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Selective and sustained occupancy of prostatic α_1-adrenoceptors by oral administration of KMD-3213 and its plasma concentration in rats

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Abstract

This study examined the ex-vivo occupancy by KMD-3213 of α_1-adrenoceptors in the prostate and other tissues of rats in terms of tissue selectivity and duration of occupancy in relation to plasma concentration. Oral administration of KMD-3213 (0.2–20.2 μmol kg^{-1}, 0.5 h) dose-dependently decreased [^{3}H]prazosin binding sites (B_{max}) in the prostate (42–74 %) and submaxillary gland (54–88 %) compared with the control value. In contrast, there was only a slight change in the B_{max} values in the spleen and cerebral cortex of KMD-3213-treated rats. The α_1-adrenoceptor occupancy in the prostate and submaxillary gland was increased, with plasma free concentration of KMD-3213 at 0.5 h after oral administration of KMD-3213 (0.6–20.2 μmol kg^{-1}). The receptor occupancy in these tissues was much greater than that in the spleen, heart or cerebral cortex. After oral administration of KMD-3213 (6.1 μmol kg^{-1}), the α_1-adrenoceptor occupancy in the prostate and submaxillary gland occurred rapidly, in parallel with the rise in the plasma concentration of the drug, and it lasted for at least 24 h, despite a remarkable decrease in the plasma concentration. It is concluded that KMD-3213 may produce fairly selective and sustained occupancy of α_1-adrenoceptors in the prostate, a target organ for treatment of bladder outlet obstruction in patients with benign prostatic hyperplasia.

Introduction

α_1-Adrenoceptor antagonists are effective therapeutic agents for symptoms suggestive of urinary obstruction in patients with benign prostatic hyperplasia (Rossi et al 2001). However, α_1-adrenoceptor antagonists such as prazosin and terazosin frequently induce orthostatic hypotension as a side-effect owing to a reduction in peripheral resistance mediated by a blockade of vascular α_1-adrenoceptors. The α_1-adrenoceptor is currently classified into several subtypes (Hieble et al 1995; Michel et al 1995; Muramatsu et al 1995). A number of reports have demonstrated that human prostate predominantly contains the α_{1A}-adrenoceptor subtype, which mediates the contractile response to noradrenaline (Lepor et al 1993; Price et al 1993; Marshall et al 1995). On the other hand, it has been shown that the α_{1B}-adrenoceptor subtype mediates the noradrenaline-induced contraction of human branches of internal iliac artery (Hatano et al 1994), and Cavalli et al (1997) have demonstrated, using α_{1B}–/– mice, that α_{1B}-adrenoceptor can mediate a large portion of the vasopressor response to α_1-agonists. Thus, the α_1-adrenoceptor antagonists that have α_{1A} subtype selectivity are expected to be prostate selective therapeutic drugs with less vascular side-effects.

KMD-3213 [(–)-1-(3-hydroxypropyl)- 5-((2R)-2-{[2-({2-[(2,2,2- trifluoroethyl)oxy]-phenyl}oxy)ethyl]amino}propyl)-2,3-dihydro-1H-indole-7-carboxamide] was demonstrated as a highly selective antagonist of recombinant α_{1A}-adrenoceptor subtype and prostatic α_1-adrenoceptor in an in-vitro radioligand binding study (Shibata et al 1995; Yamagishi et al 1996; Moriyama et al 1997; Murata et al 1999). Further, Akiyama et al (1999) have demonstrated that KMD-3213 possesses inhibitory effects on the urethral pressure response, with higher functional selectivity and longer duration of action compared with hypotensive effects in a rat model. Because various pharmacokinetic and pharmacodynamic factors are not taken into account under in-vitro conditions, in-vivo

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drug–receptor binding characteristics under physiological conditions may provide more practical information for the evaluation of tissue selectivity and duration of pharmacological effects of novel drugs (Beauchamp et al 1995; Yamada et al 1998; 1999). Recently, we have demonstrated that [^{3}H]KMD-3213 selectively bound to a_1-adrenoceptors in the rat prostate and submaxillary gland, which predominantly contain the a_{1A}-adrenoceptor subtype, and the binding lasted for at least 4 h after the intravenous injection of tracer levels of the radioligand (Yamada et al 2001). It is important to characterize in-vivo a_1-adrenoceptor binding of KMD-3213 after oral administration in relation to its pharmacokinetics, because vascular side-effects of a_1-adrenoceptor antagonists such as prazosin often occur at the absorptive phase when patients take the initial dose orally (Hoffman & Lefkowitz 1996). In addition, it is important to estimate the duration of in-vivo receptor occupancy after oral administration. The ex-vivo binding technique is very useful to simultaneously measure a_1-adrenoceptor occupancy in various tissues by oral administration of unlabelled a_1-adrenoceptor antagonists at doses that showed pharmacological effects in the lower urinary tract (Ohkura et al 1998).

The purpose of this study was to characterize the in-vivo a_1-adrenoceptor occupancy by KMD-3213 in rat prostate in terms of tissue selectivity and duration of the receptor occupancy in relation to plasma concentration after the oral administration using an ex-vivo binding technique. In addition to the prostatic a_1-adrenoceptor occupancy by KMD-3213, we also measured a_1-adrenoceptor occupancy in the submaxillary gland and spleen, which contain predominantly a_{1A}- and a_{1B}-adrenoceptor subtypes, respectively (Han et al 1987; Gross et al 1988; Michel et al 1989; Testa et al 1993; Shibata et al 1995), and the heart and cerebral cortex, which contain a couple of subtypes of a_1-adrenoceptors (Terman et al 1990; Michel et al 1993; Zhang et al 1999).

Materials and Methods

Materials

[^{3}H]Prazosin (2753 GBq mmol^{-1}) was purchased from Dupont-NEN Co. Ltd (Boston, MA, USA). KMD-3213 and KMD-3278 [(–)-1-(3-hydroxybutyl)-5-((2R)-2-[[2-[[2-[(2,2,2-trifluoroethyl)oxy]phenyl]oxy)ethyl]amino]propyl-2,3-dihydro-1H-indole-7-carboxamide] were donated by Kissei Pharmaceutical Company (Matsumoto, Japan). All other chemicals were purchased from commercial sources.

Animals

Male Sprague-Dawley rats, approximately 250 g, were housed three or four per cage with free access to food and water and maintained on a 12-h light–dark cycle in a room with controlled temperature (24±1°C) and humidity (55±5%). This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of the University of Shizuoka.

Drug administration

Rats were fasted for 16 h before drug administration and were administered orally with KMD-3213 (0.2–20.2 l mol kg^{-1}) suspended in a 0.5% methylcellulose aqueous solution through a gastric tube. The oral doses of KMD-3213 were chosen as 0.2–20.2 l mol kg^{-1} because they produced dose-dependent inhibition of the phenylephrine-induced increases in the urethral pressure in rats (Akiyama et al 1999). Control rats were administered the vehicle alone. At 0.5–24 h after the drug administration, rats were killed by taking the blood from the descending aorta under light anaesthesia with diethyl ether, and perfused with 0.9% saline from the aorta. Then, prostate, submaxillary gland, spleen, heart and brain were removed, and fat and blood vessels were trimmed. The plasma was separated from the blood by centrifugation, and stored at –20°C until the plasma concentration of KMD-3213 was determined.

Tissue preparation

The tissues (prostate, submaxillary gland, spleen, heart and cerebral cortex) were minced with scissors and homogenized by a Kinematica Polytron homogenizer in 20–80 vols of ice-cold 50 mM Tris-HCl buffer (pH 7.5). The homogenates were centrifuged at 40 000 *g* for 20 min. The pellet was resuspended in the ice-cold buffer, and the suspension was centrifuged again at 40 000 *g* for 20 min. The resulting pellet was finally suspended in the buffer for the binding assay. All steps were performed at 4°C. Protein concentration was measured according to the method of Lowry et al (1951).

[^{3}H]Prazosin binding assay

The binding assay for a_1-adrenoceptors was performed using [^{3}H]prazosin as described previously (Yamada et al 1987). The homogenates (200–700 l g of protein) prepared from rat tissues were incubated with different concentrations (0.03–0.5 nM) of [^{3}H]prazosin in 50 mM Tris-HCl buffer (pH 7.5). Incubation was carried out for 30 min at 25°C. The reaction was terminated by rapid filtration (Cell Harvester; Brandel Co., Gaithersburg, MD, USA) through Whatman GF/B glass fiber filters, and the filters were rinsed 3 times with 3 mL of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid (2 L toluene, 1 L Triton X-100, 15 g 2,5-diphenyloxazole, 0.3 g 1,4-*bis*[2-(5-phenyloxazolyl)]benzene), and the radioactivity was determined by a liquid scintillation counter. Specific [^{3}H]prazosin binding was determined experimentally from the difference between

counts in the absence and presence of 10 1 M phentolamine. All assays were conducted in duplicate.

Determination of KMD-3213 in plasma

The concentration of KMD-3213 in plasma was measured by HPLC with KMD-3278 as an internal standard. Briefly, standard solution of KMD-3278, 4 mL saturated sodium bicarbonate solution and diethyl ether/dichloromethane (6:4, v/v) were added to 0.2-mL plasma samples, and the mixture was shaken and centrifuged. The organic layer was transferred and 0.01 M HCl was added, and the mixture was shaken and centrifuged. The aqueous layer was transferred to a test tube and samples were subjected to HPLC. The HPLC system consisted of a pump (Hitachi, L-7100), fluorescence detector (Hitachi, L-7480), autosampler (Hitachi, L-7200), column oven (Hitachi, L-7300) and interface (Hitachi, L-7000). The analysis was performed on an HPLC column (Inertsil ODS III, 4.6 mm i.d. $\times$ 150 mm). The column was maintained at 40°C. The mobile phase consisted of a mixture of 0.05 M phosphate buffer (pH 6.5) and propanol (3:1) at a flow rate of 1.0 mL min^{-1}. The column elute was monitored fluorometrically at excitation and emission wavelengths of 270 and 435 nm, respectively. The plasma free concentration of KMD-3213 was calculated by using the free fraction (20.5%) for in-vitro plasma protein binding of [^{3}H]KMD-3213 (Yamada et al 2001). The lower limit of determination of KMD-3213 by this method was 0.2 nM in plasma, which corresponds to 0.04 nM of the plasma free concentration.

Data analysis

The analysis of binding data was performed as described previously (Yamada et al 1980). The apparent dissociation constant (K_D) and maximum number of binding sites (B_{max}) for [^{3}H]prazosin were estimated by Rosenthal analysis of the saturation data (Rosenthal 1967). The ability of KMD-3213 to inhibit specific [^{3}H]prazosin (0.2 nM) binding in-vitro was estimated by the IC50 value, the molar concentration of KMD-3213 necessary to displace 50% of the specific binding (estimated by log probit analysis). A value for the inhibition constant (K_i) was calculated from the equation: $K_i = IC50/(1+L/K_D)$, where L is the concentration of [^{3}H]prazosin. The Hill coefficient for an inhibition of [^{3}H]prazosin binding by KMD-3213 was obtained from Hill plot analysis.

The a_1-adrenoceptor occupancy (%) in tissues after oral administration of KMD-3213 was calculated by the following equation (Ohkura et al 1998):

$$a_1\text{-adrenoceptor occupancy (\%)} = ((B_{max}(\text{control}) - B_{max}(\text{KMD-3213}))/B_{max}(\text{control})) \times 100$$

where B_{max}(control) and B_{max}(KMD-3213) are B_{max} values for [^{3}H]prazosin binding in tissues of vehicle- and KMD-3213-treated rats, respectively.

The elimination half-life of KMD-3213 in plasma was calculated from the elimination rate constant determined by linear regression analysis.

Statistical analysis of data was performed by one-way analysis of variance followed by Dunnett's test for multiple comparison. Statistical significance was accepted at $P < 0.05$.

Results

In-vitro effect on α_1-adrenoceptor binding in rat tissues

Specific binding of [^{3}H]prazosin (0.03–0.5 nM) in the prostate, submaxillary gland, spleen, heart and cerebral cortex was saturable, and Rosenthal analysis revealed a linear plot (data not shown), suggesting a single population of binding sites with K_D values of 39–82 pM and B_{max} values of 32–119 fmol (mg protein)$^{-1}$. KMD-3213 (0.01–10 nM) competed concentration-dependently with [^{3}H]prazosin for binding sites in the prostate, submaxillary gland and spleen. The K_I values for inhibition of [^{3}H]prazosin binding by KMD-3213 in the prostate and submaxillary gland were, respectively, 8.7 and 10.4 times lower than that in the spleen (Table 1). The Hill coefficients for KMD-3213 in these tissues were close to unity.

Effect of oral administration of KMD-3213 on α_1-adrenoceptor binding in rat tissues

Following oral administration of KMD-3213 (0.2–20.2 1 mol kg^{-1}) in rats, there were dose-dependent decreases (42–74%) in B_{max} values for specific [^{3}H]prazosin binding in the prostate, with no change in the K_D values (Table 2). Greater decreases (54–88%) in B_{max} values for [^{3}H]prazosin binding in the submaxillary gland were seen. On the other hand, there was no, or only a slight, change in the B_{max} values in the spleen and cerebral cortex of KMD-3213-treated rats. The B_{max} values in the heart decreased significantly (31–39%), but a dose-dependent reduction was not seen. There were significant increases in K_D values for [^{3}H]prazosin binding in the submaxillary gland, spleen and heart at the highest dose of KMD-3213 tested (20.2 1 mol kg^{-1}).

Table 1 In-vitro inhibition by KMD-3213 of specific [^{3}H]prazosin binding in rat tissues.

Tissues	Hill coefficient	K_i (nM)
Prostate	0.95±0.12	0.88±0.13
Submaxillary gland	1.20±0.12	0.74±0.19
Spleen	1.17±0.07	7.67±2.34

Each value represents mean±s.d. of three rats.

Table 2 Effects of oral administration of KMD-3213 (0.2–20.2 l mol kg^{-1}, 0.5 h later) on values of K_D and B_{max} for [^{3}H]prazosin binding in rat tissues.

Tissues	Doses (μmol kg^{-1})	K_D (pM)	B_{max}(fmol (mg protein)$^{-1}$)
Prostate	Control	68.3±9.9	31.5±5.8
	0.2	68.8±5.0	18.4±1.2***
	0.6	51.6±11.4	14.3±2.6***
	2.0	57.4±15.0	11.1±1.8***
	6.1	56.8±15.6	11.9±2.0***
	20.2	67.4±23.7	8.2±2.1***
Submaxillary gland	Control	82.1±10.3	119±14
	0.2	62.9±13.5	54.6±23.4***
	0.6	75.3±12.0	33.9±9.1***
	2.0	66.3±11.1	19.2±2.9***
	6.1	84.8±4.6	22.3±3.5***
	20.2	109±18*	14.5±3.4***
Spleen	Control	38.9±6.9	61.7±11.3
	0.2	33.0±1.3	45.8±4.8
	0.6	32.9±5.1	51.6±5.2
	2.0	36.5±0.6	48.9±4.7
	6.1	43.6±5.3	50.4±2.1
	20.2	125±8***	56.6±11.9
Heart	Control	46.4±10.0	54.3±11.8
	0.2	35.2±3.2	32.9±2.5**
	0.6	36.2±3.0	37.7±5.0*
	2.0	34.0±2.8	34.0±8.9*
	6.1	39.4±5.5	42.3±1.8
	20.2	64.5±5.7**	37.1±4.4*
Cerebral cortex	Control	54.6±1.3	105±6
	0.2	58.0±3.5	115±6
	6.1	52.5±1.6	93.2±8.8
	20.2	49.4±5.6	86.6±14.6

Values are mean±s.d. of three to nine rats. Rats received 0.2–20.2 l mol kg^{-1} of KMD-3213 orally, and were sacrificed at 0.5 h after administration. Specific binding of [^{3}H]prazosin (0.03–0.5 nM) in rat tissues was measured. Asterisks show a significant difference from control values; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Figure 1 shows the time course of B_{max} values for [^{3}H]prazosin binding in the prostate after oral administration of KMD-3213 (0.2 or 6.1 l mol kg^{-1}). After KMD-3213 administration at a dose of 0.2 l mol kg^{-1}, the B_{max} values for [^{3}H]prazosin binding in the prostate reduced by 42, 55, 43 and 52%, respectively, 0.5, 3, 6 and 12 h later; the B_{max} value then recovered to the control level 24 h later. After KMD-3213 administration at a dose of 6.1 l mol kg^{-1}, a significant decrease (60–69%) in the B_{max} value in the prostate was sustained until 24 h later.

α_1-Adrenoceptor occupancy in relation to plasma concentration of KMD-3213

Plasma concentrations of KMD-3213 after the oral administration were determined in rats used for measurement of a_1-adrenoceptor binding. Plasma concentrations of KMD-3213 were 0.4±0.7, 3.6±1.7, 50±24 and 890±781 nM (mean±s.d., n = 3–4) at 0.5 h after oral administration of KMD-3213 at doses of 0.6, 2.0, 6.1 and 20.2 l mol kg^{-1}, respectively. At a dose of 0.2 l mol kg^{-1}, the plasma concentration was below the limit of determination (0.2 nM) at 0.5–24 h after oral administration. Figure 2 shows the plasma free concentration of KMD-3213 calculated using the plasma free fraction of 0.205 (Yamada et al 2001), and a_1-adrenoceptor occupancy in the prostate, submaxillary gland, spleen, heart and cerebral cortex at 0.5 h after oral administration of KMD-3213 (0.6–20.2 l mol kg^{-1}). a_1-Adrenoceptor occupancy in the prostate and submaxillary gland significantly increased by 55–74% and 72–88%, respectively, with increasing plasma free concentration of KMD-3213 from 0.08 to 182 nM. The a_1-adrenoceptor occupancy in the spleen (12–21%), heart (22–37%) and cerebral cortex (0–17%) was much lower than that in the prostate or submaxillary gland.

Following the oral administration of KMD-3213 (6.1 l mol kg^{-1}) in rats, a_1-adrenoceptor occupancy in the prostate and submaxillary gland reached maximum levels (65 and 83%, respectively) 0.5 h later (Figure 3A) when the peak plasma free concentration of KMD-3213 was seen (Figure 3B). The plasma free concentration then decreased rapidly and KMD-3213 in plasma was not detected 24 h later. The elimination half-life of KMD-3213 was 1.3 h. In contrast, a_1-adrenoceptor occupancy by KMD-3213 in the

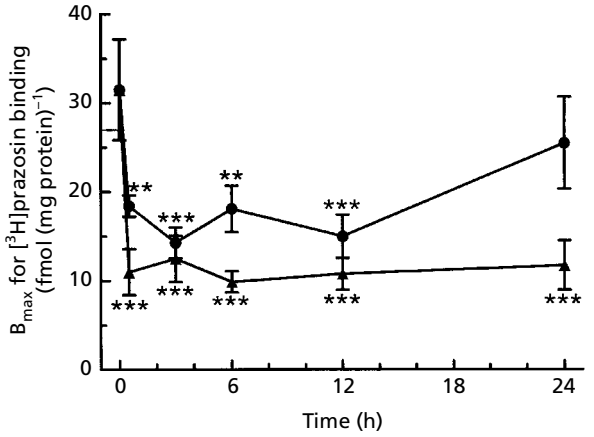

Figure 1 Changes in B_{max} values for [^{3}H]prazosin binding in the rat prostate at 0.5–24 h after oral administration of KMD-3213 at the dose of 0.2 (●) or 6.1 (▲) l mol kg^{-1}. Each point represents mean±s.d. of four to six rats. $^{**}P < 0.01$ and $^{***}P < 0.001$, significantly different compared with the control value at time 0.

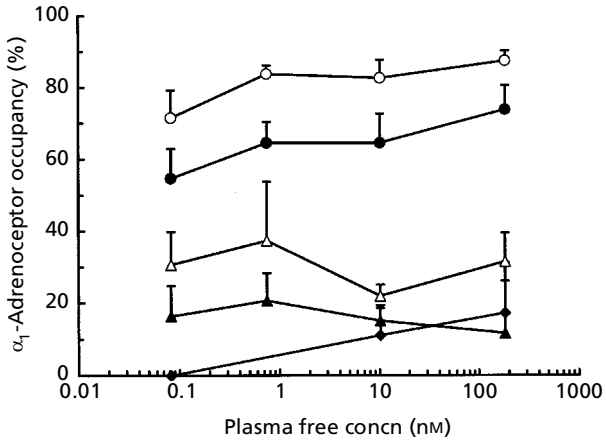

Figure 2 a_1-Adrenoceptor occupancy in rat tissues as a function of increasing plasma free concentration of KMD-3213 0.5 h after oral administration of KMD-3213 (0.6–20.2 l mol kg^{-1}) in rats. The a_1-adrenoceptor occupancy (%) in the submaxillary gland (○), prostate (●), heart (△), spleen (▲) and cerebral cortex (◆) was calculated by the equation: $((B_{max}(control) - B_{max}(KMD\text{-}3213))/B_{max}(control)) \times 100$. Each point represents mean±s.d. of three to four rats.

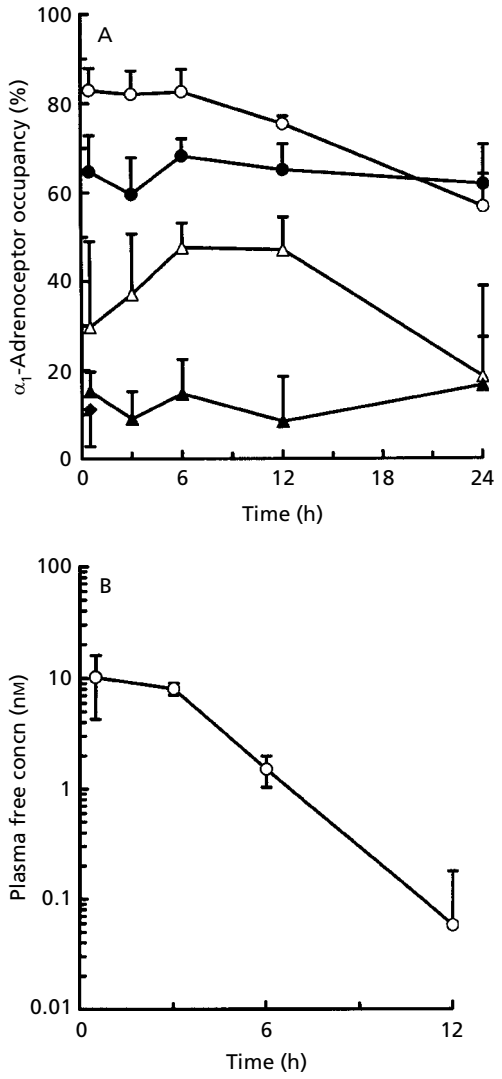

Figure 3 Time course of a_1-adrenoceptor occupancy in tissues (A) and plasma free concentration of KMD-3213 (B) after oral administration of KMD-3213 (6.1 l mol kg^{-1}) in rats. The a_1-adrenoceptor occupancy (%) in the submaxillary gland (○), prostate (●), heart (△), spleen (▲) and cerebral cortex (◆) after oral administration of KMD-3213 was calculated by the equation: $((B_{max}(control) - B_{max}(KMD\text{-}3213))/B_{max}(control)) \times 100$. Each point represents mean±s.d. of three to four rats.

prostate and submaxillary gland was maintained at relatively high levels (62 and 57%, respectively) until 24 h later. The a_1-adrenoceptor occupancy in other tissues at 0.5–24 h after the oral administration was 19–48% in heart, 8–17% in spleen, and 11% in cerebral cortex, 0.5 h later.

Discussion

The major findings of this study are that: (i) orally administered KMD-3213 more selectively occupied a_1-adrenoceptors in the prostate and submaxillary gland of rats than those in the spleen, heart and cerebral cortex; and (ii) its receptor occupancy was considerably sustained in spite of a rapid disappearance of KMD-3213 from the plasma.

KMD-3213 competed with [^{3}H]prazosin for the binding sites in the prostate, submaxillary gland and spleen of rats in-vitro. KMD-3213 was 9 and 10 times more potent in inhibiting specific [^{3}H]prazosin binding in the prostate and submaxillary gland, respectively, than in the spleen. The technique of ex-vivo receptor binding is useful for predicting in-vivo potency, organ selectivity and duration of action of drugs in relation to the pharmacokinetics and pharmacodynamics (Igari et al 1985; Ohkura et al 1998; Yamada et al 1999). This technique was used in this study to simultaneously examine in-vivo occupancy of a_1-adre-

noceptors by KMD-3213 in the prostate and other tissues of rats. The oral administration of KMD-3213 (0.2–20.2 l mol kg^{-1}) brought about dose-dependent decreases in B_{max} values for [^{3}H]prazosin in the prostate (42–74%) and submaxillary gland (54–88%) compared with the control value. In contrast, there was a slight decrease in [^{3}H]prazosin binding in the spleen and cerebral cortex of KMD-3213-treated rats. Figure 2 shows the plasma free concentration of KMD-3213 and a_1-adrenoceptor occupancy in each tissue at 0.5 h after oral administration of KMD-3213 (0.6–20.2 l mol kg^{-1}). Over the range of plasma free concentration of KMD-3213 from 0.08 to 182 nM, a_1-adrenoceptor occupancy in the prostate and submaxillary gland increased concentration dependently, and it was higher than in the spleen, heart and cerebral cortex. This selective a_1-adrenoceptor occupancy in the prostate and submaxillary gland by orally administered KMD-3213 corresponds well with selective in-vivo a_1-adrenoceptor binding of [^{3}H]KMD-3213 in these tissues after intravenous injection (Yamada et al 2001). Thus, it is considered that KMD-3213 binds more selectively to a_1-adrenoceptors in the prostate and submaxillary gland than those in the spleen, heart and cerebral cortex after the oral administration in rats.

Currently, a_1-adrenoceptors are classified pharmacologically into a_{1A}, a_{1B} and a_{1D} subtypes (Han et al 1987; Hieble et al 1995; Michel et al 1995). It has been shown that the a_{1A} subtype exists predominantly in the prostate and submaxillary gland of rats (Michel et al 1989; Testa et al 1993; Yazawa & Honda 1993; Shibata et al 1995; Ford et al 1996), whereas the a_{1B} subtype exists in the spleen (Han et al 1987; Gross et al 1988; Michel et al 1993). A couple of subtypes coexist in the heart and cerebral cortex (Terman et al 1990; Michel et al 1993; Zhang et al 1999). It is known that KMD-3213 is a selective antagonist of the a_{1A} subtype relative to a_{1B} or a_{1D} subtypes in-vitro (Shibata et al 1995; Yamagishi et al 1996; Moriyama et al 1997; Murata et al 1999). In the present study, compared with the spleen, a_1-adrenoceptors in the prostate and submaxillary gland of rats were more sensitive to the blockade by orally administered KMD-3213, and the blockade in the heart was intermediate. As KMD-3213 competed with [^{3}H]prazosin for the binding sites with higher affinity in the prostate and submaxillary gland of rats than in the spleen in-vitro, it is considered that the difference in the in-vivo sensitivity of KMD-3213 among these tissues may be attributable to the distinct distribution of a_1-adrenoceptor subtypes. Therefore, the present study clearly shows that KMD-3213 maintains the a_1-adrenoceptor selectivity in the prostate and submaxillary gland under in-vivo conditions, which includes pharmacokinetic factors. There was no significant decrease in the cerebral cortical [^{3}H]prazosin binding by KMD-3213, although both a_{1A} and a_{1B} subtypes exist equally in rat brain (Terman et al 1990; Michel et al 1993). Little occupancy of a_1-adrenoceptors in the cerebral cortex by KMD-3213 may be consistent with poor transport of this drug across the blood–brain barrier.

The relationship between the time course of a_1-adrenoceptor occupancy by KMD-3213 in tissues and its plasma free concentration was examined. KMD-3213 was absorbed rapidly after oral administration (6.1 l mol kg^{-1}) in rats, with the plasma free concentration decreasing rapidly to under detectable limits (0.04 nM) after 24 h. The a_1-adrenoceptor occupancy in the prostate and submaxillary gland appeared to attain the maximum level (65 and 83%, respectively) at 0.5 h, when the peak plasma concentration of KMD-3213 was seen. An almost similar extent of the receptor occupancy in both tissues was maintained until 24 h, despite a marked decrease in the plasma free concentration to less than in-vitro K_i values for KMD-3213 (0.88 nM in the prostate and 0.74 nM in the submaxillary gland). Thus, the receptor occupancy by this drug may be characterized by a slow dissociation from the receptor sites. In ex-vivo binding studies, the drugs that represent slow dissociation from the receptor inhibit radioligand binding with a change in B_{max} values rather than in K_D values (Zernig et al 1996; Ohkura et al 1998). The ex-vivo blockade of [^{3}H]prazosin binding in each tissue by KMD-3213 was mainly related to a marked decrease in B_{max} values. This also suggests that KMD-3213 dissociates from the a_1-adrenoceptors at a slow rate. This kinetic property of KMD-3213 is in agreement with the slow dissociation of [^{3}H]KMD-3213 from rat prostatic a_1-adrenoceptors in-vitro and in-vivo (Yamada et al 2001). Akiyama et al (1999) demonstrated long-acting pharmacological effects of KMD-3213 in-vivo in rats. Figure 4 shows the relationship between prostatic a_1-adrenoceptor occupancy in this study and inhibition of phenylephrine-induced increase in intraurethral pressure reported by Akiyama et al (1999) after oral administration of KMD-3213 (0.2 l mol kg^{-1}). The pharmacological effect of KMD-3213 in the lower urinary tract correlates well with prostatic a_1-adrenoceptor occupancy. It is considered that the long-

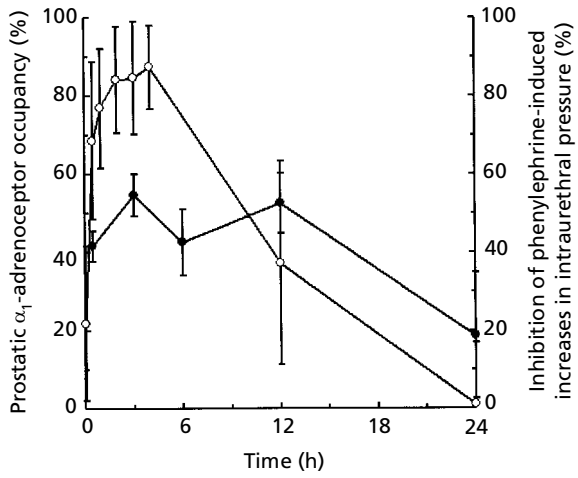

Figure 4 Time course of prostatic a_1-adrenoceptor occupancy (●) and inhibition of phenylephrine-induced increase in intraurethral pressure (○) after oral administration of KMD-3213 (0.2 l mol kg^{-1}) in rats. The prostatic a_1-adrenoceptor occupancy (%) after oral administration of KMD-3213 was calculated by the equation: $((B_{max}(\text{control}) - B_{max}(\text{KMD-3213}))/B_{max}(\text{control})) \times 100$. Pharmacological data were derived from literature (Akiyama et al 1999). Each point represents mean ± s.d. of three to eight rats.

acting pharmacological effect of KMD-3213 in the lower urinary tract of rats is owing to sustained occupancy of prostatic a_1-adrenoceptors by KMD-3213.

In conclusion, the present study strongly suggests that oral administration of KMD-3213 in-vivo produces reasonably selective and sustained occupancy of a_1-adrenoceptors in the prostate, a target organ for treatment of bladder outlet obstruction in patients with benign prostatic hyperplasia.

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