Depressor Effect of Blocking Angiotensin Subtype 1 Receptors in Anterior Hypothalamus
Ren-Hui Yang, Hongkui Jin, James Michael Wyss, and Suzanne Oparil

Previous studies have shown that the anterior hypothalamic area participates in the centrally mediated pressor response to exogenous angiotensin II. The current study was designed to test the hypothesis that endogenous anterior hypothalamic angiotensin II plays a significant role in blood pressure control. Type I angiotensin II receptors in the anterior hypothalamic area were blocked by local microinjection of DuP 753 (2-n-butyl-4-chloro-5-(hydroxymethyl)-1-(2'-((1H-tetrazol-5-yl)biphenyl-4-yl)methyl)imidazole, potassium salt), a highly selective nonpeptide antagonist. DuP 753 (20 or 40 μg; in 100 nl artificial cerebrospinal fluid) or vehicle alone was microinjected into the anterior hypothalamic area of conscious NaCl-sensitive spontaneously hypertensive rats and Wistar-Kyoto controls. DuP 753 caused significant dose-related decreases in mean arterial pressure (maximal decrease, 22.5±1.8 mm Hg) with unchanged heart rate in NaCl-sensitive spontaneously hypertensive rats but effected no change in Wistar-Kyoto rats. Injections of equal volumes of artificial cerebrospinal fluid into the anterior hypothalamic area had no effect in either strain. Further, microinjection of DuP 753 into the posterior hypothalamic area produced no significant effect on blood pressure or heart rate in NaCl-sensitive spontaneously hypertensive rats. Microinjection into the anterior hypothalamic area of the selective type 2 angiotensin II receptor antagonist PD 123319 did not affect blood pressure or heart rate in NaCl-sensitive spontaneously hypertensive rats. These data provide the first demonstration that endogenous angiotensin II in the anterior hypothalamic area participates in the tonic control of blood pressure in salt-sensitive spontaneously hypertensive rats but not normotensive Wistar-Kyoto rats and that this effect is mediated by type I angiotensin II receptors. (Hypertension 1992;19:475-481)

KEY WORDS • angiotensin II • angiotensin antagonists • angiotensin receptors • microinjections • central nervous system • essential hypertension

Several lines of evidence have implicated brain angiotensin II (Ang II) in the pathogenesis of hypertension in the spontaneously hypertensive rat (SHR). Compared with normotensive Wistar-Kyoto (WKY) rats, SHR have elevated renin activity and angiotensin-like immunoreactivity in the brain and increased levels of angiotensin-like material in the cerebrospinal fluid. Brain Ang II levels are significantly higher in SHR than in WKY rats even before the development of hypertension, suggesting that the increase in brain Ang II may play an etiologic role in the development of hypertension rather than being a consequence of the blood pressure elevation. Further, acute or chronic intracerebroventricular administration of either saralasin or captopril at doses that are minimally effective in lowering blood pressure when given intravenously markedly reduces blood pressure in adult SHR and attenuates the development of hypertension in young SHR but has a minimal effect on blood pressure in age-matched WKY rats.

Previous studies have demonstrated that an anterior hypothalamic knife cut that isolates the anterior hypothalamic area (AHA) from other cardiovascular control areas in the brain stem and hypothalamus eliminates both the pressor response to centrally administered Ang II and the central component of the pressor response to intravenous Ang II in normotensive rats. Thus, neurons in AHA appear to participate in the centrally mediated pressor response to exogenous Ang II. We have previously shown that the AHA plays an important role in blood pressure regulation in the NaCl-sensitive SHR (SHR-S). SHR-S obtained from Taconic Farms (IBU-3 colony, Germantown, N.Y.) exhibit a significant increase in blood pressure, increased peripheral sympathetic nervous system activity, and reduced norepinephrine release from nerve terminals in AHA when fed high NaCl diets. NaCl-resistant SHR (SHR-R) (Charles River Breeding Laboratories, Wilmington, Mass.) and WKY rats do not show these pressor and neurochemical responses to dietary NaCl supplementation.

The current study was designed to test the hypotheses that endogenous Ang II in AHA plays a role in blood pressure regulation and that blockade of type 1 Ang II (AT₁) receptors in the AHA by microinjection of DuP 753, a selective nonpeptide AT₁ receptor antagonist, lowers blood pressure in SHR-S. We hypothe-
sized that neither microinjections of artificial cerebrospinal fluid (ACSF; vehicle) into AHA nor microinjections of DuP 753 into the posterior hypothalamic area (PHA) of SHR-S would alter arterial pressure. To test the specificity of the AT1 receptor in this response, PD 123319, a selective type 2 Ang II (AT2) receptor antagonist,19-22 was microinjected into AHA. PD 123319 is a tetrahydroimidazopyridine that binds selectively to AT2 sites by a mechanism that is insensitive to or slightly enhanced in the presence of dithiothreitol.23-25 The AT2 binding site has been shown to be distinct from the AT1 site in molecular weight, binding properties, and coupling to second messenger systems. It appears not to be coupled to guanine nucleotide–binding proteins and its occupation by either PD 123319 or Ang II has no effect on cell growth, mitogenesis, or hypertrophy or on phosphatidylinositol turnover, tyrosine kinase activity, cyclic AMP levels, or release of arachidonic acid.21,22 Thus, the transmembrane signaling mechanisms and function of the AT1 receptor remain undefined. To confirm that the depressor effect of microinjection of Ang II into AHA was related to local blockade of Ang II, in a separate set of experiments, SHR-S and WKY were pretreated with 10 nmol DuP 753 into AHA, and the effects of DuP 753 on the pressor and bradycardic responses to subsequent microinjection of Ang II into AHA were tested.

Methods

SHR-S and normotensive WKY control rats were obtained from Taconic Farms, IBU-3 colony, Germantown, N.Y., at 9 weeks of age. All rats were maintained four per cage at constant humidity (60±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM). All rats were provided a standard rat diet (Ralston Purina 5001, Richmond, Ind.) and free access to food and water. For 1 week before acute study, rats were acclimatized to these diet and housing conditions. All procedures followed in these experiments are in accordance with institutional guidelines and were approved by the University of Alabama at Birmingham Animal Use Review Committee.

Two days before the acute experiment, each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.), and a catheter (polyethylene PE-10 fused with PE-50) was implanted into the abdominal aorta through the right femoral artery. The rat was then placed into a stereotaxic apparatus, the skin overlying the midline of the skull was incised, and a small hole was drilled through the appropriate portion of the skull. A guide cannula (16-gauge stainless steel tubing) was lowered to a position 1.0 mm dorsal to the intended site of microinjection, either AHA or PHA. A 32-gauge obturator (stainless steel wire) was inserted into the guide cannula after implantation.

Forty-eight hours after surgery, the arterial catheter was connected to a model CP-01 pressure transducer (Century Technology Company, Inglewood, Calif.) coupled to a polygraph (model 7, Grass Instruments Co., Quincy, Mass.). Mean arterial pressure (MAP) and heart rate (HR) were measured simultaneously. After a 40-minute stabilization period, the obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless steel tubing) filled with the agent to be administered. The tip of the inner cannula extended 1 mm beyond the guide cannula. The inner cannula was attached to a 0.5 μl Hamilton syringe through tubing (polyethylene PE-20) filled with saline. A small air bubble separated the saline from the injection solution. Rats were randomly injected with either 20 μg or 40 μg of DuP 753 (E.I. du Pont de Nemours and Company, Wilmington, Del.) in 100 nl ACSF or ACSF vehicle alone. Injections were delivered over a period of a few seconds. Each rat received only a single injection on a given day. Only rats that received ACSF vehicle injections were studied on a second day. The injection was administered after the rat’s vital signs returned to baseline (generally 2-5 minutes after placement of the inner cannula). All microinjection experiments were carried out in conscious freely moving rats. The two doses (20 and 40 μg) of DuP 753 used in the current study were approximately 2% and 4% of the peripheral intravenous dose (1 mg) shown to produce significant reductions in blood pressure in previous studies by Wong et al.14 In a pilot study these doses produced significant reductions in MAP in SHR-S when microinjected into AHA but had no effect on MAP or HR when administrated intravenously. The 40-μg dose was chosen for further study because it was the highest dose that could be administered into AHA without producing agitation and aversive behavior.

In parallel experiments, PD 123319 (50 μg) (Warner-Lambert Company, Ann Arbor, Mich.), a selective AT1 receptor antagonist, was injected into AHA of SHR-S, and its effects on MAP and HR were monitored. This dose of PD 123319 was shown in a pilot study to be the highest dose that did not produce behavioral changes (agitation and aversive behavior) when microinjected into AHA of SHR-S.

As an additional control, in a third protocol, DuP 753 (40 μg) was microinjected into PHA of conscious SHR-S. Surgery, arterial cannulation, and brain cannula implantation were performed as above, except that the outer cannula was positioned 1 mm above the PHA.

A fourth series of experiments tested whether pretreatment with microinjection of DuP 753 into AHA and with intravenous injection of DuP 753 can block or attenuate the pressor and bradycardic responses to subsequent microinjection of Ang II into AHA and to intravenous injection of Ang II, respectively, in conscious SHR-S and WKY rats. Surgery, arterial cannulation, and brain cannula implantation in AHA were performed as above except that the right femoral vein was also cannulated for intravenous administration of agents. DuP 753 (40 μg) or ACSF vehicle was injected into the AHA or into the femoral vein. Thirty minutes later, Ang II (2 μg) (Bachem, Torrance, Calif.) was injected into the AHA or into the femoral vein. MAP and HR were monitored before and after injections. A pilot study showed that microinjection of Ang II into AHA caused dose-related increases in MAP in SHR-S; 2 μg Ang II was the lowest dose that produced a reliable and significant pressor effect when administered into AHA.

At the conclusion of each experiment, 100 nl of 1% methylene blue in distilled H2O were administered into AHA or PHA through the cannula. The rat was then anesthetized with sodium pentobarbital (60 mg/kg i.p.), decapitated, and the cannula was removed from the
FIGURE 1. Photomicrograph of a lightly counterstained coronal section from a rat in which a 100 nl microinjection of DuP 753 (40 μg) elicited a depressor response. Darkly stained area in anterior hypothalamic area (AHA) marks the methylene blue-labeled injection site (indicated by the arrow). PVN, paraventricular nucleus; SO, supraoptic nucleus; ot, optic tract.

Results

Fifty-nine SHR-S and 30 WKY rats were studied. Histological examination confirmed that cannulas were properly placed in the anterior and central portions of AHA as defined by Saper et al in 44 SHR-S and 28 WKY rats and in PHA in five SHR-S rats. In three SHR-S, the cannula damaged the sagittal sinus; in two SHR-S, the cannula tip was in the third ventricle. In one WKY rat, cannula placement was in the dorsal hypothalamic area, and in a second WKY rat, the cannula damaged the sagittal sinus. These seven rats were excluded from the analysis of experimental results. Examination of 1% thionin-stained sections revealed that the 100 nl injection of methylene blue spread to an area =600 μm in diameter (Figure 1).

SHR-S had significantly higher basal MAP and lower body weight than WKY rats at the time of study (Table 1). There was no difference in basal HR between SHR-S and WKY rats (Table 1). Microinjection of DuP 753 into the AHA resulted in significant dose-related depressor responses (p<0.01, two-way ANOVA) but did not affect HR in SHR-S (Figure 2). The MAP response to DuP 753 began almost immediately after injection, reached maximal levels in 10–40 minutes, and returned to baseline 60 minutes after injection. The maximal fall in MAP in response to 40 μg DuP 753 was 15.3±1.7 mm Hg (Figure 2). In contrast, in WKY rats microinjection into the AHA of DuP 753 at the higher dose (40 μg) did not alter MAP or HR (Figure 3). Microinjection of vehicle into AHA did not alter MAP or HR significantly in either strain (Figures 2 and 3). Administration of DuP 753 (40 μg) into the PHA did not cause significant alterations in MAP or HR in

### Table 1. Basal Levels of Mean Arterial Pressure, Heart Rate, and Body Weight

<table>
<thead>
<tr>
<th>Strain</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S (n=59)</td>
<td>169.5±1.4*</td>
<td>384.8±4.2</td>
<td>229.1±1.0*</td>
</tr>
<tr>
<td>WKY (n=30)</td>
<td>117.1±1.4</td>
<td>375.0±5.2</td>
<td>267.8±1.8</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; BW, body weight; SHR-S, NaCl-sensitive spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. *p<0.01 compared with the respective WKY group.
SHR-S or WKY rats (Figure 4). Microinjection of PD 123319 (50 μg) into AHA did not affect MAP or HR in SHR-S.

Microinjection of Ang II (2 μg) into AHA caused significant increases in MAP and decreases in HR in both SHR-S and WKY rats (Figure 5). Pretreatment (microinjection into AHA) with DuP 753 (40 μg) almost completely abolished pressor and bradycardic responses to Ang II microinjection into AHA in both strains. Intravenous injection of Ang II (2 μg) produced significant increases in MAP and decreases in HR in both SHR-S and WKY rats (Figure 6). The pressor and bradycardic responses to intravenously administered Ang II were much more rapid in onset and greater in magnitude than the responses to the same dose of Ang II microinjected into the AHA. Pretreatment (intravenous injection) with DuP 753 (40 μg) did not affect the pressor and bradycardic responses to intravenously administered Ang II in either strain.

Discussion

The current study demonstrated that microinjection of DuP 753, a highly selective AT1 receptor antagonist, into AHA caused significant dose-related depressor responses in conscious unrestrained SHR-S but not in WKY rats. The depressor response was not associated with a significant change in HR. Control injections of equal volumes of the vehicle into AHA had no effect on MAP or HR in either strain. Further, microinjection of DuP 753 into the PHA of SHR-S did not significantly alter MAP or HR. Microinjection of the AT1 receptor antagonist PD 123319 into the AHA did not alter MAP or HR in SHR-S. These data provide the first demonstration that endogenous Ang II in the anterior hypothalamus participates in the tonic control of blood pressure in SHR-S, but not in normotensive WKY rats and that this effect is mediated by AT1 receptors.

The Ang II–sensitive neuronal groups in rat brain are concentrated in the lamina terminalis of the forebrain, a periventricular structure surrounding the optic recess of the third ventricle that stretches from the anteroventral third ventricle (AV3V) region of hypothalamus, including the organum vasculosum of the lamina terminalis, to the subfornical organ (SFO).27 Ang II binding sites in this brain region, which includes the AHA and other hypothalamic nuclei that participate in cardiovascular regulation (such as the dorsal medial hypothalamus, paraventricular and periventricular nuclei, and median preoptic nucleus) are predominantly of the AT1 type and bind well to DuP 753.28 Both the AV3V region and the SFO communicate within the lamina terminalis via the median preoptic region and send a common effector pathway through the AHA. From AHA, the pathway courses either through the medial periventricular hypothalamus and projects to the brain stem or through the paraventricular and supraoptic nuclei and projects to the neurohypophysis.12,29 Horizontal knife cuts in the medial preoptic area and AHA severing the connection from the lamina terminalis to the medial basal hypothalamus eliminate the pressor response to centrally administered Ang II in rats, supporting the functional significance of this pathway in blood pressure control.12 The current finding that pretreatment (microinjection

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FIGURE 3. Line plots show effects of microinjection of DuP 753 or artificial cerebrospinal fluid (ACSF) vehicle into the anterior hypothalamic area on mean arterial pressure (MAP) and heart rate (HR) in Wistar-Kyoto (WKY) rats.

FIGURE 4. Line plots show effects of microinjection of DuP 753 (40 μg) into the posterior hypothalamic area (PHA) on mean arterial pressure (MAP) and heart rate (HR) in NaCl-sensitive spontaneously hypertensive rats (SHR-S).
FIGURE 5. Line plots show effects of pretreatment with microinjection of DuP 753 into the anterior hypothalamic area (AHA) on the pressor and bradycardic responses to angiotensin II microinjection into AHA in NaCl-sensitive SHR (SHR-S) and Wistar-Kyoto (WKY) rats. MAP, mean arterial pressure; HR, heart rate.

Several previous studies have suggested that the endogenous renin-angiotensin system in AHA plays an important role in the development of hypertension in SHR. Phillips and Kimura\(^4\) have measured endogenous Ang II levels in several brain regions of 2-, 4-, 14-, and 20-week-old SHR and WKY rats. A consistent selective elevation of Ang II levels was found in hypothalamus of SHR compared with WKY rats in all age groups. Further, it has been shown that the content of an enzyme that can generate angiotensin I from angiotensinogen, and is thus reninlike, is elevated in AHA but not in medial or posterior hypothalamic areas in SHR compared with the age-matched WKY rats.\(^{29}\) In the current study, blockade of Ang II receptors in AHA by local microinjection of DuP 753 lowered MAP dose-dependently in SHR-S but not in WKY rats, and microinjection of DuP 753 into PHA did not affect MAP in SHR-S. Our observations are thus consistent with previous reports and provide functional evidence that the enhanced activity of renin-angiotensin system in AHA of SHR is related to the pathogenesis of hypertension in this model.

DuP 753 is a highly selective, competitive, nonpeptide AT\(_1\) receptor antagonist.\(^{15-17}\) Unlike saralasin or the angiotensin converting enzyme inhibitors, DuP 753 does not have partial agonist or bradykinin-potentiating effects.\(^{16}\) The acute antihypertensive effect of DuP 753 is greater than that of captopril in SHR, is specifically dependent on an active renin-angiotensin system, and does not require activation of vasodilator prostaglandins.\(^{16}\) In addition to its therapeutic usefulness, this class of Ang II receptor antagonists provides a valuable new experimental tool to evaluate the roles of both central and peripheral renin-angiotensin systems in the regulation of blood pressure and in the pathogenesis of hypertension. Koepke et al\(^{18}\) studied the central actions of IMI (4'-[2-butyl-4-chloro-5-(hydroxymethyl)-1H-imidazol-1-yl][methyl]-[1,1'-biphenyl]-2-carboxylic acid), a nonpeptide imidazole-containing Ang II receptor antagonist, in conscious Sprague-Dawley rats. They found that intracerebroventricular injection of IMI inhibited the dipsogenic and pressor responses to intracerebroventricular Ang II, whereas IMI alone had no effect on drinking or blood pressure when administered either intravenously or intracerebroventricularly. Our observations that microinjection of DuP 753 into AHA nearly abolished the pressor and bradycardic responses to subsequent microinjection of Ang II into the same site but that microinjection of DuP 753 alone into AHA did not alter MAP in normotensive WKY rats are consistent with their findings. Both studies suggest that...
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**FIGURE 6.** Line plots show effects of pretreatment with intravenous injection of DuP 753 on the pressor and bradycardic responses to intravenous injection of angiotensin II in NaCl-sensitive spontaneously hypertensive rats (SHR-S) and Wistar-Kyoto (WKY) rats. MAP, mean arterial pressure; HR, heart rate.

Tonic activation of AT₁ receptors in brain does not play a role in the maintenance of blood pressure in normotensive rats. Substances microinjected into circumscribed neuronal groups in brain can diffuse from the injection site into surrounding areas, and the microinjection procedure can damage surrounding neurons. In our experiments, histological examination showed that the diffusion area after 100-nl injections was very small and associated with little damage to local neurons. The observation that injection of 100 nl ACSF into AHA did not affect MAP in SHR-S rules against the interpretation that the blood pressure responses to DuP 753 or to Ang II were related to nonspecific mechanical stimulation of neurons in this brain area. Further, injection of DuP 753 into the PHA had no effect on blood pressure or HR despite the fact that the PHA lies as close to the third ventricle as AHA. This observation, plus the rapid onset of the depressor response, rules against the interpretation that the depressor response to microinjection of DuP 753 into AHA was dependent on leakage of the drug into the cerebroventricular system. Intravenous injection of DuP 753 in a dose of 40 µg did not alter either basal blood pressure or the pressor response to intravenous Ang II in SHR-S, indicating that the depressor effect of microinjecting DuP 753 into the AHA observed in the current study was not due to leakage of the drug into the systemic circulation. The pressor response to intravenously administered Ang II was much more rapid in onset and greater in magnitude than the response to the same dose of Ang II injected into the AHA, suggesting that the pressor responses to exogenous Ang II in AHA and in the peripheral circulation are mediated by independent mechanisms. Thus, the current data give strong evidence that AT₁ receptors in AHA participate in blood pressure control in both hypertensive SHR-S and normotensive WKY rats and that tonic activation of these receptors contributes to the maintenance of hypertension in SHR-S.

**References**


Depressor effect of blocking angiotensin subtype 1 receptors in anterior hypothalamus.
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