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Identification of α_1-adrenoceptor subtypes in the dog prostate

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Summary. The α_1-adrenoceptor subtypes of dog prostate were characterized in binding and functional experiments. In saturation experiments, [^{3}H]prazosin bound to α_1-adrenoceptors with high affinity. In the displacement experiments, unlabelled prazosin and WB4101 biphasically inhibited the binding of 400 pM [^{3}H]prazosin, suggesting the presence of at least two distinct affinity sites for prazosin or WB4101. The proportion of high-affinity sites was approximately 10%. HV723 also recognized two distinct affinity sites but the proportion of high-affinity sites was approximately 20%. From these results the presence of three distinct α_1-adrenoceptor subtypes was suggested: presumably subtypes α_{1A} (high affinity for prazosin and WB4101), α_{1N} (high affinity for only HV723) and α_{1L} (low affinity for the three antagonists) according to the recently proposed α_1-adrenoceptor subclassification. The density of subtype α_{1L} was much higher than that of subtypes α_{1A} and α_{1N} subtypes. In the functional experiments, prazosin, WB4101 and HV723 competitively antagonized the contractile response to noradrenaline with low affinities close to those estimated for the α_{1L} subtypes. These results suggest that the contractile response to noradrenaline in the dog prostate is mediated predominantly through α_{1L} subtype α-adrenoceptors.

Key words: α_1-Adrenoceptor subtype – Dog prostate

The secretory and contractile functions of the prostate are under the control of the autonomic nervous system [13, 24]. α-Adrenergic agonists produce a prominent contraction of the isolated prostate and increase urethral resistance of the prostatic region in vivo in the dog and human [3, 5, 8, 14, 23]. The α-adrenoceptors of the prostate are predominantly of α_1 subtype rather than α_2 subtype [4, 11].

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Radioligand binding studies with [^{3}H]prazosin or [^{125}I]BE2254 (2-[β-4-hydroxy-3-[^{125}I]iodophenyl)-ethylaminoethyl]-tetralone) originally demonstrated at least two separate populations of α_1-adrenoceptors in various tissues, which were designated α_{1A} and α_{1B}, respectively [9, 17]. The α_{1A} subtype has high affinity for WB4101, benoxathian and phentolamine, while the α_{1B} subtype has a lower affinity for the competitive antagonists. However, the two subtypes cannot be discriminated by prazosin and yohimbine [10]. In contrast, subsequent functional studies with blood vessels have suggested another subclassification, in which α_1-adrenoceptors can be classified into two (α_{1H}, α_{1L}) or three (α_{1H}, α_{1L} and α_{1N}) subtypes by their different affinities for prazosin and HV723 (α-ethyl-3,4,5-trimethoxy-α-[3-([2-(2-methoxyphenoxy)ethyl]-amino)-propyl]benzeneacetonitrile fumarate) [7, 19]. This evidence strongly suggests the existence of more than two original α_1-adrenoceptor subtypes (α_{1A} and α_{1B}). More recently, a reconciliation of these different subclassifications was proposed, whereby α_{1A} and α_{1B} subtypes can be included in the α_{1H} subtype of the α_{1H}, α_{1L}, α_{1N} subclassification [12, 20–22], because α_{1A} and α_{1B} subtypes can be identified as a single site with a high affinity for prazosin. Criteria for the α_1-adrenoceptor subclassification using competitive antagonists are shown in Table 1.

Table 1. A generalized α_1-adrenoceptor subclassification, showing the relative affinities of the subtypes for representative competitive antagonists

α_1 Subtype	Relative affinity		
	Prazosin	WB4101	HV723
α_{1H}			
α_{1A}	High	High	Medium or low
α_{1B}	High	Low	Low
α_{1L}	Low	Low	Low
α_{1N}	Low	Low	High

In the present study we have characterized the α_1-adrenoceptor subtypes of dog prostate in binding and mechanical experiments.

Materials and methods

Binding study

Prostates were isolated from mongrel dogs (10–15 kg) while they were under pentobarbital anaesthesia. The prostate was homogenized in 10 volumes of buffer (TRIS-HCl 50 mM, NaCl 100 mM, EDTA 2 mM, pH 7.4) using a polytron (setting 8, 15 s × 3) and glass potter (10 min). The homogenates were centrifuged at 10000 *g* for 10 min at 4°C. The supernatant was centrifuged at 80000 *g* for 40 min and the pellets resuspended in the same volume of assay buffer (TRIS-HCl 50 mM, EDTA 1 mM, pH 7.4). The pellet was centrifuged again and the resulting pellet resuspended in 10 volumes of buffer. All procedures for the preparation of membranes were conducted at 4°C, and ice-cold buffers were used. The final pellet was resuspended in assay buffer and used for the binding assay. The membranes were incubated with [^{3}H]prazosin for 30 min at 25°C. Incubation volume was 1 ml in all experiments. Reactions were terminated by rapid filtration through a Brandel cell harvester onto Whatman GF/C filters. The filters were then washed 4 times with 4 ml ice-cold 50 mM TRIS-HCl buffer (pH 7.4) and dried; the filter-bound radioactivity was determined. Non-specific binding was defined as binding in the presence of 1 μM prazosin or 10 μM phentolamine. Assays were conducted in duplicate.

Binding data were analysed by the weighted least-squares iterative curve-fitting program LIGAND [18]. The data were fitted first to a one-site and then to a two-site model, and if the residual sums of squares were statistically smaller for a two-site fit of the data than for a one-site fit, as determined by *F*-test comparison, then the two-site model was accepted. *P* values less than 0.05 were considered significant.

Proteins were assayed according to the method of Bradford using bovine serum albumin as standard [2].

Functional experiments

Dog prostate was isolated and cut into strips (2 mm wide, 2 mm thick, 15 mm long). The strips were mounted vertically in organ baths containing 20 ml Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, $NaHPO_4$ 1.2, glucose 11.5. The medium was maintained at 37°C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. A resting tension of 0.5 g was applied and the responses were recorded isometrically through a force-displacement transducer. The preparations were equilibrated for 90 min before starting the experiments.

Concentration-response curves for noradrenaline were obtained by adding the drug directly to the bathing media in cumulative fashion. Desmethylimipramine (0.1 μM), deoxycorticosterone acetate (5 μM) and propranolol (3 μM) were present throughout this series of experiments in order to block neuronal and extraneuronal uptake of noradrenaline and to block β-adrenoceptors, respectively. α-Adrenoceptor antagonists were present for 30 min before and during the time concentration-response curves were obtained.

The pK_B value was estimated according to Arunlakshana & Schild [1]. Briefly, the concentration of noradrenaline necessary to give a half-maximal responses in the presence of α-adrenoceptor antagonist was divided by the concentration giving a half-maximal response in the control to determine the agonist concentration ratio (CR). Data were plotted as –log antagonist concentration (M) vs log (CR–1), and pA_2 values were calculated from Schild plots along mean slope and 95% confidence limits (95% CL); straight lines were drawn by least squares linear regression. When the straight line had a slope of unity, the pA_2 value estimated was represented as pK_B [1].

Statistical analyses

Experimental values are given as the mean ± SEM. Results were analysed by Student's *t*-test and a probability of less than 0.05 was considered significant.

Drugs

The following drugs were used: [^{3}H]prazosin (specific activity 76.6 Ci/mmol; NEN, Boston, Mass., USA), prazosin hydrochloride (Taito-Pfizer, Tokyo, Japan), phentolamine mesylate (Ciba, Basel, Switzerland), WB4101 hydrochloride (2-(2,6-dimethoxy-phenoxy-ethyl)-aminomethyl-1,4-benzodioxane hydrochloride, Funakoshi, Tokyo, Japan) and HV723 (Hokuriku Seiyaku, Katsuyama, Fukui, Japan), desmethylimipramine hydrochloride (Sigma, St. Louis, Mo., USA), (–)-noradrenaline bitartate, deoxycorticosterone acetate and (±)-propranolol hydrochloride (Nacalai, Kyoto, Japan).

Results

Saturation experiments with [^{3}H]-prazosin

[^{3}H]prazosin at concentrations ranging from 20 to 3000 pM was used to label α_1-adrenoceptors of dog prostate. The specific binding was approximately 60% of the total binding at 400 pM [^{3}H]prazosin and showed a biphasic saturation curve at the concentrations tested (Fig. 1). A Scatchard plot of the binding data did not appear to be linear, but LIGAND analysis fitted the data to a one-site model better than a two-site model; the resultant affinity constant (pK_D) and number of maximal binding sites (B_{max}) were 9.20 ± 0.14 and 23.1 ± 9.9 fmol/mg protein, respectively. However, since the binding at lowest two points was scattered and two distinct affinity sites for prazosin were detected in displacement experiments (see below), we analysed the binding data obtained at higher concentrations of [^{3}H]prazosin (Fig. 1, inset dashed line) this resulted in lower estimates for the pK_D value (8.86 ± 0.17) and a higher B_{max} (127.8 ± 31.5 fmol/mg protein).

Effects of competitive antagonists on [^{3}H]prazosin binding

The pharmacological profile for [^{3}H]prazosin binding sites was examined in displacement experiments. When binding of 400 pM [^{3}H]prazosin was displaced by unlabelled prazosin and WB4101, shallow displacement curves were seen. Computerized analyses revealed that the antagonists discriminated between two distinct affinity sites (Table 2). Thus, the pK_I values for prazosin deviated from the single pK_D value (9.20 ± 0.14) obtained in the saturation experiments with [^{3}H]prazosin. The proportion of low-affinity sites for prazosin and WB4101 amounted to approximately 90% of the total binding sites

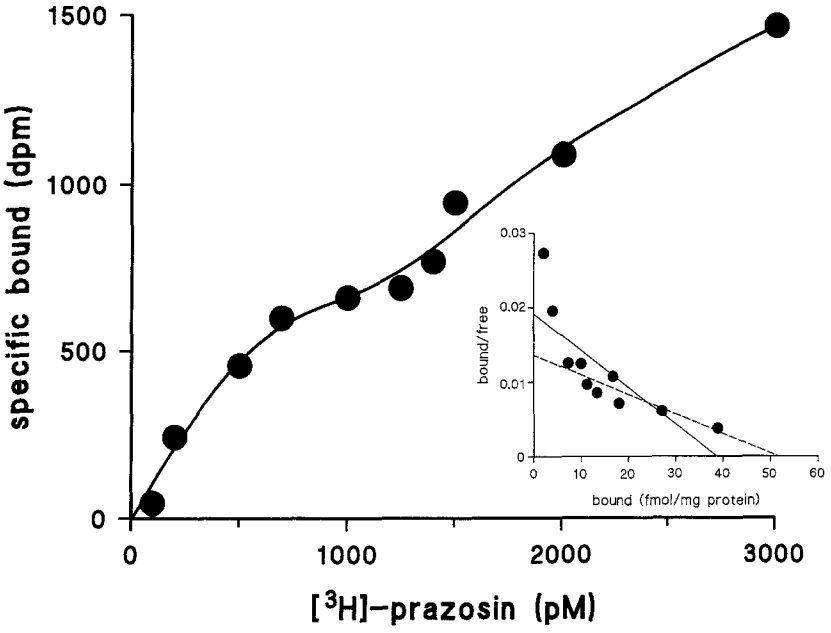

Fig. 1. Saturation curve of the specific [^{3}H]prazosin binding to dog prostate membranes and the Scatchard plot (*inset*). [^{3}H]prazosin 20–3000 pM was used. The data were obtained from a single experiment where each *point* is the mean of duplicate determinations. *Straight lines* and *dashed lines* in the *inset* were drawn according to a one-site model from all the data or the data other than the lowest two points, respectively (see text for explanation)

Table 2. Inhibition of 400 pM [^{3}H]prazosin binding to α_1-adrenoceptor of dog prostate

Antagonist	*n*	Slope factor	$pK_{I\,high}$	$pK_{I\,low}$	% low
Prazosin	6	0.51	10.02 ± 0.26	8.35 ± 0.39	92.5
WB4101	5	0.58	10.55 ± 0.11	8.29 ± 0.43	90.2
HV723	3	0.75	10.67 ± 0.27	8.14 ± 0.51	83.6

Data shown are mean ± SEM; *n*, number of experiments. Displacement experiments were done with 400 pM [^{3}H]prazosin
$pK_{I\,high}$ and $pK_{I\,low}$, negative log of the equilibrium dissociation constants (−log M) at prazosin high- and low-affinity sites for antagonists tested; % low, population binding at the low-affinity site compared with the total specific binding sites

Table 3. α_1-Adrenoceptor affinities for prazosin, WB4101 and HV723 in dog prostate (functional study)

Antagonist	pK_B	Slope (95% CL)
Prazosin	7.90 ± 0.07	1.071 (0.780 - 1.363)
WB4101	8.38 ± 0.23	1.293 (0.608–1.979)
HV723	8.13 ± 0.11	1.249 (0.872–1.626)

CL, Confidence limits

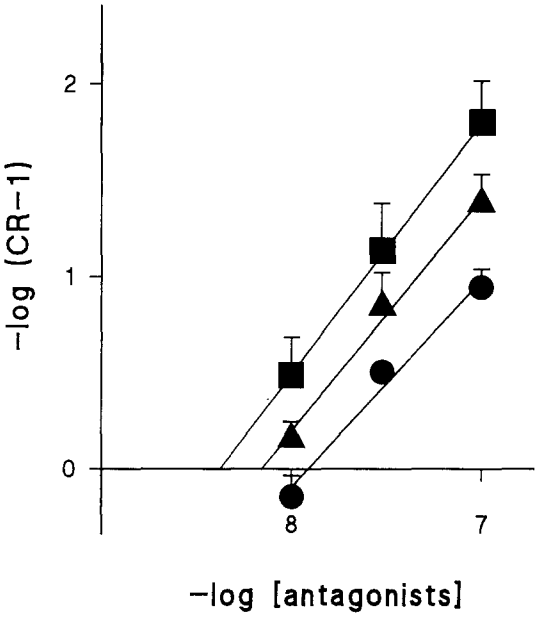

Fig. 2. Schild plots for the competitive inhibition of noradrenaline-induced contractions by prazosin (●), WB4101 (■) and HV723 (▲) in the dog prostate. Each *point* is the mean of data obtained from six to eight preperations and *vertical lines* show the SEM. For pK_B values and slopes see Table 3

(Table 2). HV723 also detected two distinct affinity sites, but the proportion of the low-affinity sites (approximately 80%) was lower than those for prazosin and WB4101.

Effects of prazosin, WB4101 and HV723 on noradrenaline-induced contractions in the dog prostate

Noradrenaline at concentrations in excess of 10^{-7} M produced a concentration-dependent contraction in the isolated dog prostates ($pD_2 = 5.91 \pm 0.09$, $n = 22$). The contractile responses to noradrenaline were attenuated by prazosin, WB4101 and HV723. Figure 2 shows the Schild plots. The slopes were close to unity for all the antagonists tested, indicating that the three antagonists competitively inhibited the contractile responses to noradrenaline. pK_B values ranging from 7.9 to 8.4 were estimated for the antagonists tested; the values agreed well with the low pK_I values obtained in the displacement experiments (Tables 2, 3).

Discussion

[^{3}H]Prazosin bound to the dog prostatic membranes with a high affinity and the binding was inhibited by three representative α_1-adrenoceptor antagonists (prazosin, WB4101 and HV723). These results indicate that the [^{3}H]prazosin binding sites display an α_1-adrenoceptor specificity.

In the saturation experiments a single affinity site for prazosin was recognized, whereas two distinct affinity sites for prazosin were detected in the displacement experiments. This discrepancy may be due to the proportion of prazosin high-affinity sites being low; accurate quantitation of a subpopulation of less than 10% is impossible with the computer analysis used [6, 15]. Therefore, the pK_D value (9.20 ± 0.14) obtained with a one-site model seems to be an intermediate value between the two pK_I values obtained in the displacement experiments. Since a predominance of prazosin low-affinity was found in the displacement experiments, we roughly estimated the binding parameters of the low-affinity site from the data obtained with high concentrations of [^{3}H]prazosin in the saturation experiments. As a result, a lower affinity constant ($pK_D = 8.86 \pm 0.17$) consistent with a low pK_i value (8.35 ± 0.39) in the displacement experiments and a higher density of α_1-adrenoceptors (127.8 ± 31.5 fmol/mg protein) were estimated. The density of α_1-adrenoceptors in dog prostate is close to the value for human prostate reported by Chapple et al. [4] but lower than the value reported by Yamada et al. [25, 26].

α_1-Adrenoceptors are not homogeneous and it has been suggested that they exist as several subtypes [7, 9, 10, 19]. As mentioned in the Introduction, two original subclassifications (the α_{1A}, α_{1B} subclassification and the α_{1H}, α_{1L}, α_{1N} subclassification) were recently reconciled because the α_{1A} and α_{1B} subtypes were recognized to be single high-affinity site for prazosin (α_{1H}) in the α_{1H}, α_{1L}, α_{1N} subclassification (Table 1) [21]. According to the generalized subclassification, the presence of three distinct α_1-adrenoceptor subtypes can be read from the results in Table 2. The proportions of α_{1A} (prazosin and WB4101 high-affinity site), α_{1N} (HV723 high-affinity site) and α_{1L} (low-affinity site for all the antagonists used) subtypes are estimated at 10%, 20% and 70% respectively of the total specific binding sites recognized by 400 pM [^{3}H]prazosin (Table 2). The high density of the α_{1L} subtype implies it has a more important role in the physiological response.

Functional studies reveal that prazosin, WB4101 and HV723 competitively antagonize the contractile response to noradrenaline with low affinities (pK_B) ranging from 7.9 to 8.4. Therefore, there is a good agreement between the pK_B values and the $pK_{I\,low}$ values in the displacement experiments, strongly suggesting that the contractile response to noradrenaline in the dog prostate is predominantly mediated through α_{1L} subtype adrenoceptors. However, a minor contribution of α_{1A} or α_{1N} subtypes cannot be ruled out completely.

We have shown previously that the contractile responses to noradrenaline of dog mesenteric and carotid arteries were mediated through α_{1N} and α_{1B} subtypes, respectively [19, 20]. This evidence, together with the present results, suggests a regional difference in α_1-adrenoceptor subtypes involved in noradrenaline-induced contraction.

Chapple et al. [5] have reported that the α_{1B} subtype comprises the majority of α_1-adrenoceptors in the human prostate, according to the criteria of the original α_{1A}, α_{1B} subclassification [9, 17]. The pA_2 values for prazosin reported are 9.1 [4] and 8.8 [16], which are much lower than the affinity constant of prazosin high-affinity sites (α_{1H}). More recently, we also confirmed similar lower sensitivity for prazosin of human prostate α_1-adrenoceptors (pA_2 = approximately 8.3: I. Muramatsu et al., unpublished observations). These results suggest that α_1-adrenoceptors involved in noradrenaline-induced contraction of human prostate may be predominantly α_{1L} subtype according to our generalized α_1-subclassification (Table 1).

In conclusion, the present study shows the existence of three distinct α_1-adrenoceptor subtypes in the dog prostate, presumably α_{1A}, α_{1L} and α_{1N} subtypes according to the recent α_1-adrenoceptor subclassification [12, 20–22]. It is likely that α_{1L} is the main subtype involved in noradrenaline-induced contraction of the dog prostate.

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References

1. Arunlakshana O, Schild HO (1959) Some quantitative uses of drug antagonists. Br J Pharmacol 14:48
2. Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248
3. Bruchini H, Schmidt RA, Tanagho EA (1978) The male genito-urinary sphincter mechanism in the dog. Invest Urol 15:284
4. Chapple CR, Aubry ML, James S, Greengrass PM, Burnstock G, Turner-Warwick RT, Milroy EJG, Davey MJ (1989) Characterization of human prostatic adrenoceptors using pharmacology receptor binding and localization. Br J Urol 63:487
5. Chapple CR, Burt RP, Marshall I (1991) α_1-Adrenoceptor subtypes in the human prostate and inferior epigastric artery. Neurorol Urodyn 10:306
6. De Lean A, Hancock AA, Lefkowitz RJ (1982) Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. Mol Pharmacol 21:5
7. Flavahn NA, Vanhoute PM (1986) α-Adrenoceptor subclassification in vascular smooth muscle. Trends Pharmacol Sci 7:347
8. Furuya S, Kumamoto Y, Yokoyama E, Tsukamoto T, Izumi T, Abiko Y (1982) Alpha-adrenergic activity and urethral pressure in prostatic zone in benign prostatic hypertrophy. J Urol 128:83
9. Han C, Abel PW, Minneman KP (1987) α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329:333
10. Hanft G, Gross G (1989) Subclassification of α_1-adrenergic receptor recognition sites by urapidil derivative and selective antagonists. Br J Pharmacol 97:691
11. Hieble JP, Caine M, Zalaznik E (1985) In vitro characterization of the α-adrenoceptors in human prostate. Eur J Pharmacol 107:111
12. Hiramatsu Y, Muraoka R, Kigoshi S, Muramatsu I (1992) 5-Methylurapidil may discriminate between α_1-adrenoceptors with a high affinity for WB4101 in rat lung. Br J Pharmacol 105:6

13. Kuru M (1965) Nervous control of micturation. Physiol Rev 45:425
14. MacGregor RJ, Diokno AC (1984) The alpha-adrenergic blocking action of prazosin hydrochloride on the canine urethra. Invest Urol 18:426
15. Molinoff PB, Wolfe BB, Weiland GA (1981) Quantitative analysis of drug-receptor interactions. II. Determination of the properties of receptor subtypes. Life Sci 29:427
16. Morita T (1991) Quantitative analyses of human prostatic α-adrenoceptors and effects of terazosin on the α-adrenoceptor activity. (abstract in English) J Smooth Muscle Res 27:149
17. Morrow AL, Creese I (1986) Characterization of α_1-adrenergic receptor subtype in rat brain: a reevaluation of [^{3}H]-WB4101 and [^{3}H]-prazosin binding. Mol Pharmacol 29:321
18. Munson PJ, Rodbard D (1980) LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 107:220
19. Muramatsu I, Kigoshi S, Ohmura T, Hashimoto S, Oshita M (1990) Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br J Pharmacol 99:197
20. Muramatsu I, Kigoshi S, Ohmura T (1992) Subtypes of α_1-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery. Jpn J Pharmacol 57:535
21. Ohmura T, Ohsita M, Kigoshi S, Muramatsu I (1992) Identification of α_1-adrenoceptor subtypes in the rat vas deferens. Br J Pharmacol 107:697
22. Oshita M, Kigoshi S, Muramatsu I (1991) Three distinct binding sites for [^{3}H]-prazosin in the rat cerebral cortex. Br J Pharmacol 104:961
23. Smith ER (1968) The stimulation of canine prostate secretion by parasympathomimetic agents. J Pharmacol Exp Ther 164:312
24. Wein AJ, Levin RM, Barrett DM (1987) Voiding function: relevant anatomy, physiology, and pharmacology. In: Gillenwater JY, Grayhack JT, Howards SS, Ducket JD (eds) Adult and pediatric urology. Year Book, Chicago, p 800
25. Yamada S, Ashizawa N, Ushizima H, Nakayama K, Hayashi E, Honda K (1987) Alpha-1 adrenoceptors in human prostate: characterization and alteration in benign prostatic hypertrophy. J Pharmacol Exp Ther 242:326
26. Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, Maruyama M, Kawabe K (1991) Binding characteristics of naftopidil and α-adrenoceptors in benign prostatic hypertrophy. Life Sci 50:127