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High-affinity specific [^{3}H]tamsulosin binding to α_1-adrenoceptors in human prostates with benign prostatic hypertrophy

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Abstract The binding of a novel radioligand, [^{3}H]tamsulosin, to human prostatic membranes with benign prostatic hypertrophy (BPH) has been characterized. [^{3}H]Tamsulosin rapidly associated with its binding sites in human prostatic membranes with BPH, and the binding reached steady state by 30 min at 25° C. The rate constants for association and dissociation of [^{3}H]tamsulosin binding were calculated to be 0.21 ± 0.05/nM per minute and 0.01 ± 0.004/min, respectively. The specific binding of [^{3}H]tamsulosin in human prostatic membranes was saturable and of high affinity ($K_d = 0.04 \pm 0.01$ nM). The density of [^{3}H]tamsulosin-binding sites (B_{max}) was 409 ± 28 fmol/mg protein. The K_d and B_{max} values for [^{3}H]tamsulosin binding in human prostates were significantly lower than those for [^{3}H]prazosin binding. [^{3}H]tamsulosin binding was remarkable for its significantly lower degree of nonspecific binding. Six α-adrenoceptor antagonists competed with [^{3}H]tamsulosin for the binding sites in the rank order: tamsulosin > WB4101 > prazosin > S-(+)-isomer > naftopidil > yohimbine. The binding affinities (pK_i) of these antagonists for [^{3}H]tamsulosin binding in human prostates closely correlated with their pharmacological potencies (pA_2) in prostates. In conclusion, [^{3}H]tamsulosin selectively labels α_1-adrenoceptors in human prostates, and thus may become a useful radioligand for the further analysis of these receptors.

Key words [^{3}H]Tamsulosin · Radioreceptor assay · Human prostates · α_1-antagonists

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Alpha$_1$-adrenoceptor antagonists have been demonstrated to be effective in the treatment of bladder outlet obstruction in benign prostatic hypertrophy (BPH). These drugs may increase urinary flow rate possibly by reducing the prostatic urethral resistance in BPH. The stimulation of prostatic α_1-adenoceptors by norepinephrine and phenylephrine causes a marked contractile response which may increase bladder outlet resistance in vivo [2, 6, 12, 23]. The biochemical characterization of prostatic α_1-adrenoceptors is important for the investigation of the etiology and pharmacological management of urinary obstruction in BPH.

Currently, several radiolabeled α_1-adrenoceptor antagonists, including [^{3}H]prazosin, [^{125}I]HEAT (iodo-2-[(β-hydroxyphenyl)-ethylaminomethyl]tetralone) and [^{3}H]bunazosin, have been introduced as selective radioligands to identify α_1-adrenoceptor sites in human prostates and also to examine binding affinities of α_1-antagonists to these receptors [5, 10, 13, 14, 16, 25]. However, one difficulty with [^{3}H]prazosin in human tissues appears to be the relatively high level of nonspecific binding found, particularly at high concentrations.

Tamsulosin (YM617, R-(−)-5-[2-[[2(*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxy-benzene-sulfonamide hydrochloride) has been shown to possess an extremely potent α_1-adrenoceptor blocking action in the aorta, lower urinary tract and prostate of rabbits [7–9]. Based on the pA_2 values for the inhibition of norepinephrine- or phenylephrine-evoked contractile responses in these tissues, tamsulosin was a 30 to 50 times more potent antagonist of α_1-adrenoceptors than prazosin. In clinical studies, the irritative and obstructive symptoms caused by BPH were decreased by tamsulosin, and urodynamic parameters were markedly improved [11]. Furthermore, this drug produced neither orthostatic hypotension nor a decrease in blood pressure in patients with mild BPH. Yamada et al. [25–27] and Lepor et al. [15] have demonstrated that tamsulosin is a potent inhibitor of α_1-adrenoceptor-binding sites in human prostates with BPH labeled by [^{3}H]prazosin and [^{3}H]bunazosin. Thus, it is of great interest to investigate directly the binding properties

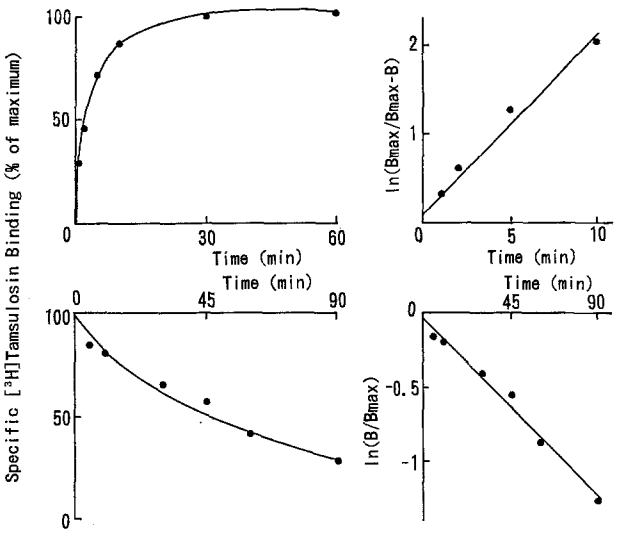

Fig. 1 Time course of association and dissociation of specific [^{3}H]tamsulosin binding to human prostates. [^{3}H]tamsulosin (0.28 nM) binding was quantified as function of time from addition of ligand. Also, dissociation of [^{3}H]tamsulosin receptor complex was monitored by addition of phentolamine. Each point represents the average of four different tissues

of tamsulosin to prostatic α_1-adrenoceptors. Very recently, Yazawa et al. [28] have characterized specific binding of [^{3}H]tamsulosin with high specific activity in hippocampus and spleen of rats. Although [^{3}H]tamsulosin was selective and of high affinity for α_1-adrenoceptors in rat tissues, there is little information available about the binding characteristics of this radioligand in human prostates. By using the new radioligand, we have attempted to characterize α_1-adrenoceptors in human prostates with BPH, in comparison with [^{3}H]prazosin.

Materials and methods

Preparation of prostatic membranes

Prostatic adenoma specimens were obtained from ten men (58–74 years old) with symptomatic BPH undergoing open prostatectomy. The prostatic tissues were placed in ice-cold saline and transferred into a freezer (−70°C) for storage. The tissue fragments (2–3 g) were blotted, minced with scissors and homogenized in 19 volumes of ice-cold 50 mM TRIS-HCl buffer containing 10 mM $MgCl_2$ (pH 7.5) by a Polytron homogenizer. The homogenates were preincubated for 15 min at 37°C and then centrifuged at 400 *g* for 10 min at 4°C. The supernatant fraction, after filtration through four layers of cheesecloth, was centrifuged at 40000 *g* for 20 min at 4°C. The pellet, after suspension in the cold buffer, was centrifuged further at 40000 *g* for 20 min at 4°C, and the resulting pellet was finally resuspended in the cold buffer to utilize in the radioligand binding assay. All steps were performed at 4°C.

The chloroethylclonidine treatment in human prostates was performed according to the previous method for rat tissues [4, 20]. Briefly, aliquots of prostatic membrane preparation were incubated for 10 min at 37°C in 50 mM TRIS-HCl buffer with or without chloroethylclonidine (10 μM). The reaction was stopped by the addition of ice-cold buffer and centrifugation at 40000 *g* for 20 min at 4°C. The resulting pellet was washed twice by resuspension in the incubation buffer followed by further centrifugation.

[^{3}H]Tamsulosin-binding assay

The binding assay of [^{3}H]tamsulosin in human prostatic membranes was performed by a similar method described previously for the [^{3}H]prazosin-binding assay [27]. The membranes (approximately 50 μg protein) prepared from human prostates were incubated with [^{3}H]tamsulosin (0.28 nM) in 50 mM TRIS-HCl buffer containing 10 mM $MgCl_2$ (pH 7.5). The incubation was carried out for 30 min at 25°C. The reaction was terminated by rapid filtration (Cell Harveser for radioreceptor binding assay, Brandel, Gaithersburg, MD., USA) through Whatman GF/B glass fiber filters, and filters were rinsed three times with 4 ml ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintilation fluid (21 toluene, 11 Triton X-100, 15 g 2,5-diphenyloxazole and 0.3 g 1,4-bis[2-(5-phenyloxazolyl)]-benzene) and the radioactivity was determined by a liquid scintillation counter. Specific binding of [^{3}H]tamsulosin was determined experimentally from the difference between counts in the absence and presence of 3 μM phentolamine. All assays were conducted in duplicate. Protein concentration was measured according to the method of Lowry et al. [19] with bovine serum albumin as standard.

Analysis of data

The analysis of binding data was performed as described previously [1, 24]. The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]tamsulosin were estimated by Scatchard analysis of the saturation data over concentration ranges of 0.03–1.5 nM. The ability of antagonists to inhibit specific [^{3}H]tamsulosin binding was estimated by IC_{50} values, which are the molar concentrations of unlabeled drugs necessary for displacing 50% of the specific binding (determined by log probit analysis). A value for the inhibition constant, K_i, was calculated from the equation $K_i = IC_{50}/(1 + L/K_d)$, where L equals the concentration of [^{3}H]tamsulosin. The Hill slopes for saturation of [^{3}H]tamsulosin and inhibition by antagonists were obtained by Hill plot analysis. The rate constants were determined from association and dissociation velocities. The binding data of [^{3}H]prazosin (0.06–1.7 nM) were analyzed as described for [^{3}H]tamsulosin binding.

Drugs

[^{3}H]Tamsulosin (56.3 Ci/mmol) was specially synthesized by Amersham (Tokyo, Japan). [^{3}H]Prazosin (76.2 Ci/mmol) was purchased from Dupont-NEN, Boston, Mass., USA. The following drugs were kindly donated by the companies indicated: prazosin hydrochloride, Pfizer (Tokyo); tamsulosin and its optical enantiomer, Yamanouchi (Tokyo); bunazosin hydrochloride, Eizai (Tokyo); terazosin hydrochloride, Mitsubishi (Tokyo) and Dainabot (Osaka); naftopidil, Toyo Jozo (Ohito). Phentolamine hydrochloride, yohimbine hydrochloride (Sigma, St. Louis, Mo., USA), WB4101 and chloroethylclonidine (Research Biochemical, Natick, Mass., USA) were purchased from their respective commercial sources.

Results

Identification of high-affinity [^{3}H]tamsulosin binding in human prostate

The time courses of association and dissociation of [^{3}H]tamsulosin binding in human prostates were examined (Fig. 1). [^{3}H]Tamsulosin bound rapidly at 25°C to human prostatic membranes, achieving steady state by 30 min at 25°C, and the dissociation of the binding could

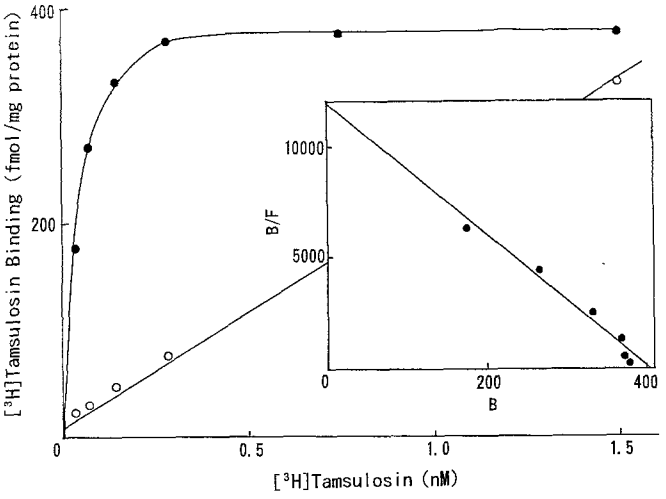

Fig. 2 Specific (●) and nonspecific (○) binding of [³H]tamsulosin to human prostates, and its Scatchard plot (*insert*). Saturation experiments of specific [³H]tamsulosin binding and its Scatchard plot were performed as described in "Materials and methods". Each point represents the average of four different tissues

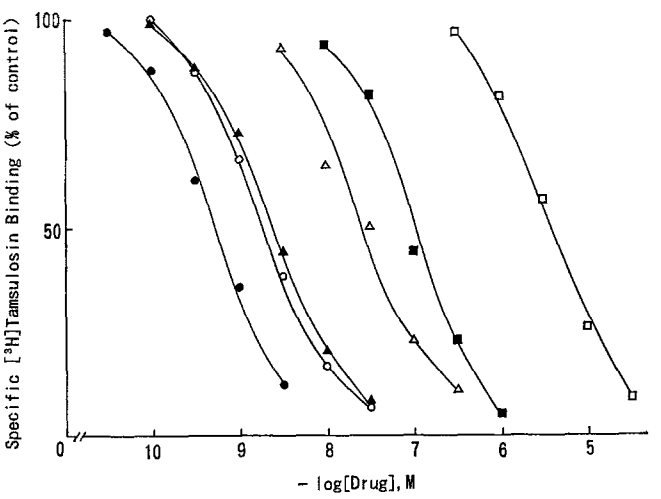

Fig. 3 Inhibition of specific [³H]tamsulosin binding to human prostates by tamsulosin (●), WB4101 (○), prazosin (▲), S(+)-isomer (△), naftopidil (■) and yohimbine (□). Competitive binding experiments of prostatic [³H]tamsulosin binding were performed in the absence and presence of five to six different concentrations of various α-antagonists. Each point represents the average of three to four different tissues

Table 1 Specific binding (K_d, B_{max} of [³H]tamsulosin and [³H]prazosin in human prostates. The saturation experiments were performed on human prostatic membranes as shown in Fig. 2. The equilibrium dissociation constant (K_d) and receptor density (B_{max}) were determined from Scatchard analysis. Each value represents the mean ± SEM of four ([³H]tamsulosin) and eight ([³H]prazosin) different tissues. A significant difference ($P < 0.001$) from b_{max} value for [³H]prazosin binding

Radioligand	Dissociation constant K_d (nM)	Receptor density B_{max} (fmol/mg protein)
[³H]Tamsulosin	0.04 ± 0.01	409 ± 28[a]
[³H]Prazosin	0.35 ± 0.03	546 ± 31

Table 2 Inhibition of specific [³H]tamsulosin binding to human prostates by α-adrenoceptor antagonists. The competitive binding experiments were performed on human prostatic membranes as shown in Fig. 3. The Hill slopes (nH) and K_i values were calculated as described in "Materials and methods". Each value represents the mean ± SEM from three to four different tissues

α-Antagonists	nH	K_i values (nM)
Tamsulosin	1.09 ± 0.04	0.06 ± 0.01
S(+)-isomer	0.91 ± 0.01	3.09 ± 0.45
Prazosin	0.98 ± 0.08	0.28 ± 0.02
WB4101	1.02 ± 0.08	0.23 ± 0.03
Naftopidil	1.12 ± 0.07	10.4 ± 0.3
Yohimbine	1.04 ± 0.05	415 ± 33

be monitored by the addition of 3 μM phentolamine ($t_{1/2} = 69$ min). The rates for association and dissociation of [³H]tamsulosin were linear when plotted on a semilogarithmic scale ($1n[B_{max}/B_{max} - B]$ or $1n[B/B_{max}]$ versus time), indicating a first-order process (Fig. 1). The rate constants for association (k_{-1}) and dissociation (k_{-1}) were 0.21 ± 0.05/nM per minute and 0.01 ± 0.004/min, respectively (mean ± SEM, $n = 4$). Thus, the dissociation rate constant ($K_d = k_{-1}/k_{+1}$) for [³H]tamsulosin receptor interaction was 0.05 nM. This kinetic K_d value agreed well with the K_d value (0.04 nM) estimated from equilibrium studies. The association rate constant for [³H]tamsulosin was similar to that for [³H]prazosin (0.30/nM per minute), but the dissociation rate constant was one-fifth of that for [³H]prazosin (0.05/min) previously reported [26].

Saturation studies revealed a high affinity and saturable binding sites for [³H]tamsulosin in human prostatic membranes ($K_d = 0.04$ nM) (Fig. 2, Table 1). In parallel studies with [³H]prazosin, the K_d of [³H]prazosin was 0.35 nM in the prostates (Table 1). The K_d value for [³H]tamsulosin was approximately one-tenth of that for [³H]prazosin. The B_{max} values for [³H]tamsulosin and [³H]prazosin were 409 and 546 fmol/mg protein respectively. The B_{max} value of [³H]tamsulosin in human prostates was significantly lower than that of [³H]prazosin under our assay conditions. The Hill slope of [³H]tamsulosin binding in this tissue was 0.93 ± 0.01. The nonspecific binding of [³H]tamsulosin increased linearly with increasing concentrations of the ligand (Fig. 2). Of particular interest was the markedly lower degree of nonspecific binding in comparison to total binding for [³H]tamsulosin. In human prostatic membranes, specific binding of [³H]tamsulosin was typically 88.0 ± 1.0% ($n = 4$) of total binding at the K_d concentration (0.04 nM) and 67.9 ± 1.5% even at an approximately 20 times higher concentration (0.75 nM), whereas specific binding of [³H]prazosin was 61.7 ± 3.9% ($n = 4$) at the K_d concentration. The percentage of specific to total binding at the k_d

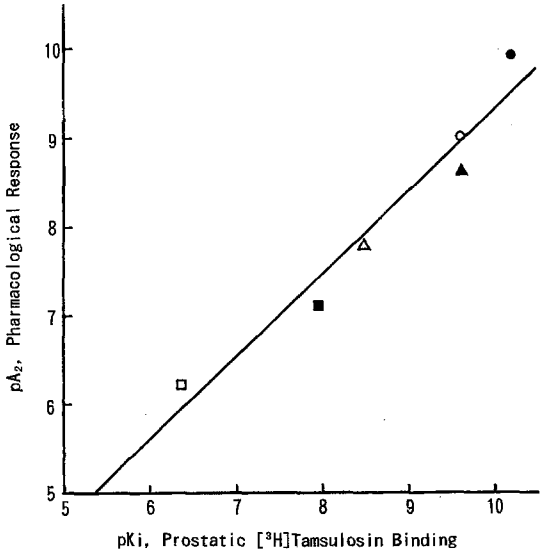

Fig. 4 Correlation between the inhibitory potencies (pKi) of [^{3}H]tamsulosin binding by α-adrenoceptor antagonists in human prostates and their pharmacological potencies (pA_2). The pKi values ($-\log[K_i]$) were calculated from drug competition experiments (Table 2). The pharmacological data (derived from pA_2 values for the inhibition of α_1-agonist-induced contractions of isolated prostatic tissues of humans or rabbits) were obtained from the literature on α-antagonists [7, 8, 12, 17, 27]. The drugs correlated were: ●, tamsulosin; ○, prazosin; ▲, WB4101; △, S-(+)-isomer; ■, naftopidil; □, yohimbine. The pK_i values for six α-antagonists from the prostatic [^{3}H]tamsulosin-binding assay were significantly ($P < 0.01$) correlated with their pharmacological potencies (pA_2). The correlation coefficient for this relationship was $r = 0.97$

concentration of [^{3}H]tamsulosin was significantly ($P < 0.001$) higher than that of [^{3}H]prazosin.

Inhibition of prostatic [^{3}H]tamsulosin binding by α-antagonists

Several α_1-adrenoceptor antagonists (tamsulosin, its enantiomer, prazosin, WB4101, naftopidil) were tested for their abilities to inhibit specific [^{3}H]tamsulosin binding in human prostatic membranes (Fig. 3, Table 2). These antagonists inhibited dose dependently specific [^{3}H]tamsulosin binding in human prostatic membranes with the rank order of: tamsulosin > WB4101 > prazosin > S(+)-isomer > naftopidil. Tamsulosin was approximately 50 times more potent than the enantiomer [S(+)-isomer], supporting the potency difference observed in physiological experiments [8]. In contrast, yohimbine, a selective antagonist of α_2-adrenoceptors, possessed a much lower potency in inhibiting prostatic [^{3}H]tamsulosin binding than α_1-antagonists (Fig. 3, Table 2). The Hill coefficients for these antagonists were close to unity.

Effects of chloroethylclonidine treatment

To investigate the selectivity of α_1-adrenoceptor subtype by tamsulosin, the effect of pretreatment with chloroethylclonidine on K_d and B_{max} values of specific [^{3}H]tamsulosin binding in human prostates was examined. The chloroethylclonidine (10 μM) treatment had a small (11%) decrease in the B_{max} value for prostatic [^{3}H]tamsulosin binding (B_{max} values for control and chloroethylclonidine treatment, 342 ± 46 and 305 ± 34 fmol/mg protein, $n = 3$) without an effect on K_d value (K_d values for control and chloroethylclonidine treatment, 0.04 ± 0.01 and 0.04 ± 0.02 nM).

Discussion

The binding of [^{3}H]tamsulosin in hypertrophied prostates of humans has been characterized. The specific binding of [^{3}H]tamsulosin to prostatic membranes was saturable, reversible and of high affinity. The equilibrium binding studies in hypertrophied prostates showed a $B_{max} = 409$ fmol/mg protein and a $K_d = 0.04$ nM. The K_i value for tamsulosin in inhibiting prostatic [^{3}H]tamsulosin binding was 0.06 nM. These values for tamsulosin in human prostates corresponded to a dissociation constant (K_B) of approximately 0.10 nM for the blockade of postsynaptic α_1-adrenoceptors evaluated by the competitive antagonism of the norepinephrine-evoked contractile responses in rabbit prostates [7, 8], indicating a close correlation in the affinity of tamsulosin to postsynaptic α_1-adrenoceptors between radioligand-binding experiments and physiological experiments. The specific [^{3}H]tamsulosin binding in human prostates also exhibited pharmacological specificity which characterized α_1-adrenoceptors. Tamsulosin, WB4101 and prazosin were relatively potent inhibitors of prostatic [^{3}H]tamsulosin binding, and yohimbine was 2000–7000 times less potent than these α_1-adrenoceptor antagonists. Furthermore, the stereoselectivity of specific [^{3}H]tamsulosin-binding sites was demonstrated by the 50 times greater potency of tamsulosin than the S-(+)-isomer. The Hill slopes for these α_1-antagonists were close to unity. As illustrated in Fig. 4, the binding affinities of these α-antagonists for prostatic [^{3}H]tamsulosin-binding sites (Table 2) correlated significantly with their pharmacological potencies (pA_2 values for the competitive inhibition of α_1-agonist-induced contractions of isolated prostatic tissues) [7, 8, 12, 17, 27]. Taken together, it has been found that [^{3}H]tamsulosin selectively binds to the physiologically relevant postsynaptic α_1-adrenoceptors in human prostates. Yazawa et al. [28] have reported that the K_d values of [^{3}H]tamsulosin in rat hippocampus and spleen were 0.17 and 0.20 nM, respectively. These values were four to five times higher than the K_d value for specific [^{3}H]tamsulosin binding in human prostates. Thus, [^{3}H]tamsulosin may bind to the α_1-adrenoceptors in human prostates with higher affinity than in rat hippocampus and spleen.

[^{3}H]Prazosin, [^{125}I]HEAT and [^{3}H]bunazosin have been introduced as radiolabeled probes to examine α_1-adrenoceptor binding in human prostates [5, 10, 13, 14, 16, 25]. The K_d value for prostatic [^{3}H]tamsulosin binding was approximately one-tenth of those for [^{3}H]prazosin and [^{3}H]bunazosin, and its dissociation rate constant (k_{-1}) was one-fifth of that of [^{3}H]prazosin. Moreover, [^{3}H]tamsulosin has shown a significantly lower level of nonspecific binding than [^{3}H]prazosin and [^{3}H]bunazosin in human prostatic membranes previously reported [26]. The low signal to noise ratio caused by high nonspecific binding decreases the accuracy of saturation isotherm determinations at higher [^{3}H]ligand concentrations. [^{3}H]Tamsulosin may be distinctively advantageous as the level of nonspecific binding is much less even at the higher radioligand concentrations. Because of the higher affinity binding and lower nonspecific binding of [^{3}H]tamsulosin than [^{3}H]prazosin and [^{3}H]bunazosin in human prostatic membranes, therefore, this radioligand would be suitable as an improved substitute for the [^{3}H]prazosin radioreceptor assays previously developed or as a tool for the in vivo labeling of prostatic α_1-adrenoceptors. Work on the in vivo labeling of α_1-adrenoceptors in rats is in progress in our laboratory.

There was some difference in the number of binding sites between [^{3}H]tamsulosin and [^{3}H]prazosin in human prostatic membranes. The B_{max} value for [^{3}H]tamsulosin-binding sites in human prostates represented approximately 75% of that (546 fmol/mg protein) for [^{3}H]prazosin-binding sites. Our previous study has shown that the B_{max} value for [^{3}H]prasozin in this tissue was similar to that for [^{3}H]bunazosin, another quinazoline derivative radioligand [26]. Thus, there is a possibility that [^{3}H]tamsulosin may label a specific subpopulation of α_1-receptor sites labeled by [^{3}H]prazosin or [^{3}H]bunazosin in human prostates.

Currently, α_1-adrenoceptors have been subclassified into two subtypes (α_{1A} and α_{1B}) based on the sensitivity to chloroethylclonidine, WB4101 and 5-methylurapidil in rat tissues [4, 20]. The genes and/or cDNA for four different subtypes of α_1-adrenoceptors have been cloned [3, 18, 21, 22]. By radioligand binding studies using WB4101 and chloroethylclonidine, Leport et al. [16] have very recently found that both α_{1A} and α_{1B} subtypes exist in human prostates and the ratio of the density of these receptor sites is approximately 1.5 (α_{1A}):1 (α_{1B}). Also, they have suggested that the pharmacological profile of the α_1-adrenoceptor-mediated contractile response of prostatic smooth muscle in humans is inconsistent with the classification as either α_{1A} or α_{1B}-receptor subtype [17]. In their studies, the incubation of prostatic tissue with chloroethylclonidine resulted in a marked (80%) reduction of the maximal contractile response produced by phenylephrine, and WB4101 was potent inhibitor of the α_1-receptor-mediated contraction. However, in our study, the chloroethylclonidine treatment (10 μM, 10 min) produced only a slight (11%) decrease in prostatic [^{3}H]tamsulosin-binding sites and WB4101 monophasically inhibited [^{3}H]tamsulosin binding with high affinity ($K_i = 0.23$ nM). A clear explanation for the discrepancy between these results with chloroethylclonidine is lacking, but it may be due to differences in concentration and preincubation time of chloroethylclonidine. Lepor et al. [16, 17] treated prostatic tissue with 100 μM chloroethylclonidine for 30 min. Thus, further detailed studies are necessary to define the subtypes of α_1-adrenoceptors labeled by [^{3}H]tamsulosin.

In conclusion, the present study has demonstrated that [^{3}H]tamsulosin is a highly specific radioligand for α_1-adrenoceptors in human prostates.

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References

1. Bennett JP (1978) Methods in binding studies. In: Yamamura HI, Enna SJ, Kuhar MJ (eds) Neurotransmitter receptor binding. Raven, New York p 59
2. Caine M, Perlberg S (1977) Dynamics of acute retension in prostatic patient and role of adrenergic receptors. Urology 9:399
3. Cotecchia S, Schwinn DA, Randall RR, Lefkowitz RJ, Caron MG, Kobilka BK (1988) Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. Proc Natl Acad Sci USA 85:7159
4. Han C, Abel PW, Minneman KP (1987) Heterogeneity of alpha$_1$ adrenergic receptors revealed by chloroethylclonidine. Mol Pharmacol 32:505
5. Hedlund H, Andersson KE, Larsson B (1985) Alpha-adrenoceptors and muscarinic receptors in the isolated human prostate. J Urol 134:1291
6. Hieble JP, Caine M, Zalaznik E (1985) In vitro characterization of the α-adrenoceptors in human prostate. Eur J Pharmacol 107:111
7. Honda K, Nakagawa C (1986) Alpha-1 adrenoceptor antagonist effects of the optical isomers of YM-12617 in rabbit lower urinary tract and prostate. J Pharmacol Exp Ther 239:512
8. Honda K, Miyata-Osawa A, Takenaka T (1985) α_1-Adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. Naunyn Schmiedebergs Arch Pharmacol 330:16
9. Honda K, Takenaka T, Miyata-Osawa A, Terai A, Shiono K (1985) Studies on YM-12617: a selective and potent antagonist of postsynaptic α_1-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 328:264
10. Kawabe K, Moriyama N, Hamada K, Ishima T (1990) Density and localization of alpha$_1$-adrenoceptors in hypertrophied prostate. J Urol 143:592
11. Kawabe K, Ueno A, Takimoto Y, Aso Y, Kato H, YM617 Clinical study group (1990) Use of an α_1-blocker, YM617, in the treatment of benign prostatic hypertrophy. J Urol 144:908
12. Kunisawa Y, Kawabe K, Niijima T, Honda K, Takenaka T (1985) A pharmacological study of alpha adrenergic receptor subtypes in smooth muscle of human urinary bladder base and prostatic urethra. J Urol 134:396
13. Lepor H, Shapiro E (1984) Characterization of alpha$_1$ adrenergic receptors in human benign prostatic hyperplasia. J Urol 132:1226
14. Lepor H, Baumann M, Shapiro E (1988) The alpha adrenergic properties of terazosin in the human prostatic adenoma and canine brain. J Urol 140:664
15. Lepor H, Baumann M, Shapiro E (1988) The stereospecificity of LY253352 for α_1 adrenoceptor binding sites in the brain and prostate. Br J Pharmacol 95:139
16. Lepor H, Tang R, Meretyk S, Shapiro E (1993) Alpha$_1$ adrenoceptor subtypes in the human prostate. J Urol 149:640

17. Lepor H, Tang R, Shapiro E (1993) The alpha-adrenoceptor subtype mediating the tension of human prostatic smooth muscle. Prostate 22:301
18. Lomasney JW, Cotecchia S,Lorenz W, Leung WY, Schwinn DA, Yang-Feng TL, Brownstein M, Lefkowitz RJ, Caron MG (1991) Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor. J Biol Chem 266:6365
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265
20. Minneman KP, Han C, Abel PW (1988) Comparison of alpha$_1$ adrenergic receptor subtypes distinguished by chloroethylclonidine and WB4101. Mol Pharmacol 33:509
21. Perez DM, Piascik MT, Graham RM (1991) Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. Mol Pharmacol 40:876
22. Schwin DA, Lomasney JW, Lorenz W, Szklut PJ, Fremeau RT Jr, Yang-Feng TL, Caron MG, Lefkowitz RJ, Cotecchia S (1990) Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. J Biol Chem 265:8183
23. Vaalasti A, Hervonen A (1980) Autonomic innervation of the human prostate. Invest Urol 17:293
24. Yamada S, Yamamura HI, Roeske WR (1980) Characterization of alpha-1 adrenergic receptors in the heart using [^{3}H]WB4101: effect of 6-hydroxydopamine treatment. J Pharmacol Exp Ther 215:176
25. Yamada S, Ashizawa N, Ushijima 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, Matsumoto Y, Kato Y, Kimura R, Maruyama M, Kawabe K (1991) [^{3}H]Bunazosin, a novel selective radioligand of alpha$_1$ adrenoceptors in human prostates. J Urol 146:877
27. Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, Maruyama M, Kawabe K, (1992) Binding characteristics of naftopidil and α_1-adrenoceptor antagonists to prostatic α-adrenoceptors in benign prostatic hypertrophy. Life Sci 50:127
28. Yazawa H, Takanashi M, Sudoh K, Inagaki O, Honda K (1992) Characterization of [^{3}H]YM617, R-(−)-5-[2-[[2[Ethoxyring(*n*)-[^{3}H](*o*-ethoxyphenoxy)-ethyl]amino]-propyl]-2-methoxybenzenesulfonamide HCl, a potent and selective alpha-1 adrenoceptor radioligand. J Pharmacol Exp Ther 263:201