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Pharmacological analysis of the novel, selective α_1-adrenoceptor antagonist, KMD-3213, and its suitability as a tritiated radioligand

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1 Pharmacological profiles of tritiated KMD-3213, a new antagonist of α_1-adrenoceptor (AR), were examined in recombinant and native α_1-AR, and compared with those of prazosin (PZ) and tamsulosin (YM-617).

2 In saturation experiments, [^{3}H]-KMD (10–2000 pM) showed high affinity for α_{1a}-AR ($pK_D = 10.5$). However, no significant binding to α_{1b}-AR and insufficient/unsaturated binding to α_{1d}-AR were observed at concentrations up to 2000 pM. In contrast, [^{3}H]-PZ and [^{3}H]-YM bound to all subtypes with high affinity ($pK_D > 9$). In competition experiments, KMD-3213 also had higher affinity for α_{1a}-AR than for other two subtypes; $pK_i = 10.4$, 8.1 and 8.6 for α_{1a}-, α_{1b}- and α_{1d}-AR, respectively.

3 [^{3}H]-KMD also bound to the native α_{1A}-AR (rat submaxillary gland) with high affinity, but not to α_{1B}-AR (rat liver). In rat kidney which expresses α_{1A}- and α_{1B}-AR, [^{3}H]-KMD and [^{3}H]-PZ bound to a single high-affinity site ($pK_D = 10.8$ and 10.1, respectively) with distinct amount of binding sites ($B_{max} = 159$ and 267 fmol mg^{-1} protein, respectively). [^{3}H]-PZ binding sites consisted of low- and high-affinity sites for KMD-3213 ($pK_i = 7.6$ and 10.7, respectively), for WB4101 ($pK_i = 8.1$ and 10.0) and for YM-617 ($pK_i = 8.7$ and 10.8). The proportion of the high affinity site was approximately 60% in these drugs which was compatible to the ratio between B_{max} of [^{3}H]-KMD and [^{3}H]-PZ. [^{3}H]-KMD binding sites consisted of a single site for these drugs with affinities which were similar to those of the high affinity sites in [^{3}H]-PZ binding.

4 In functional experiments, KMD-3213 antagonized the contractile responses to NS-49 or noradrenaline (NA) with higher affinity in functional α_{1A}- (rat caudal artery, $pA_2 = 10.0$ against NS-49) and α_{1L}-AR (dog mesenteric artery, $pA_2 = 9.9$ against NA) than in α_{1B}- (dog carotid artery, $pA_2 = 7.7$ against NA) and α_{1D}-AR (rat thoracic aorta, $pA_2 = 8.3$ against NA).

5 These results confirm the α_{1A}-AR selectivity and high affinity of KMD-3213, and indicate that [^{3}H]-KMD can label selectively α_{1A}-AR.

Keywords: α_1-Adrenoceptor subtypes; KMD-3213; prazosin; tamsulosin

Abbreviations: AR, adrenoceptor; KMD-3213, (−)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl] amino]propyl]indoline-7-carboxamide; NA, noradrenaline; PZ, prazosin; YM-617, tamsulosin

Introduction

α_1-Adrenoceptors (ARs) constitute a heterogeneous family of receptors (McGrath, 1982). The existence of two subtypes, α_{1A}- and α_{1B}-AR, was initially suggested in pharmacological studies (Han *et al*., 1987; Minneman, 1988; Morrow & Creese, 1986). Molecular cloning techniques have revealed the existence of at least three receptor subtypes (α_{1a}-, α_{1b}- and α_{1d}-AR), and pharmacological studies indicated that these clones subtypes correspond to native α_{1A}-, α_{1B}- and α_{1D}-AR subtypes, respectively (Bylund *et al*., 1995; Hieble *et al*., 1995). The fourth subtype, α_{1L}-AR which is defined principally based on a distinct low affinity for prazosin (PZ), has been proposed mainly from functional studies (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1990; 1991; Oshita *et al*., 1991; Ford *et al*., 1996). However, the molecular identity of α_{1L}-AR still remains to be clarified (Bylund *et al*., 1998).

Various α_1-AR ligands are used for characterizing the binding profiles and functions of receptor subtypes; however, few ligands showing subtype selectivity are available. Recently, KMD-3213 [(−)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy) phenoxy] ethyl] amino]propyl]indoline - 7 - carboxamide] was reported as an α_1-AR antagonist (Shibata *et al*., 1995). In the present study, we characterized the binding profiles of KMD-3213, and its tritiated form as a radioligand, by using recombinant α_1-AR subtypes and native tissues, and compared α_1-AR subtype selectivity of KMD-3213 with other α_1-AR antagonists such as PZ and tamsulosin (YM-617). The characteristics of KMD-3213 were also compared with those of other drugs in functional experiments.

Methods

Male Wistar rats (250–350 g) and Beagle dogs (10–15 kg) were used for the experiments. Animals were sacrificed under anaesthesia with pentobarbital, and tissues were isolated immediately thereafter.

Membrane preparation

For the cloned ARs, COS-7 cells were transfected with the cDNA clones of human α_{1a}-, hamster α_{1b}- and rat α_{1d}-AR. The harvested cells were suspended in ice-cold assay buffer (Tris-HCl 50 mM, EDTA 1 mM, pH 7.4), sonicated and centrifuged at $3000 \times g$ for 10 min. The supernatant was then centrifuged

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at $80,000 \times g$ for 30 min, and the resulting pellet was resuspended in assay buffer and used for binding experiments.

For preparation of native receptor subtypes, isolated rat tissues (submaxillary gland, liver and kidney) were homogenized in 20 vol. of ice-cold homogenization buffer (mM): Tris-HCl 50, NaCl 100, EDTA 2, pH 7.4) with a Polytron (setting 8, 15 s × 3) and filtered through four layers of cheese cloth. The supernatants were centrifuged at $80,000 \times g$ for 30 min, and the resulting pellets were suspended in ice-cold assay buffer, and then again centrifuged at $80,000 \times g$ for 30 min. All centrifugation was done at 4°C. Final pellets were resuspended in assay buffer and used for the following experiments.

Radioligand binding experiments

In saturation binding experiments, the membranes were incubated with various concentrations of [^{3}H]-ligands for 45 min at 30°C. Total incubation volume for [^{3}H]-PZ, [^{3}H]-YM and [^{3}H]-KMD was 1 ml, 3 ml and 2 ~ 3 ml, respectively (5 – 30 μg protein/tube for cultured cells or 50 – 150 μg protein/tube for native tissues). Nonspecific binding was defined as binding in the presence of 1 μM YM-617 for [^{3}H]-PZ or [^{3}H]-KMD or in that of 1 μM PZ for [^{3}H]-YM, excepting the binding to rat liver and kidney (0.3 μM YM-617 for [^{3}H]-PZ and 0.3 μM PZ for [^{3}H]-KMD). In competition binding experiments, membranes were incubated with about 200 pM [^{3}H]-PZ or 70 ~ 100 pM [^{3}H]-KMD and unlabelled drugs for 45 min at 30°C. Specific binding of both radioligands (200 pM [^{3}H]-PZ and 100 pM [^{3}H]-KMD) was approximately 90% and 80% of the total binding for cloned cells and native tissues, respectively. Reactions were terminated by rapid filtration with a Brandel cell harvester onto Whatman GF/C filters presoaked in 0.3% polyethyleneimine for 15 min. The filters were then washed four times with 4 ml of ice-cold 50 mM Tris-HCl (pH 7.4) and dried. The filter-bound radioactivity was determined by liquid scintillation counting. Experiments were conducted in duplicate. Binding affinities of [^{3}H]-ligands and unlabelled drugs were expressed as negative logarithm of the equilibrium dissociation constant (pK_D and pK_i, respectively). Protein concentrations were quantified by the method of Bradford using bovine serum albumin as standard (Bradford, 1976).

Functional experiments

Freshly isolated arteries were cleaned of adherent connective tissue and cut helically, and the endothelium was removed by gentle rubbing. The strip was then mounted vertically in an organ bath containing 20 ml of Krebs-Henseleit solution (composition in mM): NaCl 112.0, KCl 5.9, $CaCl_2$ 2.0, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.5, gassed with 5% CO_2 and O_2 and maintained at 37°C. Resting tension applied was 0.5 g for rat caudal artery or 1 g for other tissues, and the responses were recorded isometrically through force-displacement transducers. All preparations were equilibrated for 90 min, and the following experiments were performed.

Concentration-response curves were obtained by adding the agonist (NS-49 for rat caudal artery or noradrenaline (NA) for other arteries) in cumulative fashion. The curves were drawn at least five times for the same strip, and the third curve was used as the control. After the control experiment, strips were incubated with α_1-AR antagonists for 30 min before, and during, the construction of the curves. All the experiments were performed in the presence of 0.1 μM desipramine and 5 μM deoxycorticosterone acetate to block neural and extraneural uptake, respectively, of NA and 3 μM propranolol to block β-AR induced responses. In rat caudal artery, 0.1 μM rauwolscine was further applied in addition to those three chemicals to avoid α_2-AR mediated responses.

Data analysis

Data were given as means ± s.e.mean. Saturation and competition binding data were first fitted to a one- and then a two-site model, and the two-site model was accepted only if it resulted in a significant improvement of the fit as judged by an F-test comparison with a $P < 0.05$. Analysis of radioligand binding data was performed with LIGAND (Munson & Rodbard, 1980), a nonlinear curve-fitting program.

In functional studies, antagonist affinity estimates were obtained by construction of Schild regressions and were constrained to a slope of unity (if not statistically different) according to the equation: $pK_B = \log(r\text{-}1) - \log[\text{antagonist}]$, where r is the concentration ratio between EC_{50} values (concentration for a half-maximal response) in the presence and absence of the antagonist. Schild plots were also constructed by plotting the $\log(r\text{-}1)$ against the log of antagonist concentration, and pA_2 values were determined from the intercept (Arunlakshana & Schild, 1959).

Drugs

The drugs used and their sources were following: (−)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy) phenoxy]-ethyl]amino]propyl]indoline-7-carboxamide (KMD-3213), tamsulosin HCl (YM-617) and (R)-(−)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethane-sulphonanilide hydrochloride (NS-49), from Kissei Pharmaceutical Co. Ltd. (Matsumoto, Japan); prazosin HCl (PZ), (−)-noradrenaline bitartrate (NA) and desipramine hydrochloride, from Sigma (St. Louis, U.S.A.); WB4101, BMY7378 and rauwolscine hydrochloride, from Research Biochemicals Inc. (Natick, U.S.A.); (±)-propranolol hydrochloride and deoxycorticosterone acetate, from Nacalai (Kyoto, Japan); [^{3}H]KMD-3213 (49 ~ 52 Ci mmol^{-1}), from Amersham (England); and [^{3}H]prazosin (77.2 Ci mmol^{-1}) and [^{3}H]-YM-617 (32.5 Ci mmol^{-1}), from NEN (Boston, U.S.A.).

KMD-3213 was dissolved in dimethylsulphoxide and diluted in assay buffer for binding studies or Hartmann's solution (composition in w v^{-1}%): NaCl 0.60, KCl 0.03, $CaCl_2$ 0.02 and sodium lacate 0.31, for functional studies. [^{3}H]-ligands were diluted in assay buffer. PZ was dissolved in 50% ethanol and diluted in distilled water. Other drugs were dissolved in and diluted with distilled water.

Results

Characteristics of tritiated KMD-3213

Chemical structures of tritiated KMD-3213, (−)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)[4,6(n)-^{3}H]-phenoxy]ethyl]amino]propyl]indoline-7-carboxamide, is shown in Figure 1a. This tritiated form of KMD-3213 ([^{3}H]-KMD) with high specific radioactivity (49 ~ 52 Ci mmol^{-1}) effectively bound to α_{1a}/α_{1A}-AR at subnanomolar concentrations. A representative saturation curve of [^{3}H]-KMD at human α_{1a}-AR is shown in Figure 1b. The binding was equilibrated by incubation at 30°C for more than 40 min (data not shown). Non-specific binding was less than 10% at 100 pM [^{3}H]-KMD. Since [^{3}H]-KMD had high affinity for α_{1a}/α_{1A}-AR, 2 ~ 3 ml of incubation volume was employed in binding experiments in

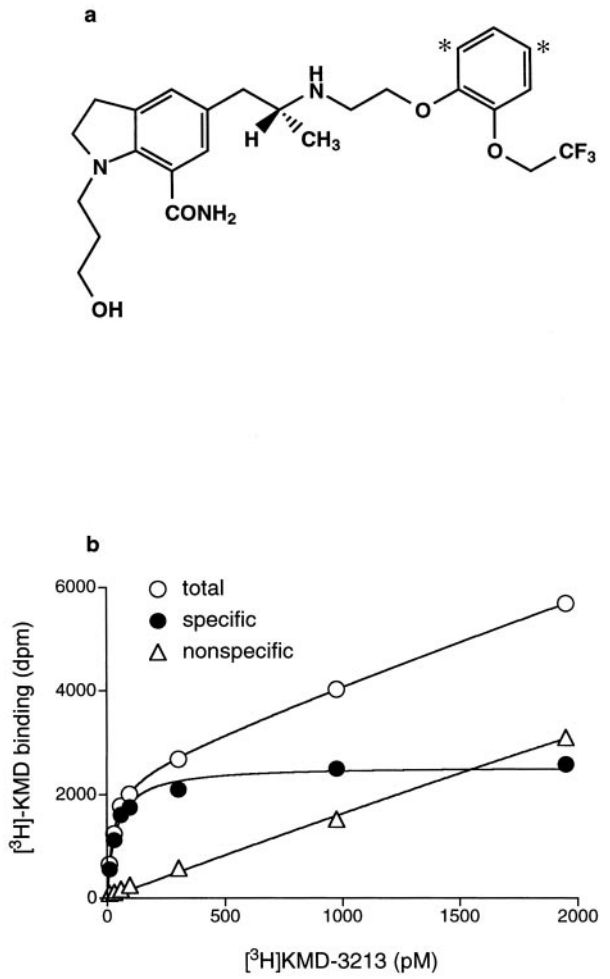

Figure 1 Characteristics of [^{3}H]KMD-3213. (a) Chemical structure of [^{3}H]KMD-3213. Asterisks show the tritiated sites. (b) Saturation of [^{3}H]KMD3213 binding to human α_{1a}-AR subtypes expressed in COS-7 cells. Each point represents the means of duplicate determinations from a single experiment. The experiments were replicated three times with similar results.

order to avoid a significant reduction of free [^{3}H]-KMD concentration during incubation.

Radioligand binding experiments for cloned α_1-AR subtypes

In saturation experiments, specific binding of the three [^{3}H]-ligands ([^{3}H]-PZ, [^{3}H]-YM and [^{3}H]-KMD) was determined by use of the same membranes in each experiment, and representative patterns are shown in Figure 2. All of the specific binding curves were saturable, except the curves for [^{3}H]-KMD binding to α_{1b}- and α_{1d}-AR. In contrast to [^{3}H]-PZ and [^{3}H]-YM which showed high affinity for the three cloned α_1-AR subtypes ($pK_D > 9$), [^{3}H]-KMD clearly showed different sensitivity to them; high affinity to α_{1a}-AR (pK_D; 10.5 ± 0.07), no significant binding to α_{1b}-AR and insufficient/unsaturated binding to α_{1d}-AR at concentrations up to 2000 pM (Table 1) were observed.

Several α_1-AR antagonists were tested in competition experiments against [^{3}H]-PZ or [^{3}H]-KMD binding to cloned α_1-AR subtypes. The competition experiment with [^{3}H]-KMD binding was done only with α_{1a}-AR, because [^{3}H]-KMD at concentrations used in this experiment did not show sufficient binding to α_{1b}- and α_{1d}-AR (Figure 2). The affinities of the α_1-

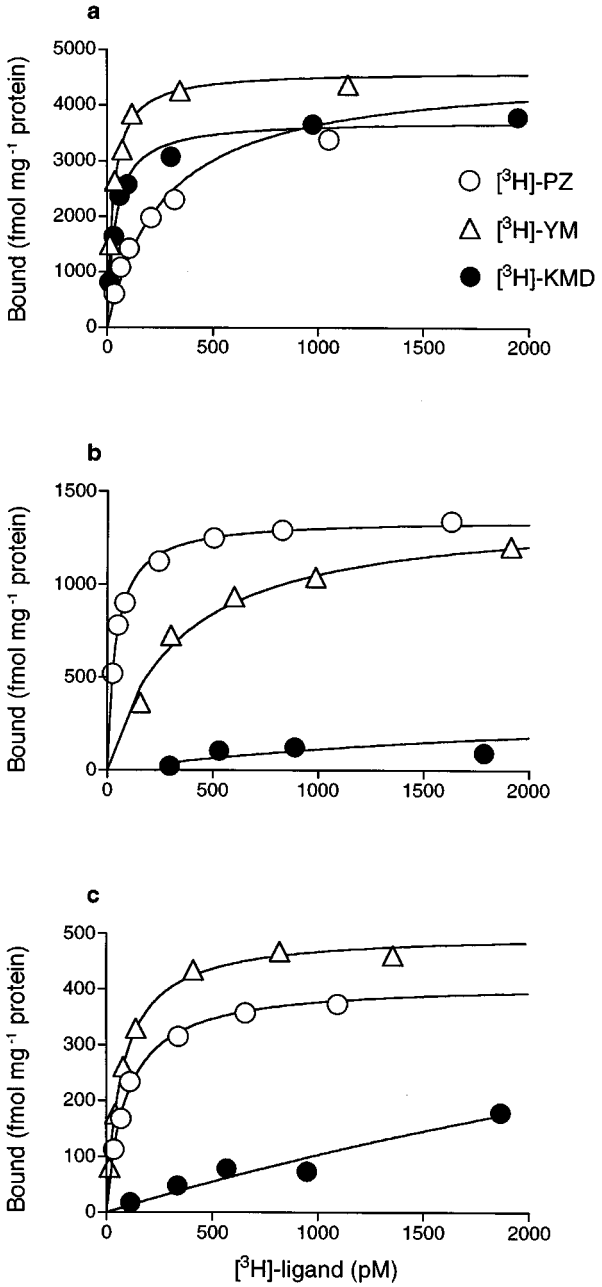

Figure 2 Saturation binding experiments of [^{3}H]-PZ, [^{3}H]-YM and [^{3}H]-KMD to recombinant α_1-AR subtypes. (a) Human α_{1a}-, (b) hamster α_{1b}- and (c) rat α_{1d}-AR subtypes were expressed in COS-7 cells. Specific binding, drawn in Figures, was determined as the difference in radioligand binding in the presence or absence of 1 μM YM-617 for [^{3}H]-PZ and [^{3}H]-KMD or 1 μM PZ for [^{3}H]-YM. All three radioligands were incubated with the same membrane in each experiment. A representative result for each subtype is shown as mean of duplicate in a single experiment (see Table 1).

AR antagonists to cloned α_1-AR subtypes are shown in Table 2. The characteristics of each antagonist were basically similar between [^{3}H]-PZ and [^{3}H]-KMD binding. Regarding the selectivity for α_1-AR subtypes, several patterns were observed. PZ showed no selectivity for the three α_1-AR subtypes. YM-617 and WB4101 showed slightly lower affinity for α_{1b}-AR than for other subtypes, and BMY7378 showed higher affinity for α_{1d}-AR than for other two subtypes. KMD-3213 had prominent selectivity, showing more than 200 and 50 times higher affinity for α_{1a}-AR than for α_{1b}- and α_{1d}-AR, respectively.

Binding characteristics for native α_1-AR subtypes

The binding of [^{3}H]-KMD was further characterized in rat submaxillary gland, liver and kidney, which are pharmacologically defined as tissues predominantly containing α_{1A}-AR, α_{1B}-AR and a mixture of α_{1A}- and α_{1B}-ARs, respectively (Michel *et al*., 1989; Feng *et al*., 1991). As shown in Table 3, [^{3}H]-PZ showed high affinity for all tissues, whereas [^{3}H]-KMD bound with high affinity to the submaxillary gland and kidney but showed no saturable binding to liver membranes at concentrations up to 2000 pM (data not shown). B_{max} values of these two tritiated compounds were similar in submaxillary gland, but in kidney B_{max} of [^{3}H]-KMD was only 60% of that of [^{3}H]-PZ.

The binding characteristics of series of antagonists for the native α_1-AR subtypes were examined in competition experiments as shown in Table 4. In submaxillary gland (α_{1A}-AR) and liver (α_{1B}-AR), the binding of [^{3}H]-PZ and [^{3}H]-KMD was displaced monophasically by the competitors used with affinities which were compatible with those shown in Table 2. In rat kidney, however, YM-617, KMD-3213 and WB4101 displaced the binding of [^{3}H]-PZ with two distinct affinity sites (Figure 3). The affinities for the high and low affinity sites corresponded well to those for α_{1A}- and α_{1B}-AR subtype, respectively. The proportion of high affinity sites in the total binding of [^{3}H]-PZ were 68, 58 and 66% for YM-617, KMD-3213 and WB4101, respectively, which matched well with the ratio (60%) between B_{max} values of [^{3}H]-KMD and [^{3}H]-PZ seen in Tables 3 and 4. The binding of [^{3}H]-KMD was uniformly displaced by a series of drugs with affinities corresponding to those for high affinity sites in the displacement of [^{3}H]-PZ binding (Figure 3 and Table 4). These results suggest that [^{3}H]-KMD recognized a single population of binding sites (α_{1A}-AR) from the mixed populations (α_{1A}- and

Table 1 Binding affinity of radioligands for cloned α_1-AR subtypes

	pK_D		
Radioligand	α_{1a}	α_{1b}	α_{1d}
[^{3}H]-prazosin	9.87±0.07	10.5±0.03	10.1±0.02
[^{3}H]-YM-617	10.7±0.07	9.44±0.06	10.2±0.03
[^{3}H]-KMD-3213	10.5±0.07	–	–

Human α_{1a}-, hamster α_{1b}- and rat α_{1d}-AR subtypes were expressed in COS-7 cells. Nonspecific binding was defined as binding in the presence of 1 μM YM-617 for [^{3}H]-PZ or [^{3}H]-KMD or in that of 1 μM PZ for [^{3}H]-YM. [^{3}H]-KMD did not bind sufficiently to α_{1b}- or α_{1d}-AR, and thus the negative logarithm of the dissociation constant (pK_D) could not be determined. Data are shown as mean±s.e.mean of 3–4 experiments.

Table 2 Profile of α_1-AR antagonist binding to cloned α_1-ARs

	pK_i			
	[^{3}H]-KMD		*[^{3}H]-PZ*	
Antagonist	α_{1a}	α_{1a}	α_{1b}	α_{1d}
Prazosin	9.73±0.05	9.82±0.08	10.6±0.06	10.1±0.04
YM-617	10.8±0.02	10.7±0.06	9.41±0.02	10.2±0.04
KMD-3213	10.7±0.07	10.4±0.09	8.12±0.02	8.64±0.04
WB4101	9.81±0.05	9.83±0.03	8.73±0.04	9.55±0.04
BMY7378	6.94±0.04	6.96±0.06	7.38±0.07	9.09±0.03

Membranes from COS-7 cells transfected with human α_{1a}-, hamster α_{1b}- and rat α_{1d}-AR subtypes were incubated with 200 pM [^{3}H]-PZ or 70 pM [^{3}H]-KMD in the presence of unlabelled drugs. Binding affinity (pK_i) of each α_1-AR antagonist is shown as mean±s.e.mean of 3–4 experiments.

Table 3 Characteristics of radioligand binding to native tissues

	[^{3}H]-PZ		*[^{3}H]-KMD*	
Rat tissue	pK_D	B_{max} (fmol mg^{-1} protein)	pK_D	B_{max} (fmol mg^{-1} protein)
Submaxillary gland	10.2±0.04	260±12.0	10.6±0.04	242±16.1
Liver	10.4±0.03	186±7.34	–	–
Kidney	10.1±0.02	267±28.4	10.8±0.03	159±4.17

Membranes were incubated with serial concentrations of [^{3}H]-ligands as described in Methods. [^{3}H]-KMD did not bind sufficiently to rat liver and so the affinity (pK_D) could not be calculated. Data are shown as mean±s.e.mean of 3–4 experiments.

Table 4 Binding affinity of α_1-AR antagonists to native tissues

	pK_i				
	Rat submaxillary gland		*Rat liver*	*Rat kidney*	
Antagonist	*[^{3}H]-PZ*	*[^{3}H]-KMD*	*[^{3}H]-PZ*	*[^{3}H]-PZ*	*[^{3}H]-KMD*
Prazosin	10.2±0.07	10.3±0.07	10.2±0.12	10.0±0.07	10.1±0.03
YM-617	10.6±0.01	10.9±0.03	8.91±0.02	10.8±0.03 8.73±0.21 (68%)	11.0±0.02
KMD-3213	10.7±0.10	10.9±0.07	7.62±0.04	10.7±0.14 7.63±0.02 (58%)	10.8±0.08
WB4101	9.88±0.04	10.1±0.08	8.24±0.08	9.96±0.10 8.14±0.14 (66%)	10.1±0.00
BMY7378	7.00±0.03	7.04±0.11	6.97±0.05	6.91±0.07	6.97±0.06

Membranes from rat tissues were incubated with 200 pM [^{3}H]-PZ or 70 ~100 pM [^{3}H]-KMD in the presence of unlabelled drugs. In two-site fitted models, percentage of high affinity site is shown in parenthesis. Data are shown as mean±s.e.mean of 3–4 experiments.

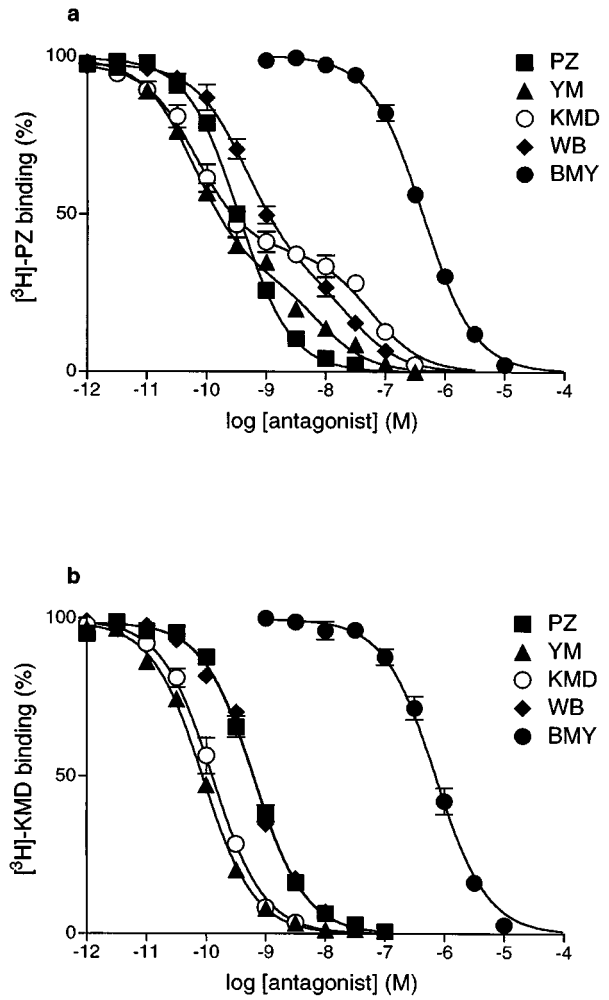

Figure 3 Competition experiments of specific [^{3}H]-PZ and [^{3}H]-KMD binding by various α_1-AR antagonists in rat kidney. (a) 200 pM [^{3}H]-PZ or (b) 100 pM [^{3}H]-KMD was incubated with rat kidney membranes in the presence of unlabelled drugs. Competition curves of YM-617, KMD-3213 and WB4101 against [^{3}H]-PZ binding were significantly fitted to two-site model ($P<0.05$), and other curves were fitted to one-site model. Data are shown as mean$\pm$s.e.mean of three independent experiments.

α_{1B}-AR) of binding sites in rat kidney because of its high affinity for α_{1A}-AR.

Functional experiments in isolated native tissues

To further characterize the selectivity of KMD-3213, we performed functional experiments using isolated rat caudal arteries, dog carotid arteries, rat thoracic aortae and dog mesenteric arteries in which agonist-induced contraction is mediated by α_{1A}-, α_{1B}-, α_{1D}- and α_{1L}-AR subtype, respectively (Lachnit *et al*., 1997; Muramatsu *et al*., 1990; 1991; Kenny *et al*., 1995). In rat caudal artery, the contractile response to NA was mediated by at least two α_1-AR subtypes; one is α_{1A}-AR and the other remains to be defined (Lachnit *et al*., 1997). We employed NS-49, an α_{1A}-AR selective agonist (Obika *et al*., 1995), to draw the response mediated by α_{1A}-AR in rat caudal artery. In other tissues NA was employed as an agonist. The representative antagonism of KMD-3213 are shown in Figures 4 and 5, and the results are summarized in Table 5. All of the antagonists shifted the agonist-induced concentration-response curves to the right in a parallel fashion, and Schild analysis revealed that slopes of all the antagonists in any tissues were

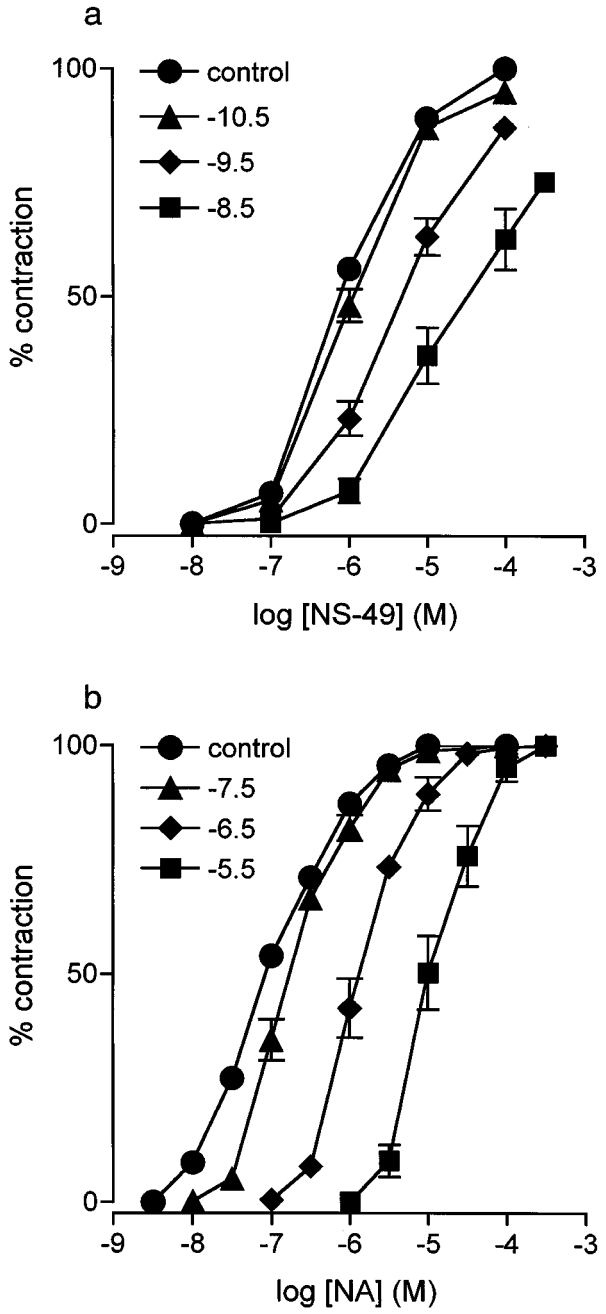

Figure 4 Antagonism of contractions to agonists by KMD-3213. (a) Concentration response curves to NS-49 in rat caudal artery are shown. ●, Control; ▲, KMD-3213 0.03 nM; ◆, 0.3 nM; ■, 3 nM. (b) Concentration response curves to NA in dog carotid artery are shown. ●, Control; ▲, KMD-3213 0.03 μM; ◆, 0.3 μM; ■, 3 μM. Data are shown as mean$\pm$s.e.mean of 5–6 independent experiments.

close to unity (Figures 4 and 5, Table 5). We, thus, concluded that the antagonist was fully equilibrated under these conditions. PZ displayed high affinity for α_{1A}-, α_{1B}- and α_{1D}-AR ($pA_2 > 9$), but low affinity for α_{1L}-AR. In contrast to PZ, KMD-3213 showed higher affinity for functional α_{1A}- and α_{1L}-AR than for α_{1B}- and α_{1D}-AR displaying pA_2 values of 10.0, 9.9, 7.7 and 8.3, respectively. The ratios of affinity of KMD-3213 for α_1-AR subtypes are approximately 200 for $\alpha_{1B}/(\alpha_{1A}$ or $\alpha_{1L})$ and 50 for $\alpha_{1D}/(\alpha_{1A}$ or $\alpha_{1L})$, which are similar values with those obtained in binding experiments. YM-617 showed high affinity for all subtypes ($pA_2 > 9$), and BMY7378 displayed α_{1D}-AR selectivity.

Discussion

KMD-3213, a recently synthesized α_{1A}-AR antagonist, displays antagonism with apparent tissue selectivity (Moriyama *et al*., 1997; Yamagishi *et al*., 1996). In the present study, we characterized the pharmacological profiles of KMD-3213 by use of its tritiated form as a radioligand and compared it with those of other α_1-AR ligands, [^{3}H]-PZ and [^{3}H]-YM.

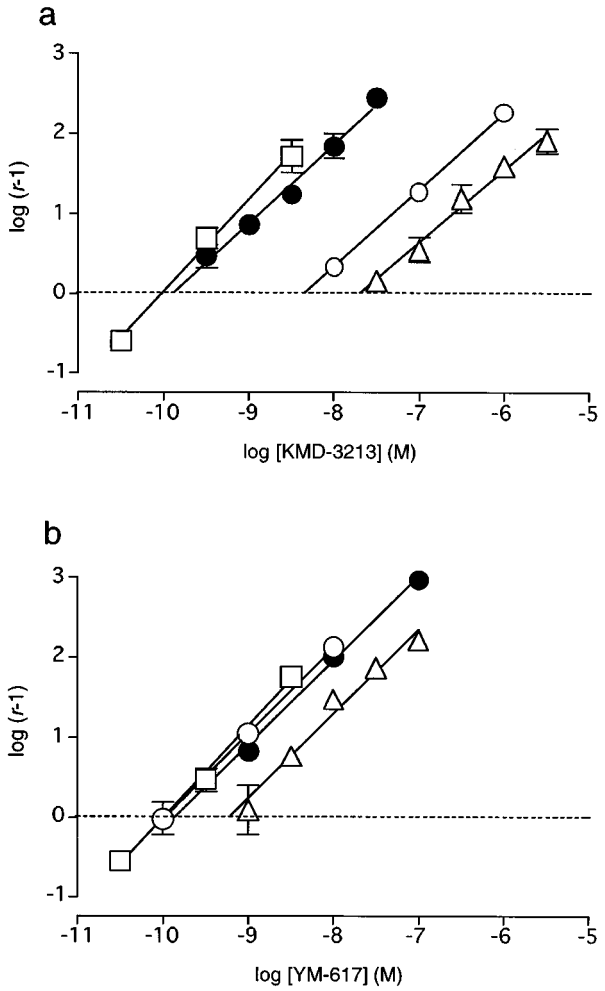

Figure 5 Comparison of Schild analyses for KMD-3213 and YM-617. Schild plots constructed with data from this Figure and Table 5 for KMD-3213 (a) or YM-617 (b) against contractile responses to NS-49 in rat caudal artery □, or to NA in dog carotid artery △, in rat thoracic aorta ○, and in dog mesenteric artery ●. Data are shown as mean±s.e.mean of 5–6 independent experiments

At first, we compared three α_1-AR specific radioligands, [^{3}H]-PZ, [^{3}H]-YM and [^{3}H]-KMD, in saturation binding experiments with recombinant α_{1a}-, α_{1b}- and α_{1d}-AR subtypes. [^{3}H]-PZ showed high affinity for all α_1-AR subtypes without apparent subtype selectivity. [^{3}H]-YM binding had slightly less affinity at α_{1b}-AR than at α_{1a}- and α_{1d}-ARs, however, relatively high affinity was observed for all subtypes as has been described previously (Han *et al*., 1995). In contrast, [^{3}H]-KMD showed high affinity for the α_{1a}-AR subtype only; [^{3}H]-KMD binding to α_{1b}- and α_{1d}-AR subtypes was insufficient and not saturated at concentrations up to 2000 pM (Table 1 and Figure 2). This high selectivity of KMD-3213 to α_{1a}-AR was also confirmed in competition binding experiments. Although YM-617 and WB4101 also showed higher affinity for the α_{1a}-AR subtype, the degree of selectivity was less than that of KMD-3213 (Table 2). These results were in good agreement with results of a previous report (Shibata *et al*., 1995), in which three recombinant subtypes of human were investigated with unlabelled KMD-3213. This suggests that the selectivity of KMD-3213 is not dependent on species and indicates that [^{3}H]-KMD could distinguish the α_{1a}-AR subtype from the other two recombinant subtypes.

At native α_1-AR subtypes, [^{3}H]-KMD showed similar subtype selectivity, i.e. high affinity for α_{1A}-AR of rat submaxillary gland and no significant binding to α_{1B}-AR of rat liver (Table 3). This selectivity was also applied to rat kidney which contains both α_{1A}- and α_{1B}-AR (Feng *et al*., 1991). Both [^{3}H]-PZ and [^{3}H]-KMD binding revealed single populations of binding sites with high affinity but showed different densities; B_{max} of [^{3}H]-KMD was 60% of that of [^{3}H]-PZ. In addition, the binding of [^{3}H]-PZ consisted of two components with high- (α_{1A}-AR) and low- (α_{1B}-AR) affinity sites discriminated by YM-617, KMD-3213 and WB4101 (Figure 3 and Table 4). The proportion of high affinity sites in the total binding sites of [^{3}H]-PZ were 68, 58 and 66% for YM-617, KMD-3213 and WB4101, respectively, which matched well with the ratio (60%) between the B_{max} values of the two [^{3}H]-ligands (Table 4). In contrast, the binding of [^{3}H]-KMD was displaced by these drugs monophasically with affinity estimates which correspond well with those for α_{1A}-AR. These results demonstrated that [^{3}H]-KMD exclusively detected an α_{1A}-AR population from the mixture labelled by ^{3}H-PZ. Since it is impossible to investigate the binding characters of α_{1D}-AR in native tissues (Yang *et al*., 1997), we conducted further functional experiments.

Rat caudal artery, dog carotid artery, rat thoracic aorta and dog mesenteric artery were employed in functional experiment as tissues in which agonist-induced contraction is mediated by α_{1A}-, α_{1B}-, α_{1D}- and α_{1L}-AR subtype, respectively (Lachnit *et al*., 1997; Muramatsu *et al*., 1990; 1991; Kenny *et al*., 1995). The

Table 5 Functional affinity of α_1-AR antagonists for native tissues

		pA_2 (Schild slope with 95% confidence limit)		
Antagonist	*Caudal artery* (rat)	*Carotid artery* (dog)	*Thoracic aorta* (rat)	*Mesenteric artery* (dog)
Prazosin	9.0±0.07 (0.9, 0.8–1.1)	9.5±0.07 (1.0, 0.9–1.2)	9.7±0.04 (1.1, 1.0–1.2)	8.1±0.04 (1.0, 0.9–1.1)
YM-617	9.9±0.06 (1.1, 1.0–1.3)	9.2±0.07 (1.1, 0.9–1.2)	9.9±0.07 (1.1, 0.9–1.2)	9.8±0.05 (1.1, 1.0–1.2)
KMD-3213	10.0±0.08 (1.2, 1.0–1.3)	7.7±0.07 (0.9, 0.8–1.1)	8.3±0.03 (1.0, 0.9–1.0)	9.9±0.05 (1.0, 0.9–1.1)
BMY7378	6.5±0.07 (1.1, 0.9–1.2)	7.1±0.06 (0.9, 0.8–1.0)	8.5±0.06 (0.9, 0.8–1.0)	6.4±0.08 (1.0, 0.8–1.2)

The effects of drugs on α_1-AR mediated contractions were examined. In rat caudal artery, NS-49 was used as an agonist. In other tissues, NA was used instead of NS-49. Affinity of antagonist (pA_2) was determined by Schild analysis. All of Schild slopes tested were not significantly different from unity. Data are shown as mean±s.e.mean of 5–6 experiments.

α_{1L}-AR, the fourth subtype (Bylund *et al*., 1998), has been proposed based on its distinct low affinity for PZ mainly in functional experiments (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1990; Ohmura *et al*., 1992). This α_{1L}-AR has not yet been identified separately as a distinct gene product and is therefore not fully characterized. Recently, Ford *et al*. (1997) suggested that the cloned α_{1a}-AR subtype, which showed typical characteristics of the α_{1a}-AR in binding experiments, exhibited pharmacological characteristics as α_{1L}-AR in the functional experiments. In fact, several investigators have indicated that there is more similarity of pharmacological characteristics between α_{1L}- and α_{1A}-ARs than between α_{1L}- and α_{1B}-/α_{1D}-ARs (Williams *et al*., 1996; Daniels *et al*., 1996). In addition, α_{1A}-AR has recently been further subdivided into four isoforms based on their structural bases derived from alternative splicing of the single gene transcript (Hirasawa *et al*., 1995; Chang *et al*., 1998). Although the pharmacological profiles of these isoforms do not differ much, as so far reported (Hirasawa *et al*., 1995; Chang *et al*., 1998), one of the isoforms may possibly represent the α_{1L}-AR. In spite of this controversy, the existence of a distinct α_1-AR with low affinity for PZ has been widely accepted especially in functional studies (Bylund *et al*., 1994; Graham *et al*., 1996; Muramatsu *et al*., 1995; Kava *et al*., 1998). Therefore, we examined the affinity of KMD-3213 for native α_1-AR subtypes including α_{1L}-AR in functional experiments. KMD-3213 showed a prominent subtype selectivity maintaining high affinity for α_{1A}- and α_{1L}-ARs. The values of pA_2 were 10.0, 9.9, 7.7 and 8.3 for α_{1A}-, α_{1L}-, α_{1B}- and α_{1D}-ARs, respectively (Table 5). In rat caudal artery, KMD-3213 sometimes gave apparently insurmountable contractile responses even at 100 μM NS-49 especially at higher concentration of the antagonist (Figure 4a). However, we thought that this is due to its high affinity to the receptor in that tissue and that the antagonism by KMD-3213 is basically competitive in nature. This α_{1A} (and/or α_{1L})-AR selectivity was not seen in both PZ and YM-617. Recently, however, several chemicals have been also developed as α_{1A}-AR selective antagonists, such as SNAP5089 (Lachnit *et al*., 1997), RS17053 (Ford *et al*., 1996), Rec15/2739 (Testa *et al*., 1996), and L762459 (O'Malley *et al*., 1998). These including KMD-3213 are targeted to medical therapeutics for diseases such as benign prostatic hypertrophy.

In conclusion, KMD-3213 is an α_1-AR antagonist that shows high affinity and selectivity for α_{1A}-AR and functional α_{1L}-AR subtypes compared with PZ or YM-617. Because of its high selectivity, KMD-3213 and its radioligand can be useful tools for analysing α_1-AR pharmacology in native tissues or recombinant receptors.

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