for phenylephrine (0.01–1000 μ M) was obtained in tissue strips from prostate ventral lobes of intact and castrated rats, the strips were equilibrated with phenoxybenzamine at 3–300 nM for 10 min. After washing the strips for 2 h in Krebs solution, with sodium thiosulphate (100 μ M) during the first hour, contraction-response curves for phenylephrine were produced. Results are expressed as mean \pm s.e. mean of four to 20 experiments. pEC_{50} value decreased before maximal contraction size was suppressed, and the decrease was observed at lower doses of phenoxybenzamine in castrates. * $P < 0.05$, ** $P < 0.01$ versus control by Dunnett's test.

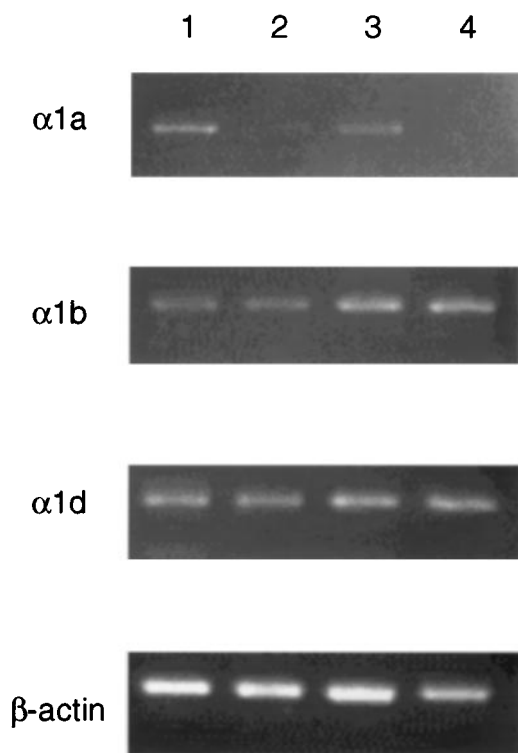


Figure 3 The effect of castration on α_1 -adrenoceptor mRNA expression in rat prostate. The expression of subtypes of α_1 -adrenoceptor mRNAs was evaluated by RT-PCR of total RNA from intact and castrated rat prostate using the primer set as described in Methods. In each panel PCR products using cDNA from rat prostate tissues as a template are shown. The band of specific primers for β -actin was used as a positive internal control, and assay without RNA or RT as a negative control. Lane 1; dorsal lobe of intact rat, lane 2; dorsal lobe of castrated rat, lane 3; ventral lobe of intact rat, lane 4; ventral lobe of castrated rat. Castration caused reduction in expression of α_{1a} -adrenoceptor mRNA but not α_{1b} or α_{1d} . The change was more pronounced in ventral lobes.

adrenoceptor predominating over other subtypes. There are, however, only a few reports of investigations on the interaction between prostate contractility and androgen level.

Using the rat castration model, Lacey *et al.* (1996) found an apparent 2 fold increase in prostatic α_1 -adrenoceptor density as a consequence of androgen deprivation in rats.

These authors speculated that this increase was an artefact due to a relative increase in the ratio of smooth muscle, which is mostly responsible for prostatic contraction, to epithelium in the prostate rather than from up-regulation of α_1 -adrenoceptors, and they concluded that α_1 -adrenoceptors are not influenced by androgen deprivation. Lin *et al.* (1995) reported a similar observation in dogs: α -adrenergic contractility was increased in castrates, which was attributed to elevation in the relative proportion of stromal tissue by the hormonal intervention. These authors also found no discernible changes in *in vivo* measurement of prostatic urethral pressure as a result of androgen deprivation. Conversely, testosterone supplementation to rats resulted in subtype non-specific reduction in α_1 -adrenoceptor density of the prostate, and again the reduction was explained simply by the decreased proportion of fibromuscular element in the entire prostate (Auger-Pourmarin *et al.*, 1998). However, castration dramatically reduced phenylephrine-induced increase in urethral pressure in rats (Akiyama *et al.*, 1999), and a neurohormonal treatment with dihydrotestosterone and prazosin increased prostate weight, its stiffness and urodynamic obstruction by the prostate (Lee *et al.*, 1998).

In our experiments, the effects of castration on α -adrenergic contractile response of the rat prostate were examined. Surgical castration resulted in a marked weight loss in both ventral and dorsal lobes of the prostate. Regarding contractile function, the maximal contractile response to K^+ and phenylephrine expressed as force per unit of tissue weight was several fold greater in castrated rats. This apparent increase in contractility is most likely explained by the change in tissue component of the prostate (Lacey *et al.*, 1996; Lin *et al.*, 1995). Upon castration, prostate epithelium undergoes degeneration, whilst stromal elements, the compartment responsible for the contractile property of the prostate, are preserved. Thus, the contraction force is greater in castrates when expressed per unit weight. Interestingly, however, we found that contraction potency, or pEC_{50} values, for phenylephrine were lowered in castrated rats. This could not be explained by preferential degeneration of epithelium, or by lower binding affinity of α -adrenoceptors, since pK_B values for prazosin in phenylephrine-induced contraction were not influenced by castration. Furthermore relatively high pK_B values for prazosin (8.78), when compared with canine (7.94, Suzuki *et al.*, 2000) or rabbit

(8.15, Martin *et al.*, 1997) prostate, would indicate that α_{1A} -adrenoceptor rather than a putative subtype, α_{1L} -adrenoceptor, is functionally important in rat prostate.

In the characterization and quantitative assay of α_1 -adrenoceptors of prostate membrane preparations, the K_d values for [3 H]-prazosin binding were 0.17 and 0.29 nM in intact and castrated rats, respectively, although this difference would not be of biological significance. The apparent increase in B_{max} values in castrates was also ambiguous because they were normalized to tissue weight or protein content, and these denominators were profoundly affected by the hormonal intervention. Inhibition assay of phenylephrine against [3 H]-prazosin binding indicated almost identical pK_i values for both treatment arms, although the functional data showing reduced sensitivity to phenylphrine by castration are more reliable and relevant to receptor affinity (Hieble *et al.*, 1997).

These results indicate that castration caused no essential changes in the affinity of phenylephrine to α_1 -adrenoceptor. We then hypothesized that spare receptors for α_1 -adrenoceptor, which have been demonstrated in the prostate (Hiraoka *et al.*, 1999), arteries (Fox & Friedman, 1987, Ruffolo & Yaden, 1984) and vas deferens (Fox & Friedman, 1987), would be present in intact rat prostate and might be down-regulated in castrated rats, explaining the lowered potency in castrates. To confirm this assumption, irreversible alkylation of α_1 -adrenoceptor was semi-quantitatively induced by phenoxybenzamine. Escalating alkylation of α_1 -adrenoceptor population from 3 nM to 300 nM produced a rightward shift in the contraction-response curves before depressing the maximal contractile force, and the suppression was readily detected at lower doses of phenoxybenzamine in castrated rats than in intact rats. The response characteristics to phenoxybenzamine treatment and the difference between intact and castrated rats may indicate that a receptor reserve for α_1 -adrenoceptor is present in intact prostate and that the reserve diminishes after castration. Subsequent RT-PCR study confirmed the expression of three types of α_1 -adrenoceptor, α_{1a} , α_{1b} and α_{1d} -adrenoceptors, in rat prostate, as has been reported previously (Roskosh *et al.*, 1994,

Scofield *et al.*, 1995). It was also shown that the expression of α_{1a} -adrenoceptor, but not α_{1b} - or α_{1d} -adrenoceptors, was down-regulated by castration. Considering the predominance of α_{1a} -adrenoceptor in the prostate in functional terms (Auger-Pourmarin *et al.*, 1998), down-regulation of α_{1a} -adrenoceptor may be of biological significance. To our knowledge this is the first report suggesting that spare receptors of α -adrenoceptors in the prostate are down-regulated by androgen deprivation and that the down-regulated spare receptors are α_{1a} -adrenoceptors.

The clinical implication of the presence of spare receptors in the prostatic smooth muscle cells, and its androgen dependency, is unclear. As far as α -adrenoceptor blocking agents in clinical use are concerned, since they are not irreversible inhibitors, the presence or absence of spare receptors should not alter the efficacy of these drugs on the contractility of prostate smooth muscle cells. In turn, the efficacy of contractility by adrenoceptor agonists may be affected by androgen deprivation. If affected, the proportion of the two components accounting for symptoms in BPH patients, a static component due to compression by the increased prostate volume and a dynamic component related to contraction of prostate smooth muscle cells (Caine, 1990), is influenced by androgen level. For example, the dynamic component of infravesical obstruction by the prostate is less important in men with a lower androgen level, i.e., in older men or men receiving antiandrogen treatment. However, extrapolating rat studies to human may be misleading, since rat prostate contains far less stromal components compared to human hypertrophied prostatic tissue, and this might have accentuated the effects of castration in rats. Clinically there was no discernible effect of a 5 α -reductase inhibitor on the therapeutic efficacy of an α_1 -adrenoceptor antagonist (Lepor *et al.*, 1996).

In conclusion, androgen deprivation suppressed α_1 -adrenergic contractility of rat prostate strips, and this suppression was associated with down-regulation of receptor reserves for the α_{1a} -adrenoceptor population. Further studies on androgen dependency of prostate contractility and the evaluation of its clinical relevance are warranted.

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