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Binding and functional characterization of α_1-adrenoceptor subtypes in the rat prostate

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Abstract

The α_1-adrenoceptor subtypes of rat prostate were characterized in binding and functional experiments. In binding experiments, [^{3}H]tamsulosin bound to a single class of binding sites with an affinity (pK_D) of 10.79 ± 0.04 and B_{max} of 87 ± 2 fmol mg^{-1} protein. This binding was inhibited by prazosin, 2-(2,6-dimethoxy-phenoxyethyl)-aminomethyl-1,4-benzodioxane hydrochloride (WB4101), 5-methylurapidil, α-ethyl-3,4,5,-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)benzeneacetonitrile fumarate (HV723) and oxymetazoline with high efficacy, resulting in a good correlation with the binding characteristics of cloned α_{1a} but not α_{1b} and α_{1d}-adrenoceptor subtypes. In functional studies, noradrenaline and oxymetazoline produced concentration-dependent contractions. These contractions were antagonized by tamsulosin, prazosin, WB4101 and 5-methylurapidil with an efficacy lower than that exhibited by these agents for inhibition of [^{3}H]tamsulosin binding. The relationship between receptor occupancy and contractile amplitude revealed the presence of receptor reserve for noradrenaline, but the contraction induced by oxymetazoline was not in parallel with receptor occupation and developed after predicted receptor saturation. From these results, it is suggested that α_{1A}-adrenoceptors are the dominant subtype in the rat prostate which can be detected with [^{3}H]tamsulosin, but that the functional subtype mediating adrenergic contractions has the characteristics of the α_{1L}-adrenoceptor subtype, having a lower affinity for prazosin and some other drugs than the α_{1A}-adrenoceptor subtype.

Keywords: Prostate; (Rat); α_1-Adrenoceptor subtype, α_{1A}, α_{1L}; Oxymetazoline

1. Introduction

The human prostate receives a dense sympathetic innervation (Chapple et al., 1991) and the tone of prostate smooth muscle is predominantly regulated through postjunctional α_1-adrenoceptors by noradrenaline released from sympathetic nerves (Furuya et al., 1982; Hieble et al., 1985; Guh et al., 1995). One of the mechanisms of the voiding dysfunction seen in patients with benign prostatic hypertrophy is contraction of smooth muscle components of the hypertrophic prostatic adenoma causing outlet obstruction. α_1-Adrenoceptor antagonists have been proven to be useful in the symptomatic relief of patients with benign prostatic hypertrophy (Caine, 1986; Kirby, 1989; Jardin et al., 1991).

Three subtypes of α_1-adrenoceptors have been characterized by using functional (pharmacological), structural and transductional criteria, i.e., α_{1A}, α_{1B} and α_{1D}-adrenoceptor subtypes. The corresponding cloned, recombinant α_1-adrenoceptors are denoted by the same subscripts but with lowercase letters, α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995; Michel et al., 1995). These three receptor subtypes have a high affinity for prazosin. An additional subtype (α_{1L}-adrenoceptor) showing low affinity for prazosin has been proposed on the basis of the results of functional and pharmacological studies (Muramatsu et al., 1990, 1995; Oshita et al., 1993; Ohmura and Muramatsu, 1995). However, to date, no gene corresponding to the α_{1L}-adrenoceptor has been cloned, and it has been proposed that the receptor represents a particular conformational state of the α_{1A}-adrenoceptor subtype (Ford et al., 1997).

Each of these subtypes has been observed to have a distinct pattern of expression in different tissues. In partic-

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ular, among the α_1-adrenoceptors with a high affinity for prazosin, the α_{1a}-adrenoceptor subtype has been shown to be preferentially expressed in the human prostate (Hirasawa et al., 1993; Price et al., 1993; Weinberg et al., 1994). Thus, some research groups have suggested that the α_{1A}-adrenoceptor might be the predominant subtype responsible for the adrenergic response in prostatic tissues (Marshall et al., 1992; Lepor et al., 1993; Michel et al., 1993). However, we previously demonstrated that the α_{1L}-adrenoceptor subtype may be the functional subtype in the prostate of humans (Muramatsu et al., 1994), rabbits (Hiraoka et al., 1995) and dogs (Ohmura et al., 1993) because of the low efficacy of prazosin and other α_1-adrenoceptor antagonists in inhibiting the contractile responses to noradrenaline (Ford et al., 1996).

In the present study, we carried out binding and mechanical experiments and characterized the α_1-adrenoceptor subtypes of the rat prostate.

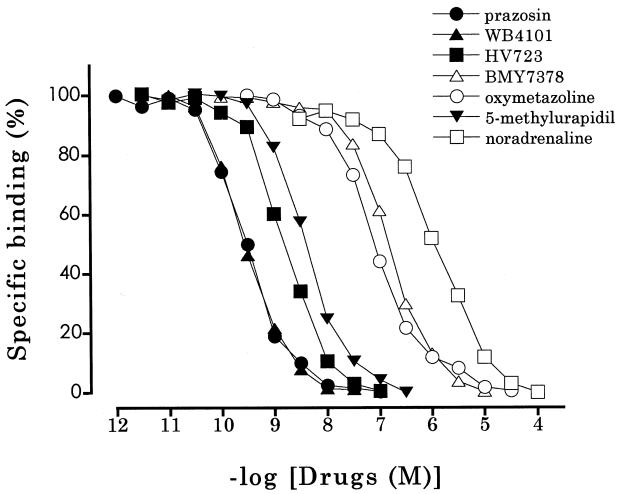

Fig. 2. Inhibition of 100 pM [^{3}H]tamsulosin binding to rat prostate membranes by various α_1-adrenoceptor agonists and antagonists. Each point was the mean of duplicate determinations. Similar results were obtained in the other 3 experiments.

2. Materials and methods

2.1. Binding study

Under pentobarbital anesthesia, the prostate glands were isolated from male Sprague–Dawley rats (220–320 g). The prostates were minced and homogenized in 10 volume of buffer (Tris–HCl 50 mM, NaCl 100 mM, EDTA 2 mM, pH 7.4) using a polytron (setting 8, 15 s $\times$ 6). The homogenate was centrifuged at 1000 $\times$ *g* for 10 min at 4°C. The supernatant was centrifuged at 80,000 $\times$ *g* for 40 min and the pellets were resuspended in the same volume of assay buffer (Tris–HCl 50 mM, EDTA 1 mM, pH 7.4). The pellet was centrifuged again as described above. The final pellet was resuspended in 10 volumes of the assay buffer and used for the binding assay. All procedures to prepare membranes were conducted at 4°C, and ice-cold buffers were used. The membranes were incubated with [^{3}H]tamsulosin for 30 min at 25°C. The incubation volume was 2 ml in all experiments. Reactions were terminated by rapid filtration through Whatman GF/C filters, using a Brandel cell harvester. The filters were washed 3 times with 4 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4) and dried, and the filter-bound radioactivity was determined. Non-specific binding was defined as binding in the presence of 0.3 μM prazosin. Assays were conducted in duplicate. Proteins were assayed according to the method of Bradford, using bovine serum albumin as standard (Bradford, 1976).

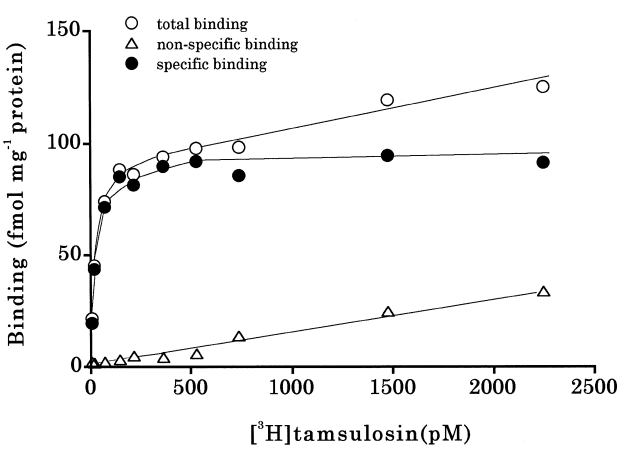

Fig. 1. Representative saturation curve of [^{3}H]tamsulosin binding to rat prostate membranes. [^{3}H]Tamsulosin at concentrations ranging from 10 pM to 2500 pM was used. Non-specific binding was defined as binding in the presence of 0.3 μM prazosin. Each point was the mean of duplicate determinations. The figure shows a representative example of 5 experiments.

2.2. Functional experiments

Rat prostates were isolated and cut into strips (2 mm wide, 2 mm depth, 10 mm length). The strips were mounted vertically in organ baths containing 20 ml of modified Krebs–Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, NaH_2PO_4 1.2, and 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 the experiments were started.

Concentration–response curves for noradrenaline and oxymetazoline were obtained by adding the drug directly to the bathing media in a cumulative fashion. Desmethylimipramine (0.1 μM), deoxycorticosterone acetate (1 μM) and propranolol (3 μM) were present throughout this series of experiments in order to block the neuronal and

Table 1
Inhibition of 100 pM [^{3}H]tamsulosin binding to α_1-adrenoceptors of rat prostate

Drug	n	pK_I	Slope factor
Prazosin	4	10.27 ± 0.02	1.01
WB4101	4	10.28 ± 0.04	0.90
HV723	4	9.62 ± 0.04	1.13
5-Methylurapidil	4	9.10 ± 0.05	0.94
BMY7378	4	7.28 ± 0.11	1.03
Oxymetazoline	4	8.19 ± 0.15	0.82
Noradrenaline	4	6.02 ± 0.08	1.04

Data shown are means ± S.E.M.
n = number of experiments.
Displacement experiments were done with 100 pM [^{3}H]tamsulosin.

extraneuronal uptake of noradrenaline and to block β-adrenoceptors, respectively. α-Adrenoceptor antagonists were present for 30 min before and while the concentration–response curves were recorded.

The pA_2 value was estimated according to the method of Arunlakushana and Schild (1959). Briefly, the concentration of noradrenaline necessary to give a half-maximal response in the presence of α-adrenoceptor antagonist was divided by the concentration giving a half-maximal response in the control condition to determine the agonist concentration ratio (CR). Data were plotted as the $-\log$ (CR-1) and pA_2 values were calculated from Schild plots, and 95% confidence limits (95% CL) and straight lines were drawn by least-squares linear regression (Arunlakushana and Schild, 1959). When the slope of the Schild plot was significantly different from unity, distinct pK_B values were estimated from the antagonism induced by two different concentrations of antagonist by the concentration-ratio method (Furchgott, 1972).

2.3. Data analysis

The saturation curves (Scatchard analysis) were analyzed using the EBDA program (Biosoft, Elsevior) (Mcpherson, 1985) to determine the dissociation constant (K_D) and maximum number of binding sites (B_{max}) for [^{3}H]tamsulosin. Displacement binding data were first analyzed by using the EBDA program. When the slope factor were close to unity, the calculated IC_{50} values were converted to K_I values by using the Cheng–Prusoff approximation (Cheng and Prusoff, 1973).

Receptor occupation was calculated with the following equation:

$$\text{receptor occupancy}\ (\%) = 100 \times [L]/(K_I + [L]),$$

where $[L]$ is the concentration of agonist which induced contraction, and K_I is the equilibrium dissociation constant for the agonist obtained in the binding experiments.

Experimental values are given as means ± S.E.M.

2.4. Drugs

The following drugs were used: [^{3}H]tamsulosin (specific activity 39.1 Ci/mmol, NEN, Boston, MA, USA), prazosin hydrochloride and desmethylimipramine hydrochloride (Sigma, St. Louis, MO, USA), 2-(2,6-dimethoxy-phenoxyethyl)-aminomethyl-1,4-benzodioxane hydrochloride (WB4101), 5-methylurapidil hydrochloride, 8-[2-[4-(2-

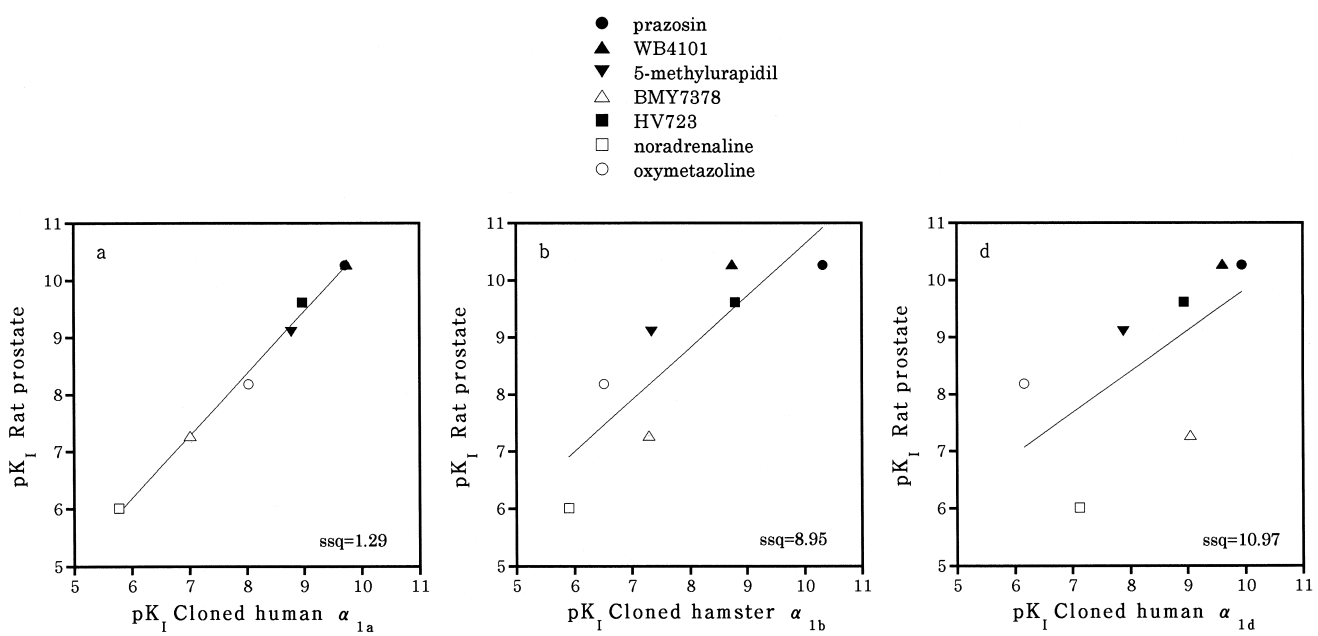

Fig. 3. Correlation plots of the binding potency of the α_1-adrenoceptor antagonists and agonists in the rat prostate membranes vs. those for the recombinant α_{1a}-adrenoceptor (left panel), α_{1b}-adrenoceptor (middle panel) and α_{1d}-adrenoceptor (right panel). The pK_I values from Table 1 were compared with the pK_I values for cloned α_1-adrenoceptor subtypes taken from the data of Suzuki et al. (1997).

methyoxy-phenyl)-L-piperazinyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY7378) and oxymetazoline hydrochloride (Research Biochemicals International, Natick, MA, USA), α-ethyl-3,4,5,-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)benzeneacetonitrile fumarate (HV723) (Hokuriku Seiyaku, Katsuyama, Fukui, Japan), L-noradrenaline bitartrate, deoxycorticosterone acetate and (±)-propranolol hydrochloride (Nacalai tesque, Kyoto, Japan).

3. Results

3.1. Saturation experiments with [^{3}H]tamsulosin

[^{3}H]Tamsulosin at concentrations ranging from 10 to 2500 pM was used to label α_1-adrenoceptors of the rat prostate membranes. Fig. 1 shows a saturation curve. The specific binding was more than 90% of the total binding at concentrations of 10–700 pM when the non-specific binding was defined as binding in the presence of 0.3 μM prazosin. Specific binding increased as the ligand concentration increased (Fig. 1, closed circle). The Scatchard plot of the data was linear, suggesting a single class of binding sites with a pK_D value of 10.79 ± 0.04, and a B_{max} value of 87 ± 2 fmol mg^{-1} protein, respectively ($n = 5$).

3.2. Effects of α-adrenoceptor antagonists and agonists on [^{3}H]tamsulosin binding

The pharmacological profile for [^{3}H]tamsulosin binding sites was examined in displacement experiments. In rat prostate membranes, prazosin, WB4101, HV723 and 5-methylurapidil inhibited the binding of 100 pM [^{3}H]tamsulosin concentration-dependently (Fig. 2). The computerized analysis revealed that prazosin, WB4101, HV723 and 5-methylurapidil competed for binding to the single class of binding sites, showing relatively high pK_I values (Table 1). BMY7378 displaced the binding of 100 pM [^{3}H]tamsulosin in a monophasic manner with relatively low pK_I value. The α_1-adrenoceptor agonists, oxymetazoline and noradrenaline, also displaced the binding of 100 pM [^{3}H]tamsulosin in a monophasic manner; the pK_I values were 8.19 ± 0.15 and 6.02 ± 0.08, respectively.

These affinities of various α-adrenoceptor antagonists and agonists were compared to those determined with [^{3}H]tamsulosin for membranes from COS-7 cells that express human α_{1a}, hamster α_{1b} or rat α_{1d}-adrenoceptors (Suzuki et al., 1997). As shown in Fig. 3, there was a good

Table 2
α_1-Adrenoceptor affinity for prazosin, WB4101, HV723, 5-methylurapidil, BMY7378 and tamsulosin in the rat prostate (functional study)

Antagonist	n	pA_2 or pK_B[a]	Slope (95% CL)
Prazosin	11	8.55 ± 0.06	0.91 (0.78–1.04)
WB4101	8	8.71 ± 0.16	0.86 (0.49–1.22)
HV723	19	8.28 ± 0.07	0.99 (0.87–1.12)
5-Methylurapidil	12	8.17 ± 0.07	1.09 (0.84–1.33)
BMY7378	10	6.62 ± 0.12	0.85 (0.78–1.04)
Tamsulosin	10	10.27 ± 0.04[a]	1.39 (1.19–1.60)

Data shown are means ± S.E.M.
n = number of experiments.
CL: Confidence limits.
[a] Since the slope factor in the Schild plot was significantly different from unity, pK_B values were estimated from the inhibitory effect of tamsulosin (0.3 and 1 nM), according to the method proposed by Furchgott (1972).

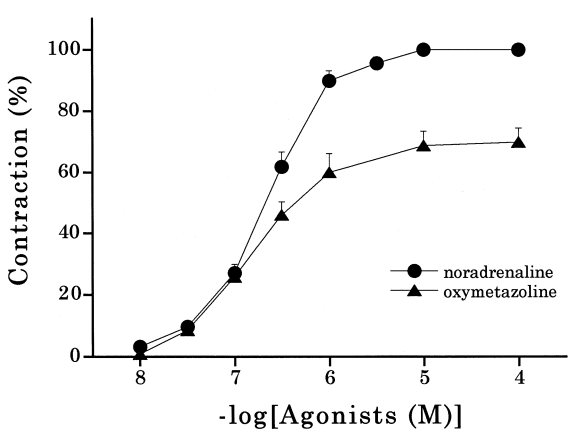

Fig. 4. Concentration–response curves for noradrenaline and oxymetazoline in the rat prostate preparations. The maximal contraction induced by noradrenaline in each preparation was taken as 100%. Each point was the mean ± S.E.M. of 14 experiments.

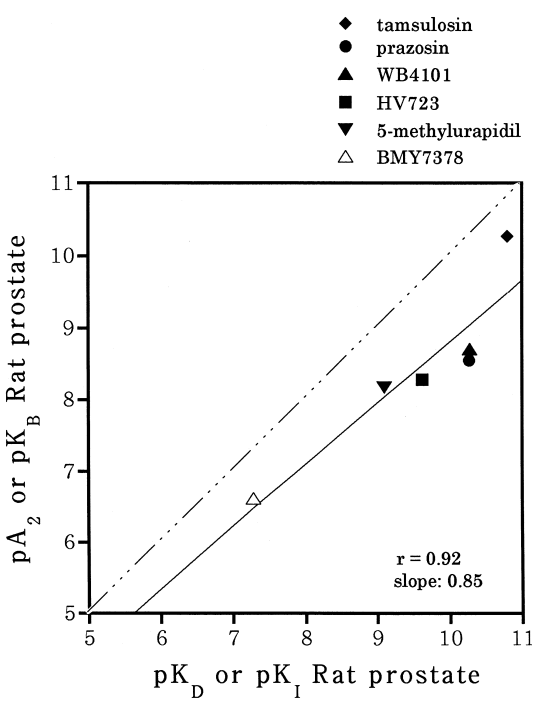

Fig. 5. Correlation between the functional potencies of the α_1-adrenoceptor antagonists and their binding affinities in the rat prostate. The functional potencies from Table 2 were plotted against the binding affinities from Table 1. In the case of tamsulosin the pK_D value of [^{3}H]tamsulosin was used as the binding affinity.

correlation between the binding affinity for the rat prostate membranes and that for human α_{1a}-adrenoceptors (sums of squares [ssq] = 1.29), as compared with α_{1b} and α_{1d}-adrenoceptors.

3.3. Functional responses to α-adrenoceptor agonists

Noradrenaline and oxymetazoline produced concentration-dependent contractions in the rat prostate (Fig. 4). The maximum contraction induced by oxymetazoline was approximately 70% of the noradrenaline-induced response in each preparation. The pD_2 values for noradrenaline and oxymetazoline were 6.57 ± 0.06 and 6.76 ± 0.05 ($n = 14$), respectively.

3.4. Effects of various antagonists on noradrenaline-induced contractions in the rat prostate

Concentration–response curves for noradrenaline were inhibited by various α_1-adrenoceptor antagonists. The slope factors in Schild plots were close to unity, except for that for tamsulosin. The resulting pA_2 values for prazosin, WB4101, HV723 and 5-methylurapidil were less than 9 (Table 2). The pA_2 value for BMY7378 was low (6.62). The slope factor in Schild plots for tamsulosin was significantly different from unity (1.39, 95% confidence limit: 1.19–1.60). An apparent pK_B value for tamsulosin was calculated from the antagonism produced by single concentrations of tamsulosin (0.3 and 1 nM), giving a value of 10.27 ± 0.04 ($n = 10$). These affinity estimates were compared with those obtained in the binding experiments with the rat prostate membranes. There was a good relationship between both estimates but affinity was lower in the functional study than in the binding study (Fig. 5). Oxymetazoline added at 30 nM for 30 min had little effect on the contractile response to noradrenaline (Fig. 6).

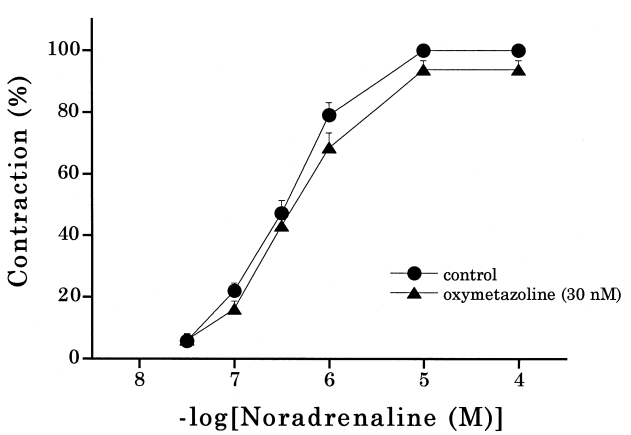

Fig. 6. Effects of oxymetazoline on the contractile response to noradrenaline in the rat prostate preparations. Rat prostate specimens were treated with 30 nM of oxymetazoline for 30 min before and during recording of the contraction elicited by noradrenaline. The maximal contraction of rat prostate induced by noradrenaline was taken as 100%. Each value was the mean ± S.E.M. of 7 experiments.

4. Discussion

In the present study, [^{3}H]tamsulosin bound to the α_1-adrenoceptors in rat prostate membranes in a monophasic manner. The affinity for [^{3}H]tamsulosin was high (pK_D: 10.79 ± 0.04), which was in good agreement with the affinities reported previously (Yazawa and Honda, 1993). However, the B_{max} value (87 ± 2 fmol mg^{-1} protein) was less than the value determined for [^{3}H]prazosin in the prostate of other species (Ohmura et al., 1993; Muramatsu et al., 1994; Hiraoka et al., 1995). Recently, Michel and Goepel (1998) also reported that in several rat tissues [^{3}H]tamsulosin had a smaller B_{max} than [^{3}H]prazosin.

[^{3}H]Tamsulosin binding sites were inhibited by prazosin, WB4101 and 5-methylurapidil, which show high (subnanomolar) affinity for the α_{1A}-subtype. Oxymetazoline also displaced [^{3}H]tamsulosin binding with a relatively high efficacy, which is consistent with its affinity at the recombinant α_{1a}-adrenoceptor subtype (Schwinn et al., 1990; Michel et al., 1995). Furthermore, there was a good correlation between the affinities of tested compounds in the rat prostate membranes and those for α_{1a}, but not for α_{1b} and α_{1d}-adrenoceptors (Fig. 3). From these results, it is considered that the [^{3}H]tamsulosin binding sites of rat prostate membranes correspond to the α_{1A}-subtype, according to recent criteria for α_1-adrenoceptor classification (Hieble et al., 1995; Muramatsu et al., 1995). Indeed, nonradioactive tamsulosin has been shown to have high affinity for α_{1A}-adrenoceptors (Hanft et al., 1989; Gracia-Sainz et al., 1995).

In the functional study, however, tamsulosin, prazosin, WB4101, 5-methylurapidil, HV723 and BMY7378 (α_{1D}-selective antagonist) antagonized the contractile response to noradrenaline with lower pA_2 values than the pK_I values obtained in the binding experiments (maximum 50-fold difference in affinity). How do we explain this difference between the functional and binding affinity of these drugs?

One explanation for the discrepancy is the existence of different α_1-adrenoceptor subtypes. An additional α_1-adrenoceptor subtype, designated α_{1L} on the basis of its relatively low affinity for prazosin, has been proposed (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990, 1995; Oshita et al., 1993; Ohmura et al., 1993). The possible involvement of the α_{1L}-adrenoceptor subtype in functional responses has been demonstrated in urinary tissues of several species (Ohmura et al., 1993; Muramatsu et al., 1994; Hiraoka et al., 1995; Kenny et al., 1996; Marshall et al., 1996; Leonardi et al., 1997; Martin et al., 1997; Testa et al., 1997; Van der Graff et al., 1997). Recently, it was demonstrated that the α_{1A}-selective antagonists RS-17053 and SNAP5089 showed a low potency in inhibiting the contractile response to noradrenaline in human prostate and urethra (Ford et al., 1996; Kenny et al., 1996; Marshall et al., 1996). However, the α_{1L}-adrenoceptor subtype was not detected in the present binding experi-

ments. The lack of binding to the α_{1L}-adrenoceptor subtype may be because of the selective labeling of the α_{1A}-adrenoceptor subtype by [^{3}H]tamsulosin, which exhibits high selectivity for the α_{1A}-adrenoceptor subtype, or because the population of α_{1L}-adrenoceptors was too small. In the present study, tamsulosin antagonized the contractile response to noradrenaline with a slightly lower potency (pK_B; 10.27 ± 0.04) than it showed in the binding study (pK_D; 10.79 ± 0.04). As with the α_{1L}-adrenoceptor subtype, there was no binding to α_{1B} and α_{1D}-adrenoceptor subtypes, even though mRNA for both subtypes has been detected (Scofield et al., 1995).

Another explanation is that, as suggested recently, the α_{1L}-adrenoceptor may be a different conformer of the α_{1A}-adrenoceptor subtype; the same α_{1A}-adrenoceptor gene product can display the pharmacological properties of both α_{1L}-adrenoceptors and α_{1A}-adrenoceptors (Ford et al., 1997). The relatively good relationship between binding and functional affinity (Fig. 5) may support this suggestion.

Oxymetazoline, which showed a high affinity for the α_{1A}-adrenoceptor subtype in the binding study, also caused a contractile response in the rat prostate. In order to further characterize the α_1-adrenoceptor subtype involved in the contraction of the rat prostate, we estimated receptor occupancy from the pK_I values obtained in displacement experiments and plotted it against the functional response. A hyperbolic relation for noradrenaline (Fig. 7(a), open circles) indicated a significant amount of receptor reserve for noradrenaline in this tissue. However, with oxymetazoline there was a poor correlation between receptor occupancy and functional response: oxymetazoline could not evoke a contraction even with 60% occupancy and the contractile response to oxymetazoline developed only after over 95% occupancy of the binding sites (Fig. 7(b), open circles). These results make it difficult to believe that the contractile response to oxymetazoline is simply mediated through α_{1A}-adrenoceptors with a high affinity for oxymetazoline. As mentioned in the results, the functional affinity of the tested drugs was consistently lower than the binding affinity but a good correlation between both values was seen. Thus, it is possible that oxymetazoline and noradrenaline also have a lower affinity in the functional state. We therefore extrapolated the functional affinity for both drugs from the relationship shown in Fig. 5 (5.41 and 7.27 for noradrenaline and oxymetazoline, respectively). However, the predicted occupancy–contraction curve (Fig. 7, open triangles) still showed a poor relationship for oxymetazoline but not for noradrenaline. This was also in contrast to the reasonable correlation between receptor occupancy and contractile amplitude (Fig. 7, closed circles) in which pK_a values (5.7 and 6.3 for noradrenaline and oxymetazoline, respectively) for the α_{1L}-adrenoceptor subtype of the rabbit aorta were used (I. Muramatsu, unpublished data). From these results it seems that adrenergic contractions of the rat prostate are not mediated through α_{1A}-adrenoceptors with high affinity for prazosin, oxymetazoline and other drugs. Indeed, oxymetazoline at 30 nM (a significantly higher than K_I at α_{1A}-adrenoceptor) failed to inhibit the contractile response to noradrenaline (Fig. 6).

What are the physiological functions of the α_{1A}-adrenoceptors which predominantly occur in the rat prostate? Chen et al. (1995) reported an inhibitory effect of α_{1A}-adrenoceptors on the growth of cultured vascular smooth muscle cells. In the rat, α_{1A}-adrenoceptors may also be involved in the growth of prostatic tissue.

In conclusion, the present study shows that the α_1-adrenoceptors of rat prostate are almost exclusively of the α_{1A}-adrenoceptor subtype, as detected with [^{3}H]tamsulo-

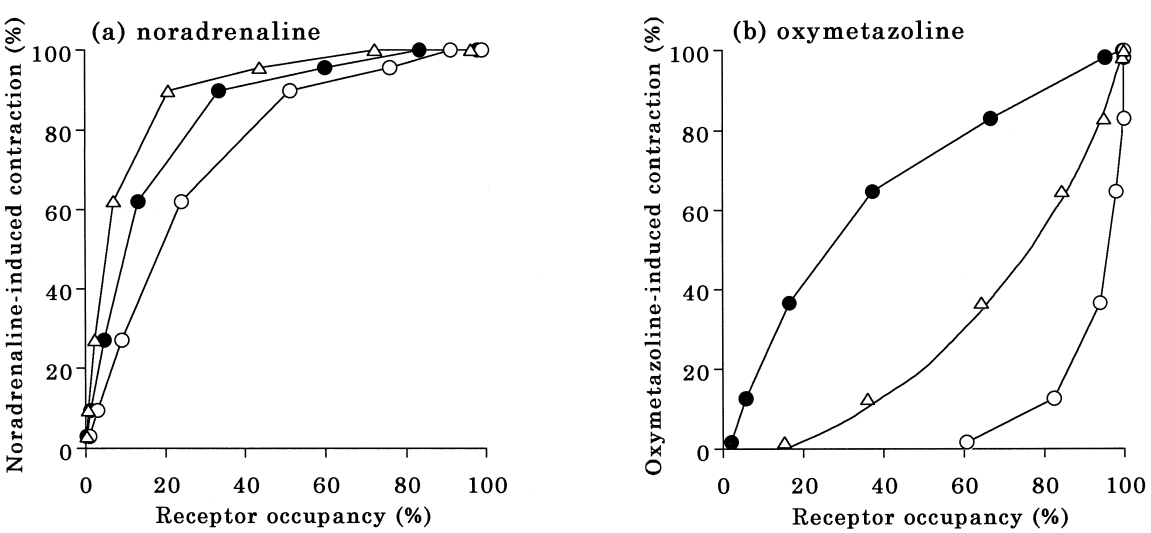

Fig. 7. Relation between receptor occupancy and the contraction induced by noradrenaline (a) and oxymetazoline (b). Each receptor occupancy was calculated by using the pK_I value from Table 1 (open circle), the affinity predicted from the relationship shown in Fig. 5 (open triangle), or the pK_a value for the α_{1L}-subtype of rabbit thoracic aorta for each agonist (closed circle), respectively. The maximal contraction of rat prostate induced by each agonist was taken as 100%. See Section 4 for further explanation.

sin, but that the functional subtype mediating adrenergic contractions has the characteristics of the α_{1L}-adrenoceptor subtype, with a lower affinity for prazosin and some drugs than the α_{1A}-adrenoceptor subtype.

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