DISPOSITION AND $\alpha_1$-ADRENOCEPTOR BINDING CHARACTERISTICS OF JTH-601 AND ITS METABOLITES IN RAT TISSUES

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(Received June 10, 2000; accepted August 18, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:
The present study was performed to characterize the disposition and $\alpha_1$-adrenoceptor binding of JTH-601, a novel $\alpha_1$-adrenoceptor antagonist, and its metabolites ($\beta$-D-glucopyranosyl uronic acid, JTH-601-G1; hydrogen sulfate, JTH-601-S1) in the rat prostate and other tissues. JTH-601, JTH-601-G1, and JTH-601-S1 inhibited competitively specific $[3H]$tamsulosin binding in the prostate, submaxillary gland, and spleen of rats in vitro, and the inhibitory effect of JTH-601 was 2.5 to 6.4 times more potent than that of its metabolites. JTH-601 and its metabolites inhibited dose dependently in vivo specific $[3H]$tamsulosin binding in the particular fraction of the prostate, aorta, submaxillary gland, and spleen of rats. Compared with that of JTH-601, the in vivo inhibitory effect of JTH-601-G1 was 1.9 to 2.9 times more potent, and the effect of JTH-601-S1 was 1.3 to 3.2 times less potent. Based on the ratios of ID$_{50}$ values, JTH-601 and JTH-601-G1 appeared to be 4.0 to 6.9 times more selective than prazosin as far as the $\alpha_1$-adrenoceptors in the prostate and submaxillary gland versus the spleen or aorta were concerned. The total radioactivity in rat tissues after i.v. injection of $[^3H]$JTH-601-G1 was considerably lower than that of $[^3H]$JTH-601. The plasma concentration of $[^3H]$JTH-601-G1 at 10 min after i.v. injection in rats was 3 times higher than that of $[^3H]$JTH-601, and conversely, the concentration in the prostate was 3 times lower. Although in vivo $[^3H]$JTH-601-G1 binding at 10 min was significantly lower than that of $[^3H]$JTH-601 in most rat tissues, there was comparable binding between these radioligands in the prostate and vas deferens. Specific binding of $[^3H]$JTH-601, at 60 min after i.v. injection compared with that at 10 min, was considerably reduced in rat tissues except the prostate and vas deferens, both of which showed relatively sustained binding. In conclusion, the present study has shown that JTH-601 and its metabolites bind to $\alpha_1$-adrenoceptors in rat tissues in vivo and that JTH-601-G1 retains the prostatic $\alpha_1$-adrenoceptor subtype selectivity of its parent compound.

$\alpha_1$-Adrenoceptor antagonists are effective therapeutic agents for urinary obstruction in patients with benign prostatic hypertrophy (BPH$^1$). However, prazosin often produces orthostatic hypotension as a side effect, due to a reduction in peripheral resistance mediated by blockade of the vascular $\alpha_1$-adrenoceptors. Previous studies have shown that the $\alpha_1A$-adrenoceptor subtype mediates the contractile response to noradrenaline in prostatic smooth muscles (Lepor et al., 1993; Price et al., 1993; Forray et al., 1994; Chapple, 1996). In addition, it has been shown that the $\alpha_{11}$-adrenoceptor subtype is involved in contraction of the prostate (Muramatsu et al., 1994; Takahashi et al., 1999). On the other hand, the $\alpha_{1B}$-adrenoceptor subtype mediates the contraction of vascular tissues produced by noradrenaline (Hatano et al., 1994).

$^1$ Abbreviations used are: BPH, benign prostatic hypertrophy; JTH-601, 3-$[N-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]-N-methylaminomethyl]-4-methoxy-2,5,6-trimethylphenol hemifumarate; JTH-601-G1, JTH-601 $\beta$-D-glucopyranosyl uronic acid; JTH-601-S1, JTH-601 hydrogen sulfate; HPLC, high performance liquid chromatography.

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its metabolites were chemically synthesized by Japan Tobacco Inc. (Osaka, Japan). All other chemicals were purchased from commercial sources.

**Animals.** Male Sprague-Dawley rats weighing about 200 g were obtained from Japan SLC Inc. (Shizuoka, Japan) and housed three to four per cage in the laboratory with free access to food and water and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%).

**In Vitro Binding Assay.** The binding of [3H]tamsulosin in rat tissues was measured using a previously described method (Yamada et al., 1994). Rat prostate, submaxillary gland, and spleen were homogenized using a Kine-
The competitive inhibition by JTH-601 and its metabolites of specific \(^{3}H\)tamsulosin binding was examined in rat tissues in vitro. Each value represents mean ± S.E. of three to four rats.

**TABLE 1**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>(K_{i}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JTH-601</td>
</tr>
<tr>
<td>Prostate</td>
<td>2.90 ± 0.23</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>4.04 ± 0.43</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.67 ± 0.65</td>
</tr>
</tbody>
</table>

**Results**

In Vivo and in Vivo Inhibitory Effect on \(\alpha_{1}\)-Adrenoceptor Binding in Rat Tissues. JTH-601 (1–100 nM), JTH-601-G1 (1–100 nM), and JTH-601-S1 (1–100 nM) competed in a concentration-dependent manner with specific \(^{3}H\)tamsulosin binding in the prostate, submaxillary gland, and spleen of rats. As shown in Table 1, the \(K_{i}\) values for JTH-601-G1 and JTH-601-S1 in each tissue were 2.5 to 6.4 times greater than the value for JTH-601. The \(K_{i}\) value for JTH-601 in the prostate was similar to that in the submaxillary gland, and the values for JTH-601 and JTH-601-G1 were 2.0 and 3.4 times, respectively, lower in the prostate than in the spleen.

A constant amount of \(^{3}H\)tamsulosin (1.3 nmol/kg, i.v.) was co-injected with increasing doses of JTH-601, JTH-601-G1, and JTH-601-S1 in rats. Intravenous injection of these compounds inhibited in a dose-dependent manner the in vivo specific \(^{3}H\)tamsulosin binding in particular fractions of the prostate, aorta, submaxillary gland, and spleen. Figure 2 illustrates the dose-dependent inhibition curves in the rat prostate. The ID\(_{50}\) values for JTH-601, JTH-601-G1, and JTH-
601-S1 differed both in terms of the drugs and tissues studied (Table 2). The ID$_{50}$ values for JTH-601-G1, compared with that for JTH-601, were 1.9 to 2.9 times smaller in rat prostate, aorta, submaxillary gland, and spleen. Conversely, the ID$_{50}$ values for JTH-601-S1 were 1.3 to 3.2 times greater than those for JTH-601 in each tissue except spleen, which was 1.5 times smaller. To examine tissue selectivity or $\alpha_1$-adrenoceptor subtype selectivity of JTH-601 and its metabolites in vivo, we compared the ratios of their ID$_{50}$ values in rat tissues. The ratios of ID$_{50}$(aorta) to ID$_{50}$(prostate) of JTH-601, JTH-601-G1, and JTH-601-S1 were 1.10, 0.80, and 0.53, respectively, and the ratios of ID$_{50}$(spleen) to ID$_{50}$(prostate) were 0.48, 0.36, and 0.12, respectively. The ratios of ID$_{50}$(spleen) to ID$_{50}$(submaxillary gland) were 0.63, 0.44, and 0.13, respectively.

Concentrations of [3H]JTH-601 and [3H]JTH-601-G1 in Plasma and Prostate. At various times (10, 60, and 120 min) following i.v. injection of [3H]JTH-601 and [3H]JTH-601-G1 at similar doses (2.4 and 2.0 nmol/kg, respectively), the radioactivity in plasma and prostate of rats was identified exclusively as the unchanged form of each radioligand, except for the appearance of a low concentration of each radioligand, which is iodo-1-methyladenosine (62.9 nmol/kg i.p.) after i.v. injection of [3H]tamsulosin represents the in vivo specific binding of the radioligand to $\alpha_1$-adrenoceptors (Yamada et al., 1999). Thus, the in vivo specific binding of [3H]tamsulosin in rat tissues was investigated. JTH-601, JTH-601-G1, and JTH-601-S1 (6.5–2176 nmol/kg), JTH-601-G1 (168–1678 nmol/kg), and JTH-601-S1 (204–2038 nmol/kg) were injected into the femoral vein together with [3H]tamsulosin (555 kBq, 1.3 nmol/kg) into the femoral vein of rats, and 10 min later, specific [3H]tamsulosin binding was measured. Each value represents mean ± S.E. of three to four rats.

**Discussion**

The $\alpha_1$-adrenoceptor binding of JTH-601 and its metabolites in rat tissues was investigated. JTH-601, JTH-601-G1, and JTH-601-S1 inhibited specific [3H]tamsulosin binding in the rat prostate, submaxillary gland, and spleen in vitro, and the inhibitory effect of JTH-601 was greater than that of JTH-601-G1 and JTH-601-S1. Moreover, the inhibitory effects of JTH-601 and JTH-601-G1 were more potent in vitro, and the inhibitory effect of JTH-601-S1 was slightly greater than that of JTH-601-G1.

We showed previously that the difference in the particulate-bound radioactivity of tissues from rats pretreated with vehicle and phenolamine (62.9 $\mu$mol/kg i.p.) after i.v. injection of [3H]tamsulosin represented the in vivo specific binding of the radioligand to $\alpha_1$-adrenoceptors (Yamada et al., 1999). Thus, the in vivo specific binding of [3H]JTH-601 and [3H]JTH-601-G1 in tissues was measured at 10 and 60 min after i.v. injection of each radioligand (2.4 and 2.0 nmol/kg, respectively) in the rats pretreated with vehicle and phenolamine (62.9 $\mu$mol/kg i.p.). As shown in Fig. 4, in vivo specific binding of [3H]JTH-601 at 10 min was observed in each tissue except the aorta, which exhibited little specific binding, and the degree of binding was relatively higher in the heart, lung, and kidney. In vivo specific binding of [3H]JTH-601-G1 was also observed in each tissue except the cerebral cortex, and the degree of binding in the submaxillary gland, heart, lung, and kidney was significantly less than that of [3H]JTH-601. Interestingly, there was a similar degree of specific binding of both radioligands in the prostate, vas deferens, and liver. Sixty minutes later, the in vivo specific binding of [3H]JTH-601 was considerably reduced in all tissues except the vas deferens and prostate, both of which showed no or only a relatively small reduction. Specific binding of [3H]JTH-601-G1 was more markedly reduced than that of [3H]JTH-601.
the prostate than in the spleen. Inasmuch as JTH-601-G1 and JTH-601-S1, albeit with a lower affinity than JTH-601, bind to \( \alpha_{1} \)-adrenoceptors in rat tissues, it is considered that both metabolites may contribute to the pharmacological effect of JTH-601 in vivo.

We have previously shown that \([^{3}H] \) tamsulosin is a useful radioligand for evaluating novel \( \alpha_{1} \)-adrenoceptor antagonists in terms of tissue selectivity and \( \alpha_{1} \)-adrenoceptor subtype selectivity under in vivo conditions (Yamada et al., 1999). Intravenous injection of JTH-601, JTH-601-G1, and JTH-601-S1 inhibited in vivo specific \([^{3}H] \) tamsulosin binding in particulate fractions of rat prostate, aorta, submaxillary gland, and spleen. Compared with the values for JTH-601, the ID\(_{50}\) values for JTH-601-G1 in these tissues were smaller and those for JTH-601-S1 were greater in tissues except the spleen. Thus, it appears that JTH-601, JTH-601-G1, and JTH-601-S1 bind to \( \alpha_{1} \)-adrenoceptors in rat tissues in vivo and the binding affinity of JTH-601-G1 is higher than that of JTH-601 and JTH-601-S1. Such in vivo data appear to contrast with the in vitro situation where JTH-601 has higher \( \alpha_{1} \)-adrenoceptor binding affinity than JTH-601-G1. Although we have no precise explanation for this discrepancy, there may be some difference between these compounds in terms of their pharmacokinetics and \( \alpha_{1} \)-adrenoceptor binding characteristics under in vivo conditions.

Prazosin is known generally as a nonselective antagonist of \( \alpha_{1} \)-adrenoceptor subtypes both in vitro and in vivo (Hanft and Gross, 1989; Aboud et al., 1993; Martin et al., 1997). It has been reported that the prostate and submaxillary gland of rats contain predominantly \( \alpha_{1A} \) subtype, whereas the spleen and liver contain the \( \alpha_{1D} \) subtype (Han et al., 1987; Michel et al., 1989; Han and Minneman, 1991; Testa et al., 1993). Therefore, to evaluate the in vivo tissue selectivity or \( \alpha_{1} \)-adrenoceptor subtype selectivity of JTH-601 and its metabolites, it may be useful to compare the ratio of ID\(_{50}\) values for both agents with the value for prazosin among different tissues. Our previous study showed that the ID\(_{50}\) values for prazosin inhibition of in vivo \([^{3}H] \) tamsulosin binding were 72.8, 12.6, 45.6, and 4.87 nmol/kg, respectively, in rat prostate, aorta, submaxillary gland, and spleen (Yamada et al., 1999). Based on the ratios of ID\(_{50}\) (prostate) to ID\(_{50}\) (submaxillary gland), JTH-601, JTH-601-G1, and JTH-601-S1 were shown to exhibit 5.7 to 6.9, 4.0 to 5.1, and 1.2 to 1.7 times greater \( \alpha_{1} \)-adrenoceptor selectivity than prazosin in the prostate and submaxillary gland versus the spleen. Similarly, they exhibited 6.5, 4.7, and 3.1 times greater \( \alpha_{1} \)-adrenoceptor selectivity than prazosin in the prostate versus the aorta. Consequently, these data are probably the first in vivo evidence that JTH-601 and JTH-601-G1 bind to the \( \alpha_{1} \)-adrenoceptor subtype with higher affinity in the prostate and submaxillary gland than in the spleen and aorta; thus, they provide a rationale for the pharmacological specificity showing that JTH-601 and JTH-601-G1 are more effective antagonists of the \( \alpha_{1} \)-agonist-induced increase in urethral pressure compared with blood pressure in anesthetized rabbits and dogs (Suzuki et al., 1999, 2000a).

The disposition and in vivo \( \alpha_{1} \)-adrenoceptor binding of JTH-601 and JTH-601-G1 in rat tissues were further examined by using radioligands with high specific activity. The total radioactivity after i.v.

**Fig. 3. Total radioactivity in rat tissues at 10 and 60 min after i.v. injection of \([^{3}H] \) JTH-601 (■) and \([^{3}H] \) JTH-601-G1 (□).\)**

\([^{3}H] \) JTH-601 (555 kBq, 2.4 nmol/kg) and \([^{3}H] \) JTH-601-G1 (555 kBq, 2.0 nmol/kg) were injected into the femoral vein, and rats were sacrificed at 10 and 60 min. Each column represents the mean ± S.E. of three rats. Asterisks show a significant difference compared with the value of \([^{3}H] \) JTH-601. *, \( P \) < .05; **, \( P \) < .01; ***, \( P \) < .001.

**Fig. 4. In vivo specific binding in rat tissues at 10 and 60 min after i.v. injection of \([^{3}H] \) JTH-601 (■) and \([^{3}H] \) JTH-601-G1 (□).**

\([^{3}H] \) JTH-601 (555 kBq, 2.4 nmol/kg) and \([^{3}H] \) JTH-601-G1 (555 kBq, 2.0 nmol/kg) were injected into the femoral vein, and rats were sacrificed at 10 and 60 min. Each column represents the mean ± S.E. of three rats. Asterisks show a significant difference compared with the value of \([^{3}H] \) JTH-601. *, \( P \) < .05; **, \( P \) < .01; ***, \( P \) < .001.
injection of [\(^3\)H]JTH-601 and [\(^3\)H]JTH-601-G1 differed among tissues, and the radioactivity in most rat tissues at 10 and 60 min after [\(^3\)H]JTH-601-G1 was considerably lower than that after [\(^3\)H]JTH-601. The lower tissue radioactivity seems to be due mainly to the relatively higher hydrophilicity of the metabolite. In contrast, the plasma concentration of [\(^3\)H]JTH-601-G1 was 3 times higher than that of [\(^3\)H]JTH-601 10 min after i.v. injection in rats, and 60 min later, the plasma concentration of JTH-601-G1 had fallen to one-fourth the concentration of [\(^3\)H]JTH-601. The fast elimination of [\(^3\)H]JTH-601-G1 from the circulation may be ascribable to its high clearance. Specific binding of [\(^3\)H]JTH-601 and [\(^3\)H]JTH-601-G1 was observed in particulate fractions of rat prostate and other tissues 10 min after i.v. injection of each radioligand. Although the in vivo specific binding of [\(^3\)H]JTH-601-G1 was significantly lower than that of [\(^3\)H]JTH-601 in most rat tissues, there was comparable binding between these radioligands in the prostate and vas deferens. This observation is of interest because the concentration of [\(^3\)H]JTH-601-G1 in the prostate after i.v. injection was 3 times lower than that of [\(^3\)H]JTH-601. Taken together, these findings allow us to speculate that JTH-601-G1, compared with the parent compound, exhibits a very high affinity to prostatic \(\alpha_1\)-adrenoceptors in vivo. This coincides with the higher potency of JTH-601-G1 than that of JTH-601 in competitive inhibition of in vivo [\(^3\)H]tamsulosin binding in the prostate. Specific binding of [\(^3\)H]JTH-601 at 60 min after i.v. injection, compared with that at 10 min, was considerably reduced in all rat tissues except the prostate and vas deferens, both of which showed relatively sustained binding. This observation is in reasonable agreement with the ex vivo binding data showing that oral administration of JTH-601 produces a long-lasting blockade of \(\alpha_1\)-adrenoceptors in the rat prostate (Ohkura et al., 1999).

In conclusion, the present study has shown that JTH-601 and its metabolites bind to \(\alpha_1\)-adrenoceptors in rat tissues in vivo and that JTH-601-G1 retains the prostatic \(\alpha_1\)-adrenoceptor subtype selectivity of the parent compound.

Acknowledgments. We thank M. Nakamoto and A. Toma for excellent technical assistance.

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