Diverse Effects of AT₁ Receptor Antagonists on Normal Blood Pressure and Regulatory System

Yoshihiro Hashimoto, Hideo Yabana, and Hiroshi Narita

An AT₁ receptor antagonist, losartan, has been reported to improve survival and quality of life in patients with congestive heart failure as angiotensin converting enzyme inhibitors do. Since many of the patients are normotensive, it may be a drawback if the compound decreases normal blood pressure. In this study, we investigated whether a novel AT₁ receptor antagonist, TA-606, which is more potent than losartan, affects normal blood pressure and its regulatory system in comparison with losartan. TA-606 (30 and 100 mg/kg, p.o.) did not change normal blood pressure, whereas losartan (100 mg/kg, p.o.) tended to decrease it. Although EXP3174 (1 and 10 mg/kg, i.v.), an active metabolite of losartan, suppressed the baroreceptor-heart rate (HR) reflex, 606A (1 and 10 mg/kg, i.v.), an active metabolite of TA-606, did not affect it. Since losartan is known to affect the L-glutamate receptor which is part of the central blood pressure regulatory system, we also investigated whether 606A affects L-glutamate receptor binding. We found that 606A did not affect the binding of the L-glutamate receptor, but EXP3174 inhibited the binding with IC₅₀ values of 13.3 μM. These findings suggest that, even having the same AT₁ receptor antagonist properties as losartan and EXP3174, TA-606 and its active metabolite do not influence normal blood pressure or its regulatory system. (Hypertens Res 1999; 22: 121-127)

Key Words: TA-606, losartan, AT₁ receptor antagonist, baroreceptor-heart rate reflex, blood pressure regulation
Methods

Animals
All studies were performed with permission from our institutional ethical committee for animal experiments, and were in conformity with “Guiding Principles for the Care and Use of Laboratory Animals” of The Japanese Pharmacological Society.

Male Sprague-Dawley rats (SD rats, Crj: CD), were obtained from Charles River Japan (Yokohama, Japan). For 1 or 2 wk before the experiment, the rats were housed under controlled environmental conditions: relative humidity (50-55%), temperature (24 ±1°C) and light (12 h light and dark cycle). Chow (CE-2, Clea Japan, Inc, Tokyo, Japan) and water were taken *ad libitum* until the experiment.

Effect on Blood Pressure in Conscious Normotensive Rats
Male SD rats (Crj: CD), 12 wk old, weighing 380-400 g were used. Systolic blood pressure (SBP) and HR were measured by the tail cuff method (KN-210, Natsume, Tokyo, Japan) with preheating of 38°C to 40°C for 15 min. The rats fasted overnight before the experiment and were given 20% sucrose solution instead of drinking water to avoid weakness. SBP and HR were measured before, and 1, 2 and 5 h after drug administration. TA-606 was suspended in 0.25% carboxymethyl cellulose (CMC) aqueous solution. Losartan was dissolved in deionized water. The drugs were administered orally at a volume of 1 ml/100 g body weight. The control group received the same volume of vehicle 0.25% CMC solution.

Baroreceptor-HR Reflex Test
Male SD rats (Crj: CD), 8 to 9 wk old, weighing 300-320 g were anesthetized with thiobutabarbital, 100 mg/kg, i.p. The rats were intubated with a tracheal cannula, and ventilated with an artificial respirator (Model-680, Harvard, South Natick, MA, USA). In all experiments, arterial blood pressure was measured with a pressure transducer (TP-400T, Nihon Kohden) triggered by arterial pressure pulses. Mean arterial pressure (MAP) and HR were simultaneously recorded on a linear recorder (WR-3300, Graphtech, Tokyo, Japan). A venous cannula was inserted into the right femoral artery. HR was also measured by a carotidachometer (AT-601G, Nihon Kohden, Tokyo, Japan) connected to a polyethylene cannula, which was inserted into the right femoral artery. HR was also measured by a carotidachometer (AT-601G, Nihon Kohden, Tokyo, Japan) triggered by arterial pressure pulses. Mean arterial pressure (MAP) and HR were simultaneously recorded on a linear recorder (WR-3300, Graphtech, Tokyo, Japan). A venous cannula for drug injection was inserted into the left femoral vein.

After MAP and HR were stabilized, four doses of phenylephrine (1-8 μg/kg) and four doses of sodium nitroprusside (1-8 μg/kg) were injected intravenously. Next, propranolol, 606A, EXP3174, or saline was injected intravenously. The same doses of phenylephrine and sodium nitroprusside were injected again 30 min after administration of the test compounds. Effects of the test compounds on the baroreceptor-HR reflex were expressed as MAP-HR curves. Steady state values of MAP and HR were calculated as:

\[ GAIN50 = -B(A-D)\ln 2 \]

where \( A \) and \( D \) are the upper and lower plateaus of the baroreflex curve (MAP-HR curve), respectively, \( C \) is MAP at the mid point of HR range (BP50), and \( B \) is a slope coefficient. Curves were fitted to the data by computer using a non-linear curve fitting program (Kaleida Graph, Hulinks, Tokyo, Japan). A gain in the Baroreceptor-HR reflex was calculated as the first derivative of the logistic function:

\[ GAIN = \frac{d(HR)}{d(MAP)} = -\left(\frac{(B(A-D)/C)(MAP/C)^{-1}}{1 + (MAP/C)^B}\right) \]

and the gain at BP50 (i.e., when MAP = C) was calculated as:

\[ GAIN_{BP50} = -B(A-D)/4C \]

The range of the baroreceptor-HR reflex was calculated as:

\[ RANGE = A - D \]

The baroreceptor-HR reflex curve for individual experiments was analyzed in this fashion and the resulting parameters were used for statistical analysis. In addition, MAP and HR data for each group were averaged, and the means were fitted to a baroreceptor-HR curve for each treatment group.

L-Glutamate Receptor Binding Assay
Details of brain membrane preparation for the L-glutamate receptor binding assay were described elsewhere (23). Male SD rats (Crj: CD), 8 to 9 wk old, weighing 300-320 g were anesthetized with thiobutabarbital (100 mg/kg, i.p.) and their brains were removed. The brain membrane was suspended in a small volume of 0.5 mM HEPES: KOH (pH = 7.0) buffer and stored at −80°C.

The binding assay was performed at a final volume of 300 μl assay buffer, which contained 50-100 μg protein. L-[3H]glutamate (1,694.6 GBq/mmol, 50 nM) in 50 mM Tris-acetate and a test compound at a given concentration were added to the incubation tubes on ice. L-glutamate was used to determine the non-specific binding. Tubes were incubated at 32°C for 30 min, and then centrifuged for 3 min in an Eppendorf microcentrifuge. The supernatant was aspirated, and the pellet was dissolved in 2% sodium dodecyl sulfate. The radioactivity was determined by scintillation counting. Protein content was assayed by the method of Lowry et al. (24), using bovine serum albumin as a standard.

Drugs
TA-606, 606A, losartan and EXP3174 were synthesized at Discovery Research Laboratory, Tanabe Seiyaku Co. Ltd. (Saitama, Japan). Phenylephrine hydrochloride was obtained from Kowa (Nagoya, Japan). Sodium nitroprusside and propranolol hydrochloride were obtained from Nacalai Tesque (Kyoto, Japan). L-[3H]glutamate was obtained from New England Nuclear (Boston, USA). Other chemicals of the highest grade were purchased commercially.
Data Analysis
All data were expressed as means ± SEM. Statistical analyses for the experiments with conscious normotensive rats and basal values of the baroreceptor-HR reflex test were performed by repeated measures analysis of variance (ANOVA) with Bonferroni correction. In the baroreceptor-HR reflex test, data were analyzed using one-way analysis of variance for repeated measures. Where appropriate, the paired t test was also used. When p < 0.05, the result was considered to be significant.

Results
Effect of AT1 Receptor Antagonists on the Blood Pressure in Conscious Normotensive Rats
The basal SBP and HR in conscious normotensive rats were 131.9 ± 0.6 mmHg and 373.1 ± 3.5 beats/min, respectively. As shown in Fig. 1, TA-606 did not influence the blood pressure at all (30, 100 mg/kg, p.o.), whereas losartan at a dose of 100 mg/kg, p.o., tended to lower it. The peak blood pressure decrease of losartan during the experiment was −7.3 ± 0.6 mmHg at 2 h after the administration. Neither drugs affected the HR in this model.

Effect of AT1 Receptor Antagonists on the Baroreceptor-HR Reflex
Baseline values of MAP and HR in the baroreceptor-HR reflex test are shown in Table 1. In the control group, the baroreflex curves obtained before and after vehicle administration did not differ (Fig. 2A, Table 2).

The effect of propranolol (1 mg/kg, i.v.) on the baroreflex is shown in Fig. 2B and Table 2. Propranolol induced a decrease in the upper plateau of the baroreflex curve without changing the lower plateau. The gain50 and range of the baroreflex were also decreased.

Table 1. Effects of Test Compounds on Basal Mean Arterial Pressure (MAP) and Heart Rate (HR) in the Baroreflex-HR Test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Before</td>
<td>128.0 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>126.3 ± 6.4</td>
</tr>
<tr>
<td>Propranolol (1 mg/kg)</td>
<td>Before</td>
<td>128.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>127.5 ± 6.6</td>
</tr>
<tr>
<td>606A (1 mg/kg)</td>
<td>Before</td>
<td>124.0 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>121.2 ± 5.8</td>
</tr>
<tr>
<td>606A (10 mg/kg)</td>
<td>Before</td>
<td>128.3 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>122.8 ± 6.6</td>
</tr>
<tr>
<td>EXP3174 (1 mg/kg)</td>
<td>Before</td>
<td>129.5 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>120.7 ± 5.9</td>
</tr>
<tr>
<td>EXP3174 (10 mg/kg)</td>
<td>Before</td>
<td>128.7 ± 3.9</td>
</tr>
</tbody>
</table>

MAP and HR were determined before and 30 min after the intravenous administration of test compounds. Values are means ± SEM of 6 experiments. *p < 0.05: Significantly different from the corresponding value from before drug administration.
The effect of 606A (1 and 10 mg/kg, iv.) are shown in Fig. 2C and D, and Table 2. Since 606A did not affect the baroreflex curve, none of the parameters obtained from the baroreflex curves were altered. On the contrary, EXP3174 (1 and 10 mg/kg, iv.) lowered the basal MAP from 129.5 ± 5.0 to 120.7 ± 5.9 and from 128.7 ± 3.9 to 112.8 ± 4.4 mmHg, respectively. The baseline HR was not affected. As shown in Fig. 2E and F, and Table 2, EXP3174 decreased the upper plateau, and increased the lower plateau of the baroreflex curve. EXP3174 also decreased BP50, gain50 and range of the baroreflex curve in a dose dependent manner.

**Effect of ATI Receptor Antagonists on L-Glutamate Receptor Binding**

In the rat brain membrane, the binding of L-[3H]-glutamate was not inhibited by 606A (IC50 > 1 mM), whereas EXP3174 inhibited it in a concentration-dependent manner. The IC50 value of EXP3174 was 13.3 ± 12.7 μM (n = 3).

**Discussion**

It has been reported that an AT1 receptor antagonist, losartan, is a useful agent not only in the treatment of hypertension, but also for the treatment of patients with heart failure (1). Previously, we reported that a novel AT1 receptor antagonist, 606A, induced regression of cardiac hypertrophy, and augmented endothelium-dependent vascular relaxation in stroke-prone spontaneously hypertensive rats (20). Since TA-606 was the pro-drug of 606A, TA-606 may be a useful agent for patients with heart failure in addition to hypertensive subjects.

The hypotensive effects of TA-606 were 30 and 10 times more potent than those of losartan in renal hypertensive rats and spontaneously hypertensive rats, respectively (18). The antagonistic action of 606A on Ang II-induced contraction was more potent than EXP3174 in the isolated guinea pig aorta, although their binding affinities for the AT1 receptor were similar (19). Therefore, we used doses of TA-606 and 606A comparable to losartan and EXP3174 in this study to examine the possibility of influence on normal blood pressure and its regulatory system.

We observed that TA-606 had no effect on normal blood pressure or its regulatory system, whereas losartan and its active metabolite EXP3174 lowered normal blood pressure and suppressed its regulatory system. Several investigators have already studied the effect of losartan on normal blood pressure. They reported that regular oral treatment of normotensive rats with losartan for 3 wk at a dose of 10 mg/kg/d reduced the blood pressure significantly (6). Moreover, intravenous administration of losartan (10 mg/kg/d) to normal rats for 10 d blocked Ang II action and lowered the blood pressure (7). Dizziness was reported as a side effect of losartan in clinical treatment (25, 26). However, the mechanism of the losartan-induced lowering effect on normal blood pressure was not elucidated in these studies. We may need to pay attention in clinical treatment to whether losartan causes dizziness and/or ischemia of multiple organs, particularly in patients with congestive heart failure having normal blood pressure.

The baroreflex system is an important physiological mechanism for the regulation of blood pressure. The modulation of the baroreflex control of HR by Ang II has been attributed to a resetting of the cardiac baroreflex to higher pressure, a decrease in baroreflex gain, or a combination of both (27). It apparently serves to reduce the buffering action of the baroreflex, and thereby increases the pressor ac-

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**Table 2. Analysis of Cardiac Baroreflex Curves Obtained before and after the Administration of Vehicle, Propranolol, 606A and EXP3174**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Upper plateau (bpm)</th>
<th>Lower plateau (bpm)</th>
<th>Slope coefficient</th>
<th>BP50 (mmHg)</th>
<th>Gain50 (bpm/mmHg)</th>
<th>Range (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Before: 442.7 ± 3.3</td>
<td>393.6 ± 2.8</td>
<td>7.5 ± 0.8</td>
<td>135.7 ± 3.3</td>
<td>-0.68 ± 0.09</td>
<td>49.1 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>After: 442.6 ± 3.4</td>
<td>392.8 ± 2.9</td>
<td>7.6 ± 0.6</td>
<td>136.2 ± 4.1</td>
<td>-0.70 ± 0.08</td>
<td>49.9 ± 4.5</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Before: 442.9 ± 1.0</td>
<td>395.6 ± 1.1</td>
<td>8.8 ± 0.3</td>
<td>136.8 ± 0.9</td>
<td>-0.76 ± 0.02</td>
<td>47.3 ± 1.0</td>
</tr>
<tr>
<td>(1 mg/kg)</td>
<td>After: 427.9 ± 3.0***</td>
<td>386.2 ± 3.0*</td>
<td>5.4 ± 0.6***</td>
<td>137.8 ± 2.8</td>
<td>-0.41 ± 0.04***</td>
<td>41.7 ± 2.5*</td>
</tr>
<tr>
<td>606A</td>
<td>Before: 441.0 ± 1.0</td>
<td>388.1 ± 1.7</td>
<td>6.2 ± 0.6</td>
<td>135.4 ± 0.6</td>
<td>-0.60 ± 0.05</td>
<td>52.9 ± 2.3</td>
</tr>
<tr>
<td>(1 mg/kg)</td>
<td>After: 440.7 ± 0.6</td>
<td>390.4 ± 1.4</td>
<td>6.6 ± 0.8</td>
<td>134.7 ± 1.3</td>
<td>-0.61 ± 0.06</td>
<td>50.3 ± 1.4</td>
</tr>
<tr>
<td>606A</td>
<td>Before: 443.4 ± 1.2</td>
<td>394.4 ± 1.9</td>
<td>7.7 ± 0.4</td>
<td>137.7 ± 1.3</td>
<td>-0.68 ± 0.04</td>
<td>48.9 ± 1.2</td>
</tr>
<tr>
<td>(10 mg/kg)</td>
<td>After: 443.4 ± 1.3</td>
<td>395.1 ± 1.8</td>
<td>7.8 ± 0.4</td>
<td>136.5 ± 0.2</td>
<td>-0.69 ± 0.04</td>
<td>48.3 ± 1.2</td>
</tr>
<tr>
<td>EXP3174</td>
<td>Before: 442.1 ± 1.8</td>
<td>394.5 ± 0.8</td>
<td>8.8 ± 0.3</td>
<td>136.1 ± 1.5</td>
<td>-0.77 ± 0.06</td>
<td>47.6 ± 2.2</td>
</tr>
<tr>
<td>(1 mg/kg)</td>
<td>After: 437.2 ± 1.5</td>
<td>401.8 ± 1.8**</td>
<td>8.2 ± 0.9</td>
<td>130.5 ± 2.1*</td>
<td>-0.55 ± 0.06*</td>
<td>35.5 ± 2.4**</td>
</tr>
<tr>
<td>EXP3174</td>
<td>Before: 443.4 ± 1.5</td>
<td>394.2 ± 2.7</td>
<td>8.0 ± 0.1</td>
<td>138.1 ± 0.6</td>
<td>-0.72 ± 0.05</td>
<td>49.3 ± 3.2</td>
</tr>
<tr>
<td>(10 mg/kg)</td>
<td>After: 437.3 ± 0.6**</td>
<td>406.1 ± 1.8**</td>
<td>7.3 ± 0.4</td>
<td>126.4 ± 2.6**</td>
<td>-0.46 ± 0.05**</td>
<td>31.2 ± 2.1***</td>
</tr>
</tbody>
</table>

Values are means ± SEM of 6 experiments. bpm: beats/min. *p<0.05, **p<0.01 and ***p<0.001: Significantly different from the corresponding value of before drug administration.
Hashimoto et al: AT₁ Antagonists and Basal Blood Pressure

It was reported that losartan shifted the baroreflex-HR curve to the left in Wistar-Kyoto rat and rabbit models; i.e., normotensive models (27, 28). Since plasma renin activity in that study was within normal range, their observation suggests that the basal level of endogenous Ang II exerts a tonic action on the cardiac baroreflex to set the equilibrium point around normal

Fig. 2. Mean arterial pressure (MAP)-heart rate (HR) curves obtained before and after the administration of vehicle (a), propranolol, 1 mg/kg, i.v. (b), 606A, 1 mg/kg, i.v. (c), 10 mg/kg, i.v. (d), EXP3174, 1 mg/kg, i.v. (e), and 10 mg/kg, i.v. (f). Open and solid circles indicate before and 30 min after the treatment, respectively. Symbols and vertical bars represent means ± SEM of 6 experiments.
arterial pressure.

Although we used an anesthetized rat model for the baroreflex-HR test to avoid unnecessary stress, our findings on the baroreflex-HR curve, and on the effect of propranolol on it, were consistent with those reported in conscious models (27, 32). Therefore, we consider that our model was appropriate to investigate the effects of drugs on the baroreceptor-HR reflex.

Using this model, we also confirmed that EXP3174 attenuated the baroreceptor-HR reflex, in that it lowered the upper plateau of HR, increased the lower plateau of HR, reduced the gain50 and the range, and shifted the BP50 downwards. Thus, our findings were consistent with previous reports in this respect.

Wong et al. have also found that the resetting of the cardiac baroreflex by losartan was eliminated by destruction of the area postrema (27). This finding, together with the fact that the area postrema contains a high density of Ang II receptors, which are accessible to circulating Ang II (15), suggests that the resetting is mediated by an action of Ang II at this circumventricular organ.

Barosensitive and chemosensitive neurons are distributed in the medulla, which contains glutamatergic neurons (29). In addition, the nucleus tractus solitarii and the ventrolateral medulla are regions which regulate autonomic functions and blood pressure, and which contain not only Ang II receptors but also glutamatergic neurons (30). It was reported that losartan, but not [Sar1, Thr8]-Ang II, inhibited cardiovascular responses induced by L-glutamate, suggesting that the effects of losartan on normal blood pressure and its regulatory system were not mediated by Ang II receptors alone (8).

The function of glutamate via its receptor plays an important role in the regulation of blood pressure (8). Effects of losartan on normal blood pressure and on the baroreceptor-HR reflex may be due in part to inhibition of the glutamate receptor.

In our L-glutamate binding assay, EXP3174 inhibited L-glutamate binding, whereas 606A did not. This observation is consistent with the fact that only losartan affected normal blood pressure and its regulatory system. Losartan only exhibited these effects at higher doses in our study. The clinical dose of losartan is 50–100 mg, p.o. (31), and the peak plasma concentration (Cmax) of EXP3174 after oral administration of losartan, 100 mg, p.o. in humans, was approximately 1,200 ng/ml; in other words, ca. 3 μM EXP3174 can exist in human plasma (33). Thus, losartan may affect the L-glutamate receptor even at clinical doses. We may observe the same effect when adequate doses of losartan are administered repeatedly as previously reported by Dowell et al. and Collister et al. (6, 7).

A small amount of the active metabolite 606A was detected in the cerebrum (3.0 ± 1.0 ng/g, n = 3) only at 30 min after oral administration of 14C-TA-606 to male rats (1 mg/kg), and it was not detected thereafter (unpublished data), whereas 14C-losartan could be detected (60.0 ± 20.0 ng/g, n = 3) until 1 h after oral administration (34). The differences in distribution and pharmacokinetics between losartan and TA-606 are also considered to contribute to the character. Further studies are necessary to confirm whether repeated administration of lower doses of losartan could cause the same effect, in addition to clarifying the differences in distribution and pharmacokinetics between losartan and TA-606 in detail.

Our results suggest that AT1 receptor antagonists, which exert inhibitory effects on glutamate receptors, affect normal blood pressure and the baroreflex, but that a novel AT1 receptor antagonist, TA-606, may have a beneficial effect on normotensive patients with heart failure in addition to patients with hypertension since this compound lacks blood pressure and baroreflex lowering effects.

References


Goldberg A, Sweet C: Efficacy and safety of losartan. Can J Cardiol 1995; 11 (Suppl F): 27F-32F.


