Effects of Angiotensin Subtype 1 and Subtype 2 Receptor Antagonists in Normotensive Versus Hypertensive Rats

Steven P. Tofovic, Amy S. Pong, and Edwin K. Jackson

The purpose of this study was to examine in vivo the importance of angiotensin subtype 1 (AT₁) versus subtype 2 (AT₂) receptors in spontaneously hypertensive (hypertensive) versus normotensive Wistar-Kyoto (control) rats. Intravenous infusions of DuP 753, a selective AT₁ receptor antagonist, abolished the pressor responses to intravenous infusions of angiotensin II in both strains, and the potency of DuP 753 in this regard was similar in the two strains. DuP 753 also abolished angiotensin II–induced aldosterone release in both strains; however, with respect to inhibiting angiotensin II–induced aldosterone release, DuP 753 was more potent in hypertensive compared with control rats. In hypertensive but not control rats, DuP 753 inhibited angiotensin II–induced aldosterone release at doses lower than required to inhibit angiotensin II–induced pressor responses. Intramesenteric infusions of DuP 753 abolished mesenteric vascular responses to intramesenteric infusions of angiotensin II with a similar potency in both strains. In control but not hypertensive rats, angiotensin II consistently potentiated noradrenergic neurotransmission in the mesenteric vascular bed, and this effect of angiotensin II was abolished by DuP 753. High doses of PD123177, a selective AT₂ antagonist, did not influence any of the aforementioned effects of angiotensin II in either strain. These data indicate that 1) in both hypertensive and control rats, AT₁ receptors mediate the acute effects of angiotensin II on arterial blood pressure, aldosterone release, and mesenteric vascular resistance; 2) in control rats, AT₁ receptors mediate the effects of angiotensin II on noradrenergic neurotransmission; and 3) spontaneously hypertensive rats are more sensitive to DuP 753–induced blockade of angiotensin II–mediated aldosterone release compared with Wistar-Kyoto control rats, at least under the conditions of this study. (Hypertension 1991;18:774–782)

In 1982, Furukawa et al.1 described in a US patent a series of imidazole-5-acetic acid compounds that relaxed blood vessels preconstricted with angiotensin II (Ang II) and lowered blood pressure in rats receiving infusions of Ang II. In 1988, Wong et al.2 and Chiu et al.3 characterized two of these compounds, S-8307 and S-8308, as highly selective and competitive, albeit weak, nonpeptide Ang II receptor antagonists. In 1989 and 1990, a flurry of reports, originating from investigators at the E.I. du Pont de Nemours and Company, described improvements on the lead compounds that resulted in much more potent, and in some cases orally active, selective nonpeptide Ang II receptor blockers (References 4–13; see References 14–16 for review). By the end of 1990, several investigators had already used these exciting new pharmacological tools to probe the physiological roles of Ang II.17–20

An interesting and potentially important outcome of the work on nonpeptide Ang II receptor antagonists was the discovery of two distinct Ang II binding sites.21–29 Ang II subtype 1 (AT₁) receptors have a high affinity for the nonpeptide antagonist DuP 753, a low affinity for the nonpeptide antagonist PD123177, and a low affinity for the peptide antagonist CGP 42112A. In contrast, Ang II subtype 2 (AT₂) binding sites (presumably receptors) have a high affinity for PD123177 and CGP 42112A and a low affinity for DuP 753. The distribution of AT₁ and AT₂ receptors, an area that is being intensely studied by numerous investigators, shows both tissue and species variation. In the rat, AT₁ receptors predominate in the aortic vascular smooth muscle cell, liver, glomeruli, vasa recta, renal mesangial cell, renal interstitial cell, and pituitary gland, whereas AT₂
receptors are the major Ang II receptor subtype in the rat ovarian granulosa cell, whole brain, thalamus, and midbrain; both receptor subtypes are present in the adrenal cortex, adrenal medulla, hypothalamus, heart, extraglomerular renal cortical structures, and uterus.21-41

Several attempts have been made to determine the physiological roles of AT₁ receptors versus AT₂ receptors with regard to mediating the biological effects of Ang II.42-46 For the most part, these studies used DuP 753 to block AT₁ receptors and either PD123177 or CGP 42112A to block AT₂ receptors. These studies indicate that in normal rats, the acute effects of Ang II on blood pressure, aldosterone release, adrenal catecholamine release, drinking behavior, aortic tone, and phosphoinositide turnover are mediated by the AT₁ receptor. In short, no role for the AT₂ receptor yet has been discovered.

Although the roles for AT₁ receptors versus AT₂ receptors at several sites where Ang II has influence have been investigated in normotensive animals, at the time the present study was initiated data on the role of Ang II receptor subtypes mediating Ang II–induced potentiation of noradrenergic neurotransmission was lacking. Also, it is quite possible that the relative importance of Ang II receptor subtypes and the potency of nonpeptide Ang II receptor blockers could be different in normotensive versus hypertensive animals. Accordingly, the purposes of the present study were: 1) to compare in normotensive versus genetically hypertensive rats the relative importance of AT₁ receptors versus AT₂ receptors at several sites where Ang II has an impact; 2) to compare in normotensive versus genetically hypertensive rats the potency of nonpeptide Ang II receptor blockers on several of the biological actions of Ang II; and 3) to investigate which Ang II receptor subtype mediates Ang II–induced enhancement of noradrenergic neurotransmission.

Methods

Protocol 1: Determination of Infusion Rate and Time to Steady-State Blockade

Before initiating a study with nonpeptide Ang II receptor blockers in hypertensive versus normotensive rats, a preliminary set of experiments was conducted to explore what infusion rate of DuP 753 was required to inhibit the effects of Ang II and how long a particular infusion rate had to be delivered before a steady-state level of inhibition was achieved. A similar dose and time ranging study with PD123177 was not possible since no effect of Ang II was known to be blocked by PD123177.

In this preliminary study, 32 normal Sprague-Dawley rats (Sasco, Omaha, Neb.) weighing 336±3 g (mean±SEM) were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A polyethylene (PE) 240 cannula (Clay Adams, Parsippany, N.J.) was inserted into the trachea to facilitate breathing, three PE 50 cannulas were placed in the left jugular vein for delivery of drugs, and a PE 50 cannula was advanced into the left carotid artery to measure arterial blood pressure with a pressure transducer (model P23ID, Statham Division, Gould Inc., Oxnard, Calif.) that was connected to a polygraph (model 79D, Grass Instruments, Quincy, Mass.).

After the surgery was completed, an infusion (50 µl/min) of 0.9% saline was initiated into two of the jugular canulas with an infusion pump (Braintree Scientific, Braintree, Mass.), and the animals were given a converting enzyme inhibitor (captopril, 30 mg/kg i.v.) and a ganglionic blocker (chlorisondamine, 10 mg/kg s.c.). After a 90-minute rest period, the rats were randomly assigned to receive either vehicle (0.9% saline) or 0.1, 1, or 10 µg/min/50 µl of DuP 753. In those rats randomly assigned to receive DuP 753, one jugular infusion line was switched to an infusion of DuP 753. Sixty, 150, 240, and 330 minutes into the infusions of DuP 753 (or its vehicle), dose–response curves to Ang II (acute pressor response) were elicited by infusing Ang II at increasing doses (10, 33, and 100 ng/min for 10 minutes at each dose) via the second jugular infusion line. The third jugular cannula was used to deliver supplementary doses of anesthetic.

Protocol 2: Effects of DuP 753 and PD123177 on the Acute Blood Pressure and Aldosterone Response to Angiotensin II

Twenty spontaneously hypertensive rats (SHR) and 20 Wistar-Kyoto (WKY) normotensive rats were obtained from Taconic Farms, Germantown, N.Y. SHR and WKY rats weighed 315±9 g and 402±12 g (mean±SEM), respectively, and were aged matched (15–25 weeks old). Animals were anesthetized and instrumented exactly as described for the experiments in protocol 1 (see above). After the surgery, an intravenous infusion of 0.9% saline was initiated into jugular canulas A and B (20 µl/min). In addition, a dose of captopril (30 mg/kg) was administered through jugular canula C. Jugal cannula C was also used to deliver supplementary doses of anesthetic. On a given day, one or two experimental rats were prepared for study. To replace the blood volume removed during blood sampling, a blood donor rat (male Sprague-Dawley) was also prepared by placing canulas in the trachea, carotid artery, and jugular vein and by administering 1,500 units of heparin.

The protocol was divided into four 80-minute periods. The animals were randomly assigned to one of three groups, and each group received a different infusion protocol via jugular canula A. The time-control group received only a saline infusion throughout all four 80-minute periods. The DuP 753 and PD123177 groups received saline during the first period and 3, 10, and 100 µg/min of DuP 753 or PD123177 during periods 2, 3, and 4, respectively. All infusions through jugular canula A were administered at 20 µl/min.
One hour into each 80-minute period, the saline infusion in jugular cannula B was switched to Ang II (100 ng/min; 50 µl/min; dissolved in saline), and this infusion was maintained for 20 minutes before switching back to saline (20 µl/min). Just before initiating and terminating the infusions of Ang II, mean arterial blood pressure was recorded and an arterial blood sample (500 µl) was taken for measurement of plasma aldosterone level. After each blood sampling, 500 µl donor blood was administered to the experimental animal. Blood samples for plasma aldosterone were collected into 10 µl of 10% EDTA and centrifuged at 4°C; the plasma was stored at −20°C until assayed for aldosterone using a Coat-A-Count aldosterone radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, Calif.).

Protocol 3: Effects of DuP 753 and PD123177 on the Mesenteric Vascular Response to Angiotensin II and on Angiotensin II–Induced Potentiation of Mesenteric Vascular Responses to Periarterial Nerve Stimulation

Twenty-one SHR and 23 WKY rats obtained from Taconic Farms were used in this protocol. SHR and WKY rats weighed 268±4 g and 338±5 g (mean±SEM), respectively, and were aged matched (15–20 weeks old). Animals were anesthetized with pentobarbital (50 mg/kg i.p.) and were prepared for in situ perfusion of their mesenteric vascular bed as described by Jackson and Campbell.47 With the modifications of Zimmerman et al.48 Briefly, a PE 240 cannula was placed in the trachea to facilitate breathing, a jugular cannula (PE 50) was inserted for administration of supplementary anesthetic, an extracorporeal shunt was established between the abdominal aorta and superior mesenteric artery, and the mesenteric vasculature was perfused at a constant blood flow of 3 ml/min with a peristaltic pump (model 1203, Harvard Apparatus, South Natick, Mass.). All animals were treated with heparin (1,500 units) to prevent coagulation of blood in the extracorporeal shunt. Inasmuch as perfusion rate was held constant, mesenteric perfusion pressure was proportional to mesenteric vascular resistance. The extracorporeal shunt was constructed of Silastic tubing and contained access ports that permitted intramesenteric infusion of drugs. Access ports in the shunt also allowed measurement of arterial blood pressure and mesenteric perfusion pressure via Statham pressure transducers that were located appropriately in the shunt and were connected to a Grass polygraph. A bipolar platinum electrode was also placed around the superior mesenteric artery and attached to a Grass stimulator (model SD9) for stimulating the periarterial sympathetic nerves. After the surgery was completed, animals were treated with captopril (30 mg/kg i.v.), and two intramesenteric infusions of 0.9% saline (20 µl/min each) were initiated into the extracorporeal shunt.

After a 60-minute stabilization period, the experiment was begun. The protocol consisted of five experimental periods separated by 45 minutes. At the beginning of the first experimental period, the infusion rate of intramesenteric infusion line A was increased to 50 µl/min. Five minutes later, periarterial infusion of PNS was delivered for 20 seconds (pulse duration, 1 msec; waveform, biphasic; frequency, 5 Hz; 34 V). Three minutes later, the stimulation was repeated, and the average mesenteric vascular response to these two stimulations was taken as the response to PNS in the absence of Ang II. Next, intramesenteric infusion line B was switched to Ang II (30 ng/min; 50 µl/min), and five minutes later, another two 20-second periods of PNS separated by 3 minutes were delivered. The average mesenteric vascular response to these two stimulations was taken as the response to PNS in the presence of Ang II. The change in baseline mesenteric perfusion pressure caused by Ang II was also recorded.

Both intramesenteric infusion lines A and B were restored to 0.9% saline (20 µl/min), and a 45-minute rest period was allowed. Animals were then randomly assigned to either the time-control group, the DuP 753 group, or the PD123177 group. In the time-control group, the second, third, fourth, and fifth experimental periods were exactly like the first experimental period. In the DuP 753 and PD123177 groups, the subsequent experimental periods were like the first experimental period with the exception that intramesenteric infusion line A delivered 0.3, 1, 3, and 10 µg/min of either DuP 753 or PD123177 during periods two, three, four, and five, respectively.

Statistical Analysis

Data were analyzed by analysis of variance and paired and unpaired Student's t tests on an IBM PS/2 (model 30) using the Number Crunchers Statistical System, Kaysville, Utah.

Results

Protocol 1: Determination of Infusion Rate and Time to Steady-State Blockade

DuP 753 was infused intravenously into male Sprague-Dawley rats for longer than 5½ hours while dose–response curves to Ang II (pressor responses) were assessed periodically. Data were analyzed with a three-factor analysis of variance (factor A, dose of Ang II; factor B, time into infusion; factor C, control group or DuP 753–treated group) with repeated measures on factors A and B. When infused at 0.1 and 1 µg/min, DuP 753 did not alter significantly acute pressor responses to Ang II, although responses tended to be reduced (p=0.0543) with the 1 µg/min dose (data not shown). As shown in Figure 1, when infused at 10 µg/min, DuP 753 significantly attenuated, but did not abolish, acute pressor responses to Ang II (p<0.0001). With this dose of DuP 753, inhibition was achieved by 60 minutes into the infusion; the degree of inhibition was stable and did not change significantly over the 5½-hour observation period. Based on these studies, in subsequent investigations with intravenously administered DuP 753, doses of 3, 10, and 100 µg/min were used to provide a
range of blockade, and infusions were sustained for at least 1 hour after initiating a change of dose.

Protocol 2: Effects of DuP 753 and PD123177 on the Acute Blood Pressure and Aldosterone Response to Angiotensin II

Figures 2 and 3 illustrate the effects of Ang II (100 ng/min) on mean arterial blood pressure in WKY rats (Figure 2) and SHR (Figure 3) in a time-control group (top row), a group treated with increasing doses of PD123177 (middle row), and a group treated with increasing doses of DuP 753 (bottom row). In both WKY rats and SHR, in the time-control and PD123177 groups, Ang II significantly increased mean arterial blood pressure during all four experimental periods. Also in both WKY rats and SHR, in the DuP 753 group, Ang II significantly increased mean arterial blood pressure during all four experimental periods.

It appeared that SHR, compared with WKY rats, were more sensitive to DuP 753-induced blockade of Ang II–mediated aldosterone release. In SHR, but not WKY rats, it seemed that DuP 753 blocked Ang II–induced aldosterone responses at lower doses than were required to block Ang II–induced pressor responses. To determine whether these conclusions were correct, responses (Δ) induced by Ang II were calculated for the time-control groups and DuP 753–treated groups during experimental periods 2, 3, and 4 so that responses in the DuP 753–treated groups could be directly compared with responses in the corresponding periods of the time-control groups.

both WKY rats and SHR, in the time-control and PD123177 groups. Ang II significantly increased plasma levels of aldosterone during all four experimental periods. In WKY rats in the DuP 753 group, Ang II significantly increased plasma levels of aldosterone during the control period and when DuP 753 was infused at 3 and 10 μg/min, but not when DuP 753 was infused at 100 μg/min. However, in SHR both 10 and 100 μg/min of DuP 753 abolished the effects of Ang II on plasma levels of aldosterone. Thus in both WKY rats and SHR, DuP 753, but not PD123177, abolished the effects of Ang II on plasma aldosterone levels.

It appeared that SHR, compared with WKY rats, were more sensitive to DuP 753–induced blockade of Ang II–mediated aldosterone release. In SHR, but not WKY rats, it seemed that DuP 753 blocked Ang II–induced aldosterone responses at lower doses than were required to block Ang II–induced pressor responses. To determine whether these conclusions were correct, responses (Δ) induced by Ang II were calculated for the time-control groups and DuP 753–treated groups during experimental periods 2, 3, and 4 so that responses in the DuP 753–treated groups could be directly compared with responses in the corresponding periods of the time-control groups.

Figure 2. Bar graphs show effects of intravenous infusions of PD123177 and DuP 753 on angiotensin II–induced changes in mean arterial blood pressure in normotensive Wistar-Kyoto rats. Baseline (B) measurements were made, angiotensin II (100 ng/min i.v.) was infused for 20 minutes, and measurements were taken again (ANGII). This protocol was repeated during four experimental periods. In time-control animals (top panel), only vehicle (0.9% saline) was infused during all four periods. In the PD123177 (PD) group (middle panel) and DuP 753 (DuP) group (bottom panel), vehicle was administered during the first period, and 3, 10, and 100 μg/min of antagonist were infused during the second, third, and fourth experimental periods, respectively. Each dose of each antagonist was infused for 1 hour before challenging with angiotensin II. *p<0.05 by paired Student’s t test compared with baseline value.

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FIGURE 3. Bar graphs show effects of intravenous infusions of PD123177 and DuP 753 on angiotensin II-induced changes in mean arterial blood pressure in spontaneously hypertensive rats. Baseline (B) measurements were made, angiotensin II (100 ng/min i.v.) was infused for 20 minutes, and measurements were taken again (ANGII). This protocol was repeated during four experimental periods. In time-control animals (top panel), only vehicle (0.9% saline) was infused during all four periods. In the PD123177 (PD) group (middle panel) and DuP 753 (DuP) group (bottom panel), vehicle was administered during the first period, and 3, 10, and 100 μg/min of antagonist were infused during the second, third, and fourth experimental periods, respectively. Each dose of each antagonist was infused for 1 hour before challenging with angiotensin II. *p<0.05 by paired Student’s t test compared with baseline value.

FIGURE 4. Bar graphs show effects of intravenous infusions of PD123177 and DuP 753 on angiotensin II-induced changes in plasma aldosterone levels in normotensive Wistar-Kyoto rats. Baseline (B) measurements were made, angiotensin II (100 ng/min i.v.) was infused for 20 minutes, and measurements were taken again (ANGII). This protocol was repeated during four experimental periods. In time-control animals (top panel), only vehicle (0.9% saline) was infused during all four periods. In the PD123177 (PD) group (middle panel) and DuP 753 (DuP) group (bottom panel), vehicle was administered during the first period, and 3, 10, and 100 μg/min of antagonist were infused during the second, third, and fourth experimental periods, respectively. Each dose of each antagonist was infused for 1 hour before challenging with angiotensin II. *p<0.05 by paired Student’s t test compared with baseline value.

(Tables 1). Compared with the corresponding periods in the time-control groups, in both WKY rats and SHR, a significant inhibition of the blood pressure response to Ang II was achieved only with the 100 μg/min dose of DuP 753. Similarly, in WKY rats 100 μg/min of DuP 753 was required to attenuate significantly the aldosterone response to Ang II. In contrast, in SHR the aldosterone response to Ang II was significantly reduced by DuP 753 at 3 μg/min and was abolished by DuP 753 at 10 μg/min. Thus, the aldosterone response in SHR was more sensitive to inhibition by DuP 753 than was the aldosterone response in WKY rats or the blood pressure response in both WKY rats and SHR.

Protocol 3: Effects of DuP 753 and PD123177 on the Mesenteric Vascular Response to Angiotensin II and on Angiotensin II–Induced Potentiation of Mesenteric Vascular Responses to Periarterial Nerve Stimulation

In both WKY rats (Figure 6) and SHR (Figure 7), intramesenteric infusions of DuP 753 abolished the direct vasoconstrictive effects of Ang II, whereas PD123177 had no effect. When expressed as percentage inhibition of baseline response to Ang II, the DuP 753 dose–response curves in WKY rats and SHR for inhibition of Ang II–induced vasoconstriction were practically superimposable (data not shown). In WKY rats, Ang II potentiated vascular responses to PNS. This enhancement of noradrenergic neurotransmission by Ang II in WKY rats was abolished by DuP 753 but was unaffected by PD123177 (Figure 8). In SHR, the effects of Ang II on vascular responses to PNS were too inconsistent to quantitate, so it was not possible to determine the effects of either DuP 753 or PD123177 on noradrenergic neurotransmission in vivo in SHR in this study.

Discussion

The major objectives of this study were to determine whether the importance of AT₁ versus AT₂ receptors in mediating some of the effects of Ang II was different in SHR versus WKY rats, to determine whether the potency of nonpeptide Ang II receptor blockers on several effects of Ang II was different in SHR compared with WKY rats, and to determine which Ang II receptor subtype mediates Ang II–induced enhancement of noradrenergic neurotransmission.

Regarding the first objective, the acute effects of Ang II on blood pressure, aldosterone levels, and mesenteric vascular tone were abolished by DuP 753 in both SHR and WKY rats. PD123177 did not
Figure 5. Bar graphs show effects of intravenous infusions of PD123177 and DuP 753 on angiotensin II–induced changes in plasma aldosterone levels in spontaneously hypertensive rats. Baseline (B) measurements were made, angiotensin II (100 ng/min i.v.) was infused for 20 minutes, and measurements were taken again (ANGII). This protocol was repeated during four experimental periods. In time-control animals (top panel), only vehicle (0.9% saline) was infused during all four periods. In the PD123177 (PD) group (middle panel) and DuP 753 (DuP) group (bottom panel), vehicle was administered during the first period, and 3, 10, and 100 µg/min of antagonist were infused during the second, third, and fourth experimental periods, respectively. Each dose of each antagonist was infused for 1 hour before challenging with angiotensin II. \*p<0.05 by paired Student’s t test compared with baseline value.

Figure 6. Line graph shows mesenteric vascular response to angiotensin II (ANGII) (30 ng/min) in saline-treated (time-control group) normotensive Wistar-Kyoto (WKY) rats, DuP 753–treated WKY rats, and PD123177-treated WKY rats. All infusions were delivered directly into the mesenteric artery. \*p<0.05 by unpaired Student’s t test compared with corresponding period in saline-treated group.

Attenuate these actions of Ang II in either strain. These data strongly suggest that in both SHR and WKY rats, the acute effects of Ang II on blood pressure, aldosterone release, and vascular tone are mediated exclusively by the AT₁ receptor.

Although DuP 753 abolished the effects of Ang II on aldosterone release in both SHR and WKY rats, the two strains did differ in their sensitivity to DuP 753. In SHR, Ang II–induced increases in aldosterone levels were significantly reduced (when compared with corresponding period in time-control) by 3 µg/min of DuP 753 and were abolished with 10 µg/min of DuP 753. The same comparisons in WKY rats indicated that 100 µg/min of DuP 753 was required to abolish aldosterone responses to Ang II.

It is possible that Ang II–induced aldosterone responses in SHR were more sensitive to DuP 753 because a given infusion rate of DuP 753 provided a greater plasma level of DuP 753 in SHR compared with WKY rats. Although plasma levels of DuP 753 were not measured in the present study, it is noteworthy that pressor responses to Ang II were blocked by similar infusion rates in the two strains. If a pharmacokinetic mechanism accounted for the differential blockade of aldosterone responses in the

Table 1. Blood Pressure and Aldosterone Response to Angiotensin II in Periods 2, 3, and 4 in Control and DuP 753–Treated Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Period</th>
<th>BP response (mm Hg)</th>
<th>ALDO response (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
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</tr>
<tr>
<td>Control group (n=7)</td>
<td>34±4</td>
<td>34±2</td>
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<tr>
<td>DuP 753 group (3 µg/min; n=6)</td>
<td>26±6</td>
<td>38±5</td>
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<tr>
<td>Control group (n=7)</td>
<td>38±9</td>
<td>31±6</td>
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<tr>
<td>DuP 753 group (10 µg/min; n=6)</td>
<td>21±5</td>
<td>28±8</td>
</tr>
<tr>
<td>Period 4</td>
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<tr>
<td>Control group (n=7)</td>
<td>50±6</td>
<td>56±10</td>
</tr>
<tr>
<td>DuP 753 group (100 µg/min; n=6)</td>
<td>10±4*</td>
<td>-2±11*</td>
</tr>
</tbody>
</table>

BP, blood pressure; ALDO, aldosterone; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*Indicates significantly different compared with control group in corresponding period.
two strains, then a differential blockade of pressor responses also should have been observed, which was not the case. It is also interesting to note that in SHR, but not WKY rats, a greater infusion rate of DuP 753 was required to block pressor responses to Ang II than to block aldosterone responses. Therefore, it seems unlikely that a pharmacokinetic explanation accounts for the greater sensitivity of SHR to DuP 753-induced blockade of Ang II–mediated aldosterone release. An alternative explanation is that AT1 receptors in the SHR adrenal cortex have a greater affinity for DuP 753 than do the corresponding receptors in WKY rats. However, further studies are needed to corroborate our observation and to explore its mechanism.

The third objective of this study was to determine whether Ang II–induced potentiation of noradrenergic neurotransmission is mediated by AT1 or AT2 receptors. Ang II facilitates noradrenergic neurotransmission in part by increasing vascular responsiveness to norepinephrine, but mostly by facilitating the release of norepinephrine. In a previous study, we demonstrated that the nonpeptide Ang II receptor antagonist CV-2961 (also known as S-8308) blocked completely the enhancement of noradrenergic neurotransmission in the in situ perfused rat mesentery. However, CV-2961 is not selective for angiotensin receptor subtypes, so it is not possible to infer from that study which receptor subtype mediates Ang II–induced facilitation of noradrenergic neurotransmission. In the present study, in WKY rats Ang II potentiated vascular responses to sympathetic nerve stimulation, and this effect of Ang II was abolished by DuP 753 but was unaffected by PD123177. These data indicate that in the WKY rat, both the postjunctional and prejunctional effects of Ang II are mediated by AT1 receptors. In the present study, in SHR, the potentiation of noradrenergic neurotransmission was too inconsistent to quantitate.

While our studies were in progress, several abstracts and one full length article were published by other investigators interested in the prejunctional Ang II receptor. Wong et al reported that in the dog kidney, DuP 753 reduced vascular responses to renal nerve stimulation but not to exogenous norepinephrine and concluded that DuP 753 blocked the prejunctional effects of endogenous Ang II on sympathetic neurotransmission. However, in that study, the effects of DuP 753 on exogenous Ang II were not examined, and the effects of PD123177 were not investigated. In contrast to the study by Wong et al, Trachte et al found that the potentiating effect of exogenous Ang II on noradrenergic neurotransmission in the rabbit vas deferens was not inhibited by either DuP 753 or PD123177 and concluded that neither AT1 nor AT2 receptors mediate Ang II–induced potentiation of noradrenergic neurotransmission. In two separate studies, DuP 753 was found to block the prejunctional effects of exogenous Ang II in the isolated rat mesentery; however, in one study the inhibitory effect of DuP 753 was reversed with indomethacin by an unknown mechanism. Ang II–induced norepinephrine release in vivo in the nucleus paraventricularis and nucleus supraopticus was also blocked by DuP 753. Our present study is...
the first to examine the effects of DuP 753 on the ability of exogenous Ang II to facilitate noradrenergic neurotransmission in vivo in a vascular bed and is the first to compare DuP 753 and PD123177 on noradrenergic neurotransmission in a vascular bed.

In summary, SHR do not differ from WKY rats regarding which subtype of Ang II receptor mediates the acute effects of Ang II on arterial blood pressure, aldosterone release, and mesenteric vascular tone. However, under the conditions of this study, SHR appear to be more sensitive to DuP 753–induced blockade of Ang II–mediated aldosterone release. Finally, the prejunctional and postjunctional effects of Ang II on noradrenergic neurotransmission are mediated by AT1 receptors in vivo.

Acknowledgments

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References


**Key Words**  • angiotensin II  • DuP 753  • PD123177  • angiotensin receptors  • hypertension  • aldosterone  • blood pressure  • vascular resistance  • neuroregulators
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