Ex Vivo and in Vivo α_1 -Adrenoceptor Binding Characteristics of a Novel α_{1L} -Adrenoceptor Antagonist, JTH-601, in Rat Tissues

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In vitro, *ex vivo* and *in vivo* α_1 -adrenoceptor binding of JTH-601 (3- $\{N$ -[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]- N -methylaminomethyl}-4-methoxy-2,5,6-trimethyl-phenol hemifumarate), a novel α_{1L} -adrenoceptor antagonist, in rat tissues was investigated. JTH-601 competed in a concentration-dependent manner with [3H]prazosin for binding sites in the prostate, submaxillary gland and spleen of rats *in vitro*, and the inhibitory effect was not largely different among these tissues, as shown by the K_i values of 2–3 nM. At 0.25, 0.5 and 3 h after oral administration of JTH-601 (6.5 μ mol/kg) in rats, there was a significant (57, 64 and 28%, respectively) increase in the apparent dissociation constant (K_d) for prostatic [3H]prazosin binding, compared to the control value. The administration of a higher dose (21.8 μ mol/kg) of this agent produced greater (67–99%) increases in K_d values for prostatic [3H]prazosin binding at 0.5–12 h later. Similar significant increases in K_d values, as with the prostate, were seen in the submaxillary gland and heart 0.25–12 h after the oral administration of JTH-601 (6.5 and 21.8 μ mol/kg), but significant increases in the spleen and cerebral cortex were seen only at 0.25–3 h and 0.5 h, respectively. At 10 min of i.v. injection of [3H]JTH-601 in rats, *in vivo* specific binding was observed in the prostate, cerebral cortex, submaxillary gland, spleen and heart but not in the aorta. The binding in the prostate, submaxillary gland and heart, but not in the cerebral cortex and spleen, lasted until 120 min. It is concluded that JTH-601 may exert a considerably sustained blockade of α_1 -adrenoceptors in the prostate of rats. This finding may be important in characterizing the therapeutic effect of JTH-601 for bladder outlet obstruction in patients with benign prostatic hyperplasia.

Key words JTH-601; α_{1L} -adrenoceptor antagonist; *in vivo* α_1 -adrenoceptor binding; prostate

New α_1 -adrenoceptor antagonists are currently receiving a great deal of attention, especially in terms of developing effective agents to treat bladder outlet obstruction with fewer vascular side effects in patients with benign prostatic hyperplasia (BPH).^{1–3)} α_1 -Adrenoceptors have been classified into three distinct subtypes, α_{1A} -, α_{1B} - and α_{1D} -subtypes. α_{1A} -Adrenoceptors have been shown in radioligand binding and molecular biological studies to be predominant in the human prostate.^{4–6)} However, several studies have shown that the pharmacological profile of the α_1 -adrenoceptor in mediating a contractile response to noradrenaline in the human lower urinary tract tissues differs somewhat from the profile in binding studies of the α_{1A} -adrenoceptor subtype.⁷⁾ An additional α_1 -adrenoceptor subtype which was involved in the contraction of these tissues has been proposed and has been designated as an α_{1L} -adrenoceptor based on its low affinity for prazosin.⁸⁾ JTH-601 (3- $\{N$ -[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]- N -methylamino-methyl}-4-methoxy-2,5,6-trimethylphenol hemifumarate), a novel α_1 -adrenoceptor antagonist, showed a 10 times higher affinity for the α_{1L} -adrenoceptor, a similar affinity for α_{1A} -adrenoceptors, but more than 10 times lower affinity for the α_{1B} - and α_{1D} -adrenoceptors compared with prazosin *in vitro*.⁹⁾ Such *in vitro* receptor binding characteristics of this agent may not necessarily assure pharmacological specificity, such as tissue selectivity and subtype specificity *in vivo*, because they do not take various pharmacokinetic (absorption, distribution and elimination) and pharmacodynamic factors into account. The technique of *ex vivo* and *in vivo* receptor binding is useful in predicting *in vivo* potency, organ selectivity, and duration of action of drugs in relation to their pharmacokinetics and pharmacodynamics.^{10–12)} Thus, the purpose of

the present study was to characterize α_1 -adrenoceptor binding of JTH-601 in the prostate and other tissues of rats under physiological conditions.

MATERIALS AND METHODS

Materials [3H]Prazosin (2.75 TBq/mmol) was purchased from Dupont-NEN Co., Ltd. (Boston, MA, U.S.A.). [3H]JTH-601 (1.04 TBq/mmol) was synthesized by Amersham Int. PLC (Buckinghamshire, England). JTH-601 and terazosin hydrochloride were donated by Japan Tobacco, Inc. (Osaka, Japan) and Mitsubishi Chemical Industries (Tokyo, Japan), respectively. All other chemicals were purchased from commercial sources.

Tissue Preparation Male Sprague Dawley rats weighing about 250 g were housed three to four per cage in the laboratory with free access to food and water, and were maintained on a 12 h dark/light cycle in a room with controlled temperature (24 $\pm$ 1 $^{\circ}$ C) and humidity (55 $\pm$ 5%). They were fasted for 16 h before drug administration, then were orally administered JTH-601 (6.5 and 21.8 μ mol/kg) suspended in methyl cellulose. Control animals were administered the vehicle alone. At 0.5 to 24 h after the drug administration, rats were killed by taking the blood from the descending aorta under temporary anesthesia with diethyl ether, and were then perfused with 0.9% saline from the aorta. Then, the prostate, submaxillary gland, spleen, heart and brain were dissected, and fat and blood vessels were trimmed.

The tissues (prostate, submaxillary gland, spleen, heart and cerebral cortex) from rats were minced with scissors and homogenized by a Kinematica Polytron homogenizer in 20–80 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5). The

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homogenates were centrifuged at $40000 \times g$ for 20 min. The pellet was resuspended in ice-cold buffer, and the suspension was centrifuged again at $40000 \times g$ 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.*¹³⁾

[³H]Prazosin Binding Assay The binding assay for α_1 -adrenoceptors was performed using [³H]prazosin as previously described.¹⁴⁾ The homogenates (200–700 μg of protein) of rat tissues were incubated with different concentrations (0.03–1.0 nM) of [³H]prazosin in 50 mM of a 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, U.S.A.) through Whatman GF/B glass fiber filters, and the filters were rinsed three times with 3 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid (2 l of toluene, 1 l of Triton X-100, 15 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene), and the radioactivity was determined by a liquid scintillation counter. Specific [³H]prazosin binding was determined experimentally from the difference between counts in the absence and presence of $10 \mu\text{M}$ phentolamine. All assays were conducted in duplicate.

In Vivo [³H]JTH-601 Binding Assay The particulate bound radioactivity in rat tissues after i.v. injection of [³H]-JTH-601 was measured according to essentially the same method described recently for the *in vivo* measurement of α_1 -adrenoceptors by [³H]tamsulosin.¹²⁾ [³H]JTH-601 (555 kBq, 2.4 nmol/kg) was injected into the femoral vein of vehicle- and phentolamine (62.9 $\mu\text{mol/kg}$ i.p. 0.5 h pretreatment)-administered rats under temporary anesthesia with diethyl ether. The animals were allowed to recover, then were sacrificed by taking blood from the descending aorta under temporary anesthesia with diethyl ether at 10 min after the injection. Next, the tissues (prostate, aorta, cerebral cortex, submaxillary gland, spleen and heart) were rapidly dissected, and each tissue was homogenized in ice-cold 50 mM Tris–HCl buffer to a final tissue concentration of 10 mg/ml using a Kinematica Polytron homogenizer. Particulate bound radioactivity was determined by the rapid filtration of 1–3 ml of homogenate over Whatmann GF/C filters, which were subsequently washed with 2 ml of ice-cold buffer. The particulate-bound radioactivity was measured using a liquid scintillation counter after the addition of scintillation fluids.

Data Analysis The analysis of binding data of [³H]prazosin in *ex vivo* experiments was performed as described previously.¹⁵⁾ The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [³H]prazosin were estimated by Rosenthal analysis of the saturation data.¹⁶⁾ Statistical analysis of the data was performed by a one-way analysis of variance (ANOVA), followed by Dunnet'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 The specific binding of [³H]prazosin (0.03–1.0 nM) in the prostate, submaxillary gland, spleen, heart and cerebral cortex was saturable, and Rosenthal analysis revealed a linear

Table 1. Effects of Oral Administration of JTH-601 (6.5 $\mu\text{mol/kg}$) on the Values of K_d and B_{max} for [³H]Prazosin in the Prostate, Submaxillary Gland, Spleen, Heart and Cerebral Cortex of Rats

		K_d (pM)	B_{max} (fmol/mg protein)
Prostate	Control	68.3 $\pm$ 3.5	31.5 $\pm$ 2.0
	0.25 h	107 $\pm$ 5***	25.8 $\pm$ 1.8
	0.5 h	112 $\pm$ 7***	29.6 $\pm$ 3.5
	3 h	87.5 $\pm$ 5.7*	31.2 $\pm$ 3.0
Submaxillary gland	Control	82.1 $\pm$ 3.4	119 $\pm$ 5
	0.25 h	125 $\pm$ 16**	101 $\pm$ 3
	0.5 h	127 $\pm$ 17**	96.3 $\pm$ 13.0
	3 h	91.1 $\pm$ 2.6	102 $\pm$ 4
Spleen	Control	38.9 $\pm$ 2.3	61.7 $\pm$ 3.8
	0.25 h	144 $\pm$ 25***	52.4 $\pm$ 3.8
	0.5 h	112 $\pm$ 19***	53.0 $\pm$ 2.4
	3 h	53.0 $\pm$ 2.6	55.4 $\pm$ 2.6
Heart	Control	46.4 $\pm$ 3.3	54.3 $\pm$ 3.9
	0.25 h	103 $\pm$ 8***	56.2 $\pm$ 6.5
	0.5 h	74.0 $\pm$ 7.4**	36.9 $\pm$ 1.2
	3 h	55.2 $\pm$ 1.7	57.7 $\pm$ 6.0
Cerebral cortex	Control	54.6 $\pm$ 0.7	105 $\pm$ 3
	0.5 h	82.4 $\pm$ 5.5***	115 $\pm$ 1
	3 h	51.0 $\pm$ 2.3	110 $\pm$ 3

Values are the mean $\pm$ S.E. of three to nine rats. Rats received 6.5 $\mu\text{mol/kg}$ of JTH-601 orally, and then were killed at 0.25 to 3 h after the administration. Specific binding of [³H]prazosin (0.03–1.0 nM) in rat tissues was measured. *, ** and ***, significantly different from the control value at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

plot, suggesting a single population of binding sites with K_d values of 38.9–82.1 pM and B_{max} of 31.5–119 fmol/mg protein. JTH-601 (1–100 nM) competed in a concentration-dependent manner with [³H]prazosin binding in the prostate, submaxillary gland and spleen. The K_i values for JTH-601 were 2.06 ± 0.17 (prostate), 2.89 ± 0.85 (submaxillary gland) and 3.00 ± 0.24 (spleen) nM (mean $\pm$ S.E., $n=3$), respectively.

Ex Vivo Effects on α_1 -Adrenoceptor Binding in Rat Tissues The effects of the oral administration of JTH-601 on specific [³H]prazosin binding in the prostate, submaxillary gland, spleen, heart and cerebral cortex of rats were investigated. Following the oral administration of JTH-601 at doses of 6.5 and 21.8 $\mu\text{mol/kg}$ in rats, there was a significant increase in K_d values for specific [³H]prazosin binding in the prostate compared to the control value, as shown in Tables 1 and 2. Figure 1 shows Rosenthal plots for specific [³H]prazosin binding in the prostate 3 h after the oral administration of a vehicle and JTH-601 at doses of 6.5 and 21.8 $\mu\text{mol/kg}$. The increases in K_d values were 57, 64 and 28%, respectively, at 0.25, 0.5 and 3 h after the administration of 6.5 $\mu\text{mol/kg}$ JTH-601. Also, K_d values for specific [³H]prazosin binding increased by 99, 79, 67 and 87%, respectively, at 0.5, 3, 6 and 12 h after the oral administration of a higher dose (21.8 $\mu\text{mol/kg}$), and the K_d value 24 h later was comparable to the control value. Similar significant increases in K_d values, as in the case of the prostate, were seen in the submaxillary gland and heart at 0.25–12 h after the oral administration of JTH-601 (6.5, 21.8 $\mu\text{mol/kg}$). In the spleen of JTH-601 administered rats, there was a marked increase in the K_d values for [³H]prazosin binding at 0.25 and 0.5 h at the dose of 6.5 $\mu\text{mol/kg}$ and also at 0.5 and 3 h later at the dose of 21.8 $\mu\text{mol/kg}$ of JTH-601, and thereafter, no significant increase by this agent was seen. JTH-601 administration showed significant increases in K_d values for cerebral cortical

Table 2. Effects of Oral Administration of JTH-601 (21.8 $\mu\text{mol/kg}$) on Values of K_d and B_{max} for [^3H]Prazosin in Prostate, Submaxillary Gland, Spleen, Heart and Cerebral Cortex of Rats

		K_d (pM)	B_{max} (fmol/mg protein)
Prostate	Control	68.3 $\pm$ 3.5	31.5 $\pm$ 2.0
	0.5 h	136 $\pm$ 19***	27.5 $\pm$ 3.7
	3 h	122 $\pm$ 17**	26.8 $\pm$ 3.3
	6 h	114 $\pm$ 15*	30.0 $\pm$ 3.4
	12 h	128 $\pm$ 10***	30.2 $\pm$ 3.9
	24 h	77.9 $\pm$ 3.9	31.7 $\pm$ 4.0
Submaxillary gland	Control	82.1 $\pm$ 3.4	119 $\pm$ 5
	0.5 h	173 $\pm$ 6***	97.2 $\pm$ 13.9
	3 h	127 $\pm$ 6***	114 $\pm$ 12
	6 h	111 $\pm$ 9**	106 $\pm$ 4
	12 h	129 $\pm$ 5***	121 $\pm$ 3
	24 h	91.3 $\pm$ 2.0	102 $\pm$ 8
Spleen	Control	38.9 $\pm$ 2.3	61.7 $\pm$ 3.8
	0.5 h	239 $\pm$ 66***	72.7 $\pm$ 3.7
	3 h	149 $\pm$ 33**	60.5 $\pm$ 1.2
	6 h	74.2 $\pm$ 8.1	60.5 $\pm$ 4.2
	12 h	67.0 $\pm$ 8.5	73.7 $\pm$ 3.8
	24 h	46.1 $\pm$ 1.5	62.9 $\pm$ 4.0
Heart	Control	46.4 $\pm$ 3.3	54.3 $\pm$ 3.9
	0.5 h	129 $\pm$ 16***	55.8 $\pm$ 3.9
	3 h	82.1 $\pm$ 9.1**	46.3 $\pm$ 2.8
	6 h	69.8 $\pm$ 4.6	43.6 $\pm$ 2.8
	12 h	74.6 $\pm$ 6.3*	52.3 $\pm$ 1.4
	24 h	47.3 $\pm$ 1.0	50.4 $\pm$ 2.4
Cerebral cortex	Control	54.6 $\pm$ 0.7	105 $\pm$ 3
	0.5 h	76.8 $\pm$ 1.0***	113 $\pm$ 2
	3 h	56.7 $\pm$ 0.2	111 $\pm$ 6

Values are the mean $\pm$ S.E. of three to nine rats. Rats received 21.8 $\mu\text{mol/kg}$ of JTH-601 orally, and they were killed at 0.5 to 24 h after the administration. Specific binding of [^3H]prazosin (0.03–1.0 nM) in rat tissues was measured. *, ** and ***, significantly different from the control value at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

Fig. 1. Rosenthal Plots of Specific [^3H]Prazosin Binding in the Prostate 3 h after Oral Administration of Vehicle (O) and JTH-601 at Doses of 6.5 (●) and 21.8 (▲) $\mu\text{mol/kg}$

Rats received JTH-601 orally, and were sacrificed 3 h after the administration. Each point represents the average of four to nine rats.

[^3H]prazosin binding only at 0.5 h. The JTH-601 administration produced little significant change in B_{max} value in each tested tissue of rats.

In Vivo Specific Binding of [^3H]JTH-601 The particulate-bound radioactivity was measured in the prostate, aorta, cerebral cortex, submaxillary gland, spleen and heart at 10, 60 and 120 min after the intravenous injection of [^3H]JTH-601 at a dose of 2.4 nmol/kg (555 kBq) in both vehicle- and

Fig. 2. *In Vivo* Specific Binding in Rat Tissues 10 min after i.v. Injection of [^3H]JTH-601

[^3H]JTH-601 (555 kBq, 2.4 nmol/kg) was injected into the femoral vein of rats, and 10 min later the specific binding in each tissue was measured. Each column represents the mean $\pm$ S.E. of three rats.

Fig. 3. Time Course of *in Vivo* Specific Binding in Rat Tissues (O, Heart; ▲, Submaxillary Gland; ●, Prostate; ◆, Spleen; △, Cerebral Cortex) 10, 60 and 120 min after i.v. Injection of [^3H]JTH-601

[^3H]JTH-601 (555 kBq, 2.4 nmol/kg) was injected into the femoral vein of rats, then at 10, 60 and 120 min later, the specific binding in each tissue was measured. Each column represents the mean $\pm$ S.E. of three rats.

phentolamine (62.9 $\mu\text{mol/kg}$ i.p.)-pretreated rats. The particulate bound radioactivity in each tissue of vehicle- and phentolamine-pretreated rats after the i.v. injection of [^3H]JTH-601 was considered as total binding and nonspecific binding, respectively, of the radioligand, and the difference was defined as the *in vivo* specific binding of [^3H]JTH-601. As shown in Fig. 2, *in vivo* specific binding 10 min after the injection of [^3H]JTH-601 was observed in the prostate, cerebral cortex, submaxillary gland, spleen and heart, but not in the aorta (data not shown). Figure 3 illustrates the time course of *in vivo* specific binding in rat tissues 10, 60 and 120 min after the i.v. injection of [^3H]JTH-601. The [^3H]JTH-601 binding in each tissue decreased with time; a significant amount of specific binding in the prostate, submaxillary gland and heart was seen even at 60 and 120 min later, but binding in the cerebral cortex and spleen had disappeared by these times.

DISCUSSION

JTH-601 competed in a concentration-dependent manner with [3 H]prazosin for binding sites in the prostate, submaxillary gland and spleen of rats *in vitro*, and the inhibitory effect was not largely different among these tissues, as shown by the K_i values of 2–3 nM. Thus, JTH-601 appeared to bind α_1 -adrenoceptors in these tissues with similar affinity *in vitro*. An *ex vivo* and *in vivo* receptor binding technique was used to simultaneously examine α_1 -adrenoceptor binding characteristics in the prostate and other tissues of rats after the systemic administration of JTH-601. In these experiments, there is a possibility that this agent might dissociate partly from the receptor sites during tissue preparation (homogenization and suspension) after the oral administration of JTH-601 or the i.v. injection of [3 H]JTH-601 in rats. Yamada *et al.*¹⁵⁾ have previously shown that the dissociation of α_1 -adrenoceptor antagonists from receptor sites in the rat heart at 4 °C was extremely slow. To minimize the dissociation of JTH-601 and [3 H]JTH-601 from receptor sites, therefore, all steps for the tissue preparation were performed at 4 °C.

Oral doses of 6.5 and 21.8 μ mol/kg of JTH-601 were used in the *ex vivo* experiment, because the intraduodenal administration of this agent at these doses produced significant inhibitory effects on the phenylephrine-induced increase in the urethral pressure of anesthetized rabbits (Aisaka *et al.*, unpublished observation). The oral administration of JTH-601 at a dose of 6.5 μ mol/kg in rats brought about a significant (28–64%) increase in K_d values for [3 H]prazosin binding in the prostate at 0.25, 0.5 and 3 h later, compared to the control value. Greater (67–99%) increases in K_d values were also observed at 0.5, 3, 6 and 12 h after the administration of a higher dose (21.8 μ mol/kg) of JTH-601. Similar significant increases in K_d values were seen in the submaxillary gland and heart at 0.25–12 h after JTH-601 administration. In the spleen of these JTH-601-administered rats, there was a marked increase in K_d values for [3 H]prazosin binding at 0.25, 0.5 and/or 3 h later, and thereafter, the effect by this agent declined rapidly with time. The cerebral cortex showed a significant increase in K_d value only at 0.5 h later. These data suggest that JTH-601 produces a more sustained blockade of α_1 -adrenoceptors in the prostate, submaxillary gland and heart than in the spleen and cerebral cortex. The sustained blockade of α_1 -adrenoceptors in the prostate may result in the long-lasting pharmacological effect of this agent in the lower urinary tract. Inasmuch as JTH-601 administration had little significant effect on the $B_{\max}$ values for [3 H]prazosin binding in each tissue of the rats, it is suggested that the blockade by this agent of α_1 -adrenoceptor sites in rat tissues may be related mainly to a decrease in affinity rather than a decrease in receptor density. This mode of antagonism by the oral administration of JTH-601 contrasted with that by tamsulosin, but it was similar to that by terazosin.¹¹⁾

At 10 min after the i.v. injection of a relatively low dose of [3 H]JTH-601 in rats, *in vivo* specific binding was observed in particulate fractions of the prostate, cerebral cortex, submaxillary gland, spleen and heart, but not the aorta. The specific binding in the prostate, submaxillary gland and heart was

seen even at 60 and 120 min after the injection of [3 H]JTH-601, but not in the spleen and cerebral cortex. These data seem to coincide with the *ex vivo* observation of a more sustained blockade by JTH-601 of α_1 -adrenoceptors in the prostate, submaxillary gland and heart of rats than in the spleen and cerebral cortex. Thus, there was a significant difference among tissues in the time course of α_1 -adrenoceptor binding of JTH-601 under a physiological condition. As a possible mechanism, it is suggested that the sustained binding of JTH-601 in the prostate, submaxillary gland and heart of rats is due to the relatively slow dissociation rate of this ligand from the receptor sites. Alternatively, the observed difference in the time course of α_1 -adrenoceptor binding of JTH-601 may be ascribable to the difference among tissues in pharmacokinetic factors such as organ blood flow rate, and/or the distribution and metabolism of this agent. It is of great interest that [3 H]JTH-601 showed little *in vivo* specific binding in the rat aorta, because [3 H]prazosin, [3 H]tamsulosin and [3 H]KMD-3213 exhibited significant amounts of *in vivo* specific binding in this tissue.¹⁰⁾

In conclusion, the present study suggests that JTH-601 exerts considerably sustained blockade of α_1 -adrenoceptors in the prostate of rats under a physiological condition. This finding may be important in characterizing the therapeutic effects by JTH-601 for bladder outlet obstruction in patients with BPH.

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