Blocking Hypothalamic AT₁ Receptors Lowers Blood Pressure in Salt-Sensitive Rats

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Previous studies from our laboratory have shown that 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 of type 1 angiotensin II receptors, into the anterior hypothalamic area produces a dose-related depressor response in salt-sensitive spontaneously hypertensive rats fed a basal (1%) salt diet. The current study tested the hypothesis that the depressor response to anterior hypothalamic type 1 angiotensin II receptor blockade with DuP 753 or its metabolite EXP 3174 is enhanced by high (8%) salt feeding in this model. DuP 753 or EXP 3174 (40 μg in 100 nl artificial cerebrospinal fluid vehicle) or vehicle alone was microinjected into the anterior hypothalamic area of conscious salt-sensitive spontaneously hypertensive and Wistar-Kyoto rats that had been fed 1% or 8% salt diets for 3 weeks. Both DuP 753 and EXP 3174 caused significant decreases in mean arterial pressure in spontaneously hypertensive but not in Wistar-Kyoto rats fed either diet. The magnitude and duration of the depressor responses to DuP 753 and EXP 3174 were significantly greater in the 8% salt-fed spontaneously hypertensive rats than in 1% salt-fed rats. Vehicle injections had no effect on blood pressure in either strain-diet group. Microinjection of angiotensin II (2 μg in 100 nl artificial cerebrospinal fluid vehicle) into the anterior hypothalamic area caused significant pressor and brady-cardiac responses in all strain-diet groups; dietary salt supplementation enhanced these effects in salt-sensitive spontaneously hypertensive rats but not in Wistar-Kyoto rats. These responses were blocked by pretreatment with EXP 3174. These findings suggest that endogenous angiotensin II and type 1 angiotensin II receptors in the anterior hypothalamic area may be involved in the pathogenesis of salt-sensitive hypertension in this model. (Hypertension 1992;20:755–762)

KEY WORDS • angiotensin II • receptors, angiotensin • hypertension, sodium-dependent • microinjections • DuP 753

All of the components of the renin-angiotensin system have been identified in brain, and there is increasing evidence for generation of endogenous brain angiotensin II (Ang II).1–9 Ang II immunoreactive cell bodies and fibers, as well as specific Ang II receptors, have been localized to brain areas that participate in cardiovascular regulation, including the circumventricular organs, anterior hypothalamic region, locus coeruleus, nucleus tractus solitarius, area postrema, and ventrolateral medulla.8,10,11 Ang II binding sites in the anterior hypothalamic region, which includes the anterior hypothalamic area (AHA), dorsal medial hypothalamus, paraventricular and periventricular nuclei, and median preoptic nucleus, are predominantly type 1 (AT₁) and bind well to DuP 753.11 The presence of components of the renin-angiotensin system in primary neuronal enriched cultures and the finding that these cells synthesize immunoprecipitable Ang II, which comigrates with authentic Ang II on high-performance liquid chromatographic analysis, support the concept of local synthesis of Ang II in brain.12–15

Brain Ang II has been shown to participate in blood pressure and fluid and electrolyte regulation through mechanisms distinct from those in the periphery, including enhancement of sympathetic outflow, stimulation of vasopressin and corticotropin release, and increased catecholamine biosynthesis and turnover.5 In addition, brain Ang II elicits two behaviors, thirst and salt appetite, which are critical in body fluid and electrolyte regulation.16,17 Furthermore, there is evidence that brain Ang II may play a role in the pathogenesis of hypertension in the spontaneously hypertensive rat (SHR). In comparison to Wistar-Kyoto (WKY) rats, SHRs show increased sympathetic tone18 and increased vasopressin and corticotropin secretion.19,20 SHRs also show increased levels of Ang II–like material in brain and cerebrospinal fluid,21,22 increased turnover of Ang II in brain,22 increased numbers of Ang II receptors in some brain areas,21–25 and increased pressor and neuronal responsiveness to central administration of Ang II.26,27

A previous study from our laboratory has shown that blockade of endogenous Ang II in the AHA by local microinjection of DuP 753 (2-n-butyl-4-chloro-5-(hydroxymethyl)-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)]
methylimidazole (potassium salt), a selective nonpeptide AT\textsubscript{1} receptor antagonist, lowered blood pressure in the salt-sensitive SHR (SHR-S) maintained on a basal (1%) NaCl diet. DuP 753 did not alter blood pressure in WKY rats. In contrast, microinjection of the type 2 Ang II (AT\textsubscript{2}) receptor antagonist PD 123319 into the AHA had no effect on blood pressure in SHR-S. These data provided 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 AT\textsubscript{1} receptors.

In the peripheral circulation of the rat, DuP 753 is extensively metabolized to EXP 3174 (2-n-butyl-4-chloro-1-[2'-1H-tetrazol-5-yl]biphenyl-4-yl)methylimidazole-5-carboxylic acid), an active metabolite that likely contributes to the antihypertensive effect of DuP 753 in that species. EXP 3174 has a very potent and long-lasting blood pressure–lowering effect in the renal hypertensive rat. In this model, the intravenous dose of EXP 3174 needed to lower blood pressure by 30% is 20-fold less than the dose of DuP 753 needed to produce a similar effect. Like DuP 753, EXP 3174 is a noncompetitive antagonist of Ang II. It induces contractions in the isolated rabbit aorta, causes nonparallel rightward shifts in the dose–response curve, and reduces the maximal contractile response to Ang II. Although the conversion of DuP 753 to EXP 3174 is well documented in the periphery, this conversion has not been demonstrated in the brain.

Recent studies indicate that dietary NaCl supplementation increases arterial pressure in SHR-S, at least in part, via an alteration in AHA neurons. Furthermore, dietary NaCl supplementation in NaCl-sensitive subjects appears to alter the brain renin-angiotensin system and thereby may affect a rise in arterial pressure. Together with our previous data showing that the AHA is responsive to Ang II, this suggests that alterations in the brain renin-angiotensin system may contribute to NaCl-sensitive hypertension in SHR-S. The current study was designed to test the hypothesis that endogenous Ang II in the AHA is involved in the pathogenesis of NaCl-sensitive hypertension in SHR-S, specifically, that the depressor response to AHA AT\textsubscript{1} receptor blockade with DuP 753 and EXP 3174 is enhanced by high (8%) NaCl feeding in SHR-S. To further assess the effects of AHA AT\textsubscript{1} receptors on blood pressure control in SHR-S, exogenous Ang II was microinjected into the AHA in separate groups of SHR-S and WKY rats fed 1% or 8% NaCl diets. We found that high NaCl intake enhanced the duration of the depressor effects of DuP 753 and EXP 3174 and the pressor responses to microinjection of exogenous Ang II into the AHA in SHR-S but not in WKY rats.

Methods

SHR-S and WKY rats were obtained from Taconic Farms (IBU-3 colony, Germantown, N.Y.) at 7 weeks of age. All rats were maintained four per cage at constant humidity (60±3%), temperature (24±1°C), and light cycle (6 AM–6 PM). Two days after arrival, half of the rats in each group (SHR-S or WKY) were placed on an 8% NaCl diet (ICN Biochemicals Purina chow with 8% NaCl, Costa Mesa, Calif.), and the other half remained on the basal 1% NaCl diet (Ralston Purina diet 5001, St. Louis, Mo.). Food and water were available ad libitum throughout the study.

Nineteen days after initiation of the special diets, 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 skining the midline of the skull was incised, and a small hole was drilled through the appropriate portion of the skull. A guide cannula (26-gauge stainless-steel tubing) was lowered to a position 1.0 mm dorsal to the AHA. A 2-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 Co., Inc., Inglewood, Calif.) coupled to a model 7 polygraph (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 40 μg of DuP 753 (E.I. du Pont de Nemours and Co., Wilmington, Del.) in 100 nl artificial cerebrospinal fluid or artificial cerebrospinal fluid vehicle alone or with 40 μg of EXP 3174 (du Pont) dissolved in a solution of artificial cerebrospinal fluid: dimethyl sulfoxide (1:1 vol/vol) or artificial cerebrospinal fluid: dimethyl sulfoxide vehicle alone. Each rat received only a single injection. 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 dose (40 μg) of DuP 753 used in the current study was approximately 4% of the peripheral intravenous dose (1 mg) shown to produce significant reductions in blood pressure in previous studies by Wong et al. We had previously shown that microinjection of this dose of DuP 753 into the AHA of SHR-S fed 1% NaCl diets produced significant reductions in MAP but had no effect on MAP or HR when administered intravenously. Furthermore, this was the highest dose of DuP 753 that could be administered into the AHA without producing agitation and aversive behavior. The same dose of EXP 3174 was administered for purposes of comparison.

In parallel experiments, the effects of high NaCl intake on MAP and HR responses to microinjection of exogenous Ang II into the AHA were examined in SHR-S and WKY rats. Surgery, arterial cannulation, and cannula implantation in the AHA were performed as above. Ang II (2 μg) (Bachem, Torrance, Calif.) was microinjected into the AHA. MAP and HR were monitored before and after microinjections. A pilot study showed that microinjection of Ang II into the AHA caused dose-related increases in MAP in SHR-S; 2 μg of Ang II was the lowest dose that produced a reliable
and significant pressor effect when administered into the AHA.

A third series of experiments tested whether pretreatment with microinjection of EXP 3174 into the AHA can block or attenuate the pressor and bradycardic responses to subsequent microinjection of Ang II into the AHA in conscious SHR-S maintained on 8% and 1% NaCl. Rats were instrumented as described above. EXP 3174 (40 μg) was injected into the AHA; 120 minutes later, Ang II (2 μg) was injected into the AHA. MAP and HR were monitored before and after injections.

At the conclusion of each experiment, 100 nl of 1% methylene blue in distilled H2O was administered into the AHA through the cannula. The rat was then anesthetized with sodium pentobarbital (60 mg/kg i.p.) and decapitated, and the cannula was removed from the brain. The brain was removed from the skull and sectioned at 30 μm on a freezing microtome (Slee Medical Equipment Ltd., London). Sections were mounted and stained with 1% thionin for verification of the microinjection site.

To determine the effective area to which the injected DuP 753 diffused in these experiments, in separate groups of SHR-S on 1% (n=3) or 8% (n=3) NaCl diets, we injected 100 nl of [3H]DuP 753 (1 mCi/mg) (gift of du Pont Merck Pharmaceutical Co.) according to the above protocol. Forty minutes after the injection, the rats were decapitated; the brains were removed, frozen, and sectioned in a cryostat. The sections were picked up on slides, dried, and exposed to [3H]-sensitive ultrafilm for 8 weeks. The film was developed, and the area containing exposed silver grains was measured.

Statistical Analysis

Results are expressed as mean±SEM. Analysis of variance was performed to assess the differences in MAP and HR responses to DuP 753, EXP 3174, or Ang II among experimental groups and to compare differences over time in each group. Significant differences were then subjected to Newman-Keuls post hoc analysis. Significance was defined at a value of p<0.05.

Results

Thirty SHR-S and 15 WKY rats on 1% NaCl diets and 29 SHR-S and 15 WKY rats on 8% NaCl diets were studied. Histological examination confirmed that cannu-ulas were properly placed in the anterior and central portions of the AHA as defined by Saper et al19 in 29 SHR-S and 14 WKY rats fed 8% NaCl. In one 1% NaCl-fed SHR-S, one 8% NaCl-fed SHR-S, and one 1% NaCl-fed WKY rat, the cannula damaged the sagittal sinus; in one 8% NaCl-fed SHR-S, the cannula tip was in the third ventricle; in one 8% NaCl-fed WKY rat, cannula placement was in the dorsal hypothalamic area. These five 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 approximately 600 μm in diameter (Figure 1).

In the rats in which [3H]DuP 753 was injected, the radioactivity diffused 0.2–0.3 mm from the center of the injection (0.4–0.6 mm diameter). After injections into the center of the AHA, [3H]DuP 753 did not spread to the preoptic, paraventricular, or ventromedial hypo-

FIGURE 1. Photomicrograph of a lightly counterstained coronal section from a salt-sensitive spontaneously hypertensive rat fed 8% NaCl in which a 100-nl microinjection of DuP 753 (40 μg) elicited a depressor response. The darkly stained area in the anterior hypothalamic area (AHA) marks the methylene blue-labeled injection site. PVN, paraventricular nucleus; ot, optic tract.
Fed SHR-S. DuP 753 caused a prolonged depressor response in 8% NaCl-fed SHR-S, with a transient nadir at 10–20 minutes, followed by a deeper trough at 70–80 minutes, suggesting generation of active metabolite or metabolites. The maximal fall in MAP in response to DuP 753 was significantly greater in 8% NaCl-fed SHR-S (22.5±1.8 mm Hg; 11.2±0.77%) than in 1% NaCl-fed SHR-S (15.4±1.4 mm Hg; 8.73±0.67%; p<0.05). In contrast, in WKY rats on either diet, microinjection of DuP 753 (40 μg) into the AHA did not alter MAP or HR (Figure 3).

Microinjection of EXP 3174 (40 μg) into the AHA caused significant depressor responses in SHR-S on both diets but did not affect HR in either group (Figure 4). The magnitude and duration of the depressor response to microinjected EXP 3174 were greater in 8% NaCl-fed SHR-S than in 1% NaCl-fed rats. MAP began to fall rapidly after EXP 3174 injection in SHR-S on either diet and remained depressed for >6 hours. In the 8% NaCl-fed SHR-S, MAP was significantly decreased within 1 minute after injection of EXP 3174. In the 1% NaCl-fed rats, the onset of the depressor response was slightly delayed (3 minutes). The maximal fall in MAP in response to EXP 3174 was significantly greater in the 8% NaCl-fed SHR-S (47.0±3.3 mm Hg; 23.5±0.9%) than in 1% NaCl–fed SHR-S (33.2±3.1 mm Hg; 19.6±0.7%; p<0.05). Thus, in SHR-S the early responses to microinjection of EXP 3174 and DuP 753 into the AHA were very similar, but the duration of the depressor response to EXP 3174 was greatly prolonged, similar to the pattern seen in 8% NaCl-fed SHR-S after administration of DuP 753. Microinjection of vehicle into the AHA did not alter MAP or HR significantly in either strain on either diet (Figure 5).

Microinjection of Ang II (2 μg) into the AHA caused significant increases in MAP and decreases in HR in both SHR-S and WKY rats on either diet (Figure 6). Pretreatment (microinjection into the AHA) with EXP 3174 (40 μg) almost completely abolished the pressor and bradycardic responses to Ang II microinjection into the AHA in SHR-S on both diets (Figure 7). High NaCl intake was associated with enhanced pressor and bradycardic responses to AHA Ang II in SHR-S but not in WKY rats. There was no significant difference in either pressor or bradycardic response between SHR-S and WKY rats fed a 1% NaCl diet.

Discussion

The current study demonstrated that microinjection of DuP 753, a highly selective AT1 receptor antagonist, into the AHA caused significant depressor responses in conscious, unrestrained SHR-S fed either a basal or a high NaCl diet but not in WKY rats. Dietary NaCl supplementation enhanced the duration of the depressor effect in 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 the AHA had no effect on MAP or HR in either strain.
strain on either diet. Furthermore, microinjection of Ang II directly into the AHA caused a greater pressor response in SHR-S fed an 8% NaCl diet compared with a 1% NaCl diet. These data provide the first evidence that endogenous Ang II and AT1 receptors in the anterior hypothalamus may be involved in the pathogenesis of NaCl-sensitive hypertension in SHR-S.

The most convincing evidence that brain Ang II plays a role in the pathogenesis of hypertension in SHRs comes from the findings that central administration of an Ang II receptor antagonist or an angiotensin converting enzyme inhibitor markedly reduces blood pressure in mature SHRs and attenuates the subsequent development of hypertension in young SHRs. Acute or chronic intracerebroventricular administration of saralasin, captopril, or enalaprilat at doses that are minimally effective in lowering blood pressure when given intravenously reduces blood pressure in SHRs but has little to no effect in age-matched WKY rats. Furthermore, intracerebroventricular captopril has been shown to attenuate the development of hypertension in young SHRs without altering plasma renin activity, arginine vasopressin or aldosterone concentration, fluid intake, urine volume, urinary sodium excretion, or resting sympathetic nervous system activity. This study did not rule out a contribution of the sympathetic nervous system to the depressor effect of intracerebroventricular captopril; however, the indexes of peripheral sympathetic nervous system activity employed, plasma norepinephrine levels and the blood pressure response to ganglion blockade, were imperfect, and studies were carried out with rats in the resting state only. A subsequent study showed that SHRs treated with intracerebroventricular captopril during the developmental phase of hypertension displayed attenuated vascular reactivity to phenylephrine and arginine vasopressin and blunted reflex-mediated control of HR in response to phenylephrine infusion. SHRs receiving the same dose of intravenous captopril did not develop these alterations in vascular reactivity and baroreceptor reflex sensitivity. Furthermore, intracerebroventricular captopril-treated SHRs had a defect in central stimulation of sympathetic outflow, as signified by attenuated vasoconstrictor responses to electrical stimulation of the posterior hypothalamic area. Thus, brain Ang II appears to contribute to the development and maintenance of hypertension in SHRs, at least in part, by enhancing sympathetic vasoconstrictor tone.

Several lines of evidence suggest that the brain renin-angiotensin system is responsive to dietary NaCl supplementation in NaCl-sensitive subjects and that alterations in activity of that system contribute to NaCl-sensitive hypertension. The depressor response to centrally administered angiotensin converting enzyme inhibitors has been shown to be exaggerated in SHR-S...
During periods of oral NaCl supplementation, suggesting that NaCl-sensitive hypertension in this model is mediated, at least in part, by brain Ang II. In contrast, neither the depressor effect of intracerebroventricular captopril in WKY rats nor the pressor effect of intracerebroventricular Ang II in SHRs was enhanced by oral NaCl supplementation. Furthermore, the depressor effect of intravenous captopril was attenuated, rather than enhanced, in SHRs during chronic oral NaCl supplementation. Plasma renin concentration was suppressed during periods of enhanced NaCl intake in both SHRs and WKY rats, giving further evidence for suppression of the peripheral renin-angiotensin system in response to NaCl supplementation. These findings, along with reports that regional brain Ang II binding and angiotensin converting enzyme activity were increased in SHRs but not in WKY rats after chronic oral NaCl supplementation. These observations are consistent with previous reports and provide functional evidence that enhanced activity of the endogenous renin-angiotensin system in the AHA of SHR-S is related to the pathogenesis of hypertension in this model. The current study extended these observations by demonstrating enhancement in the duration of the depressor responses to administration of Ang II in the AHA of NaCl-fed SHR-S compared with 1% NaCl-fed animals. These findings provide the first evidence that enhanced tonic activation of AT\_1 receptors in the AHA by endogenous Ang II may participate in the dietary NaCl-induced exacerbation of hypertension in SHR-S. The observation that blood pressure responses to local (AHA) administration of either the AT\_1 receptor blocker DuP 753 nor exogenous Ang II activates a more distal mechanism coupled to the Ang II receptor.

In the current study, microinjection of DuP 753 into the AHA caused a prolonged depressor response in 8% NaCl-fed SHR-S, with an early nadir at 10–20 minutes followed by a deeper trough at 70–80 minutes, suggesting in vivo generation of an active metabolite. EXP 3174, the major active metabolite of DuP 753 in the peripheral circulation of the rat, produced protracted (6 or more hours) depressor and bradycardic responses when microinjected into the AHA of SHR-S, likely because of the noncompetitive nature of its binding to the AT\_1 receptor. The magnitude and duration of the depressor response to EXP 3174 were greater in 8% NaCl-fed SHR-S than in 1% NaCl-fed animals. This could be related to upregulation of AT\_1 receptor number and a more distal mechanism coupled to the receptor or to alterations in the metabolism or clearance of the drug in 8% NaCl-fed animals. When injected into the AHA of 8% NaCl-fed SHR-S, DuP 753 elicited a prolonged depressor response with an Ang II plateau phase resembling that seen after microinjection of EXP 3174. This contrasts with the short-duration pressor response seen after microinjection of DuP 753 into 1% NaCl-fed SHR-S and suggests that the prolonged depressor response to DuP 753 may be accounted for, at least in part, by enhanced conversion of DuP 753 to the noncompetitive AT\_1 receptor antagonist EXP 3174 in 8% NaCl-fed SHR-S. Extraction and assay of the DuP 753 and its metabolite from brain are needed to test this hypothesis directly.

Although the neuronal pathways by which the brain renin-angiotensin system regulates blood pressure have not been fully defined, there is evidence that Ang II in the AHA participates in the development of hypertension in SHRs. Phillips and Kimura have observed elevated Ang II levels in hypothalamus of SHRs compared with WKY rats both before and after the development of hypertension. Selective elevations in an enzyme that can generate angiotensin I from angiotensinogen, and is thus reninlike, have been demonstrated in the AHA of SHRs compared with age-matched WKY rats. Furthermore, a previous study from our laboratory demonstrated that blockade of AT\_1 receptors in the AHA by local microinjection of DuP 753 lowered blood pressure in SHR-S but not in WKY rats maintained on a basal (1%) NaCl diet, whereas microinjection of DuP 753 into the posterior hypothalamic area (a control region) did not affect blood pressure in SHR-S. These observations are consistent with previous reports and provide functional evidence that enhanced activity of the endogenous renin-angiotensin system in the AHA of SHR-S is related to the pathogenesis of hypertension in this model. The current study extended these observations by demonstrating enhancement in the duration of the depressor responses to administration of DuP 753 and EXP 3174 and in the magnitude of the pressor response to administration of Ang II into the AHA in high NaCl-fed SHR-S. These findings provide the first evidence that enhanced tonic activation of AT\_1 receptors in the AHA by endogenous Ang II may participate in the dietary NaCl-induced exacerbation of hypertension in SHR-S. The observation that blood pressure responses to local (AHA) administration of either the AT\_1 receptor blocker DuP 753 nor exogenous Ang II...
were altered by dietary NaCl intake in WKY rats rules out a nonspecific effect of dietary NaCl on the AHA renin-angiotensin system. Furthermore, the finding that blood pressure in WKY rats responded to AHA microinjection of Ang II but not to DuP 753 suggests that AT, receptors in the AHA participate in blood pressure control but are not tonically activated by endogenous Ang II in this NaCl-resistant normotensive rat strain.

**References**


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