High-affinity specific \([^3H]tamsulosin\) binding to \(\alpha_1\)-adrenoceptors in human prostates with benign prostatic hypertrophy

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Abstract

The binding of a novel radioligand, \([^3H]tamsulosin\), to human prostatic membranes with benign prostatic hypertrophy (BPH) has been characterized. \([^3H]tamsulosin\) 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 \([^3H]tamsulosin\) binding were calculated to be 0.21 ± 0.05/nM per minute and 0.01 ± 0.004/min, respectively. The specific binding of \([^3H]tamsulosin\) in human prostatic membranes was saturable and of high affinity (\(K_d = 0.04 ± 0.01\) nM). The density of \([^3H]tamsulosin\)-binding sites (\(B_{max}\)) was 409 ± 28 fmol/mg protein. The \(K_d\) and \(B_{max}\) values for \([^3H]tamsulosin\) binding in human prostates were significantly lower than those for \([^3H]prazosin\) binding. \([^3H]tamsulosin\) binding was remarkable for its significantly lower degree of nonspecific binding. Six \(\alpha\)-adrenoceptor antagonists competed with \([^3H]tamsulosin\) for the binding sites in the rank order: tamsulosin > WB4101 > prazosin > S-(+)-isomer > naftopidil > yohimbine. The binding affinities (pKi) of these antagonists for \([^3H]tamsulosin\) binding in human prostates closely correlated with their pharmacological potencies (pA2) in prostates. In conclusion, \([^3H]tamsulosin\) selectively labels \(\alpha_1\)-adrenoceptors in human prostates, and thus may become a useful radioligand for the further analysis of these receptors.

Key words \([^3H]tamsulosin\) · Radioreceptor assay · Human prostates · \(\alpha_1\)-antagonists

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Alpha-\(\alpha_1\)-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 \(\alpha_1\)-adrenoceptors 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 \(\alpha_1\)-adrenoceptors is important for the investigation of the etiology and pharmacological management of urinary obstruction in BPH.

Currently, several radiolabeled \(\alpha_1\)-adrenoceptor antagonists, including \([^3H]prazosin\), \([^{125}I]\)HEAT (iodo-2-[(β-hydroxyphenyl)-ethylaminomethyl]tetralone) and \([^3H]bunazosin\), have been introduced as selective radioligands to identify \(\alpha_1\)-adrenoceptor sites in human prostates and also to examine binding affinities of \(\alpha_1\)-antagonists to these receptors [5, 10, 13, 14, 16, 25]. However, one difficulty with \([^3H]prazosin\) 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-ethoxyphenoxoxy)ethyl]amino]propyl]-2-methoxy-benzene-sulphonamide hydrochloride) has been shown to possess an extremely potent \(\alpha_1\)-adrenoceptor blocking action in the aorta, lower urinary tract and prostate of rabbits [7–9]. Based on the pA2 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 \(\alpha_1\)-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 \(\alpha_1\)-adrenoceptor-binding sites in human prostates with BPH labeled by \([^3H]prazosin\) and \([^3H]bunazosin\). Thus, it is of great interest to investigate directly the binding properties...
of tamsulosin to prostatic α1-adrenoceptors. Very recently, Yazawa et al. [28] have characterized specific binding of [3H]tamsulosin with high specific activity in hippocampus and spleen of rats. Although [3H]tamsulosin was selective and of high affinity for α1-adrenoceptors in rat tissues, there is little information available about the binding characteristics of this radioligand in human prostate. By using the new radioligand, we have attempted to characterize α1-adrenoceptors in human prostates with BPH, in comparison with [3H]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 MgCl2 (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 40,000 g for 20 min at 4°C. The pellet, after suspension in the cold buffer, was centrifuged further at 40,000 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 confirmed (Fig. 1). [3H]Tamsulosin (0.28 nM) binding was quantified as function of time from addition of ligand. Also, dissociation of [3H]tamsulosin receptor complex was monitored by addition of phentolamine. Each point represents the average of four different tissues

[3H]Tamsulosin-binding assay

The binding assay of [3H]tamsulosin in human prostatic membranes was performed by a similar method described previously for the [3H]prazosin-binding assay [27]. The membranes (approximately 50 μg protein) prepared from human prostates were incubated with [3H]tamsulosin (0.28 nM) in 50 mM TRIS-HCl buffer containing 10 mM MgCl2 (pH 7.5). The incubation was carried out for 30 min at 25°C. The reaction was terminated by rapid filtration (Cell Harvester 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 scintillation 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 [3H]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 (Kd) and maximal number of binding sites (Bmax) for [3H]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 [3H]tamsulosin binding was estimated by IC50 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, KI, was calculated from the equation KI = IC50/(1 + L/Kd), where L equals the concentration of [3H]tamsulosin. The Hill slopes for saturation of [3H]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 [3H]prazosin (0.06-1.7 nM) were analyzed as described for [3H]tamsulosin binding.

Drugs

[3H]Tamsulosin (56.3 Ci/mm) was specially synthesized by Amersham (Tokyo, Japan). [3H]Prazosin (76.2 Ci/mm) 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, Eiara (Tokyo); terazosin hydrochloride, Mitsubishi (Tokyo) and Dainabot (Osaka); naftopidil, Toyo Jozoho (Osito). Phenotolamine 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 [3H]tamsulosin binding in human prostate

The time courses of association and dissociation of [3H]tamsulosin binding in human prostates were examined (Fig. 1). [3H]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