Central DuP 753 Does Not Lower Blood Pressure in Spontaneously Hypertensive Rats

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Oral administration of the angiotensin II receptor subtype 1 (AT₁) antagonist DuP 753 causes long-lasting lowering of mean arterial pressure in spontaneously hypertensive rats. We examined whether the antihypertensive action of DuP 753 is a result of inhibition of brain angiotensin II. In normal spontaneously hypertensive rats, we found that intracerebroventricular DuP 753 (10 µg) blocked the pressor action of intracerebroventricular angiotensin II (100 ng); however, intracerebroventricular DuP 753 (10 µg) had no effect on the pressor response to 300 ng/kg angiotensin II administered intravenously (48±3 mm Hg in the presence of intracerebroventricular DuP 753 versus 49±4 mm Hg in its absence). In both normal and furosemide-treated spontaneously hypertensive rats (low Na⁺ diet plus furosemide), intracerebroventricular DuP 753 alone at 10 or 100 µg caused transient but significant pressor responses; however, no significant reduction in pressure (versus controls) was observed over the next 48 hours. In contrast to its central effects, we found that oral DuP 753 (10 or 30 µg/kg) in normal spontaneously hypertensive rats resulted in sustained mean arterial pressure decreases of up to −74 mm Hg. These data suggest that, although the pressor effect of brain angiotensin II is mediated by the AT₁ receptor, blockade of these receptors does not lower blood pressure in spontaneously hypertensive rats. In the spontaneously hypertensive rat, DuP 753 depresses blood pressure by blockade of peripheral, not central, AT₁ receptors. (Hypertension 1992;19:668-671)

KEY WORDS • angiotensins • DuP 753 • angiotensin antagonists • essential hypertension

The high degree of efficacy of renin-angiotensin system (RAS) antagonists (such as DuP 753 and captopril) in spontaneously hypertensive rats (SHRs) has long puzzled many investigators, primarily because SHRs do not have elevated plasma renin or angiotensin. The explanation may be that angiotensin levels are high in one or more tissues critical for the maintenance of the hypertension. According to this hypothesis, the RAS antagonists are presumed to have their antihypertensive actions in these key tissues. Some authors (e.g., Unger et al) have suggested that one of these key tissues is the brain, proposing that the angiotensin receptors of the brain may be critical for the development and maintenance of hypertension. The evidence (reviewed in Unger et al) is 1) components of the RAS are found in the brain, although recent evidence indicates that the brain “angiotensin II” (Ang II) may not be authentic; 2) Ang II injected into the brain increases blood pressure; and 3) saralasin injection or captopril infusion into the brain lowers blood pressure.

In the present study, we sought to determine whether Ang II subtype 1 (AT₁) receptors protected by the blood–brain barrier are responsible for the efficacy and long duration of action of DuP 753. We determined that Ang II in the brain appears to increase blood pressure via AT₁ receptors but found that blockade of these receptors with DuP 753 had variable effects on blood pressure quite unlike the oral antihypertensive action of the compound.

Methods

Animals

Male SHRs, 17–21 weeks of age, were sedated with xylazine (8 mg/kg s.c.) and anesthetized with ketamine (65 mg/kg s.c.), and the left jugular vein and right carotid artery were cannulated. For intracerebroventricular injection, rats were also stereotactically instrumented with a 22-gauge guide catheter in the lateral cerebral ventricle. Rats were allowed to recover 3–5 days for intracerebroventricular studies and 24 hours for oral dosing experiments. Mean arterial pressure (MAP) was monitored in the conscious rat via the carotid artery catheter. All intracerebroventricular injections delivered a total volume of 10 or 20 µl at a rate of 10 µl/min. Proper intracerebroventricular catheter placement was confirmed at the end of a study by injection of 5 µl of 1% Evans blue and visual inspection of the sectioned tissue.

Two groups of rats were used in the study: normal SHRs and furosemide-treated SHRs. The furosemide-treated SHRs were pretreated (10 mg/kg s.c.) at 22 and 4 hours before the experiment and were not allowed access to water the night before the study. The furosemide-treated rats were also maintained on a low sodium diet beginning on the day of diuretic treatment.

Pharmacological Agents

DuP 753 was synthesized by the Medicinal Chemistry Department at Pfizer Central Research, Groton, Conn.,
and dissolved in distilled water at 5 mg/ml for oral gavage and in artificial cerebrospinal fluid at 1 or 10 mg/ml for intracerebroventricular injection. The addition of DuP 753 to artificial cerebrospinal fluid made the solution acidic; however, the pH was titrated back to pH 7 with weak base. In addition, the pH of the artificial cerebrospinal fluid vehicle control was matched to that of the DuP 753 solution. Ang II was prepared in saline at 1 μg/ml for intravenous administration and in artificial cerebrospinal fluid at 10 μg/ml for intracerebroventricular injection.

Oral Antihypertensive Effect of DuP 753

Normal SHRs were dosed orally by gavage with vehicle or DuP 753 at 10 and 30 mg/kg, MAP then was monitored continuously for 6 hours and checked again at 24 hours.

Effect of Intracerebroventricular DuP 753 on Pressor Response to Intracerebroventricular or Intravenous Angiotensin II

The pressor response to Ang II (100 ng i.c.v. or 300 ng/kg i.v.) was measured before and at 10 minutes and 24 hours after DuP 753 (10 μg i.c.v.).

Central Effects of DuP 753

Normal and furosemide-treated SHRs were administered either artificial cerebrospinal fluid vehicle or 10 or 100 μg DuP 753 i.c.v., and MAP was monitored continuously for 1 hour and again at 3, 6, 24, and 48 hours.

Statistical Analyses

Analyses used were one-way analysis of variance, Dunnett’s test for multiple comparisons, and repeated-measures analysis of variance. Because of the transient pressor response observed immediately after injection of intracerebroventricular DuP 753, the first 10 minutes of the after-dose period were excluded from the analysis to improve detection of a statistically significant decrease in MAP. Values for MAP used in the analysis were averages of 30-minute intervals. A value of p<0.05 was considered significant. All data are expressed as mean±SEM.

Results

Oral Antihypertensive Effect of DuP 753

Oral DuP 753 decreased MAP in a dose-dependent manner in normal SHRs (Figure 1). The maximal decrease in MAP observed was −74±14 mm Hg at 30 mg/kg and −45±6 mm Hg at 10 mg/kg. The antihypertensive effect lasted for at least 24 hours. These data are comparable to those reported by Timmermans et al.3

Effect of Intracerebroventricular DuP 753 on Pressor Response to Intracerebroventricular or Intravenous Angiotensin II

In normal SHRs (Table 1), Ang II (100 ng i.c.v.) resulted in an increase in MAP of 37±7 mm Hg (n=5). After pretreatment with DuP 753 (10 μg i.c.v.), the pressor response was reduced (p<0.05) to 7±2 mm Hg, which was comparable to the vehicle group (9±2 mm Hg, n=5). In contrast, the pressor response to intravenous Ang II at 300 ng/kg (49±4 mm Hg, n=4) was not altered at 10 minutes (48±3 mm Hg) or at 24 hours (47±3 mm Hg) after intracerebroventricular injection of 10 μg DuP 753.

Central Effects of DuP 753

Resting MAP (in millimeters of mercury) in normal SHRs was not significantly different between the vehicle, 10 μg, or 100 μg DuP 753 treatment groups. In furosemide-treated SHRs, resting MAP was also not significantly different between treatment groups. As shown in Figure 2, administration of vehicle or DuP 753 (10 or 100 μg i.c.v.) did not have any significant hypotensive effect in normal SHRs (p=0.73) or in furosemide-treated SHRs (p=0.12). At the end of the study, sodium depletion (i.e., renin dependency) in furosemide-treated SHRs was checked; in response to captopril (3 mg/kg i.v.), a drop in MAP of at least 25 mm Hg was required as evidence of sodium depletion.

In addition to its overall lack of any central antihypertensive effect, DuP 753 (10 or 100 μg i.c.v.) elicited a transient pressor response with a duration of less than 10 minutes in both normal and furosemide-treated SHRs. In normal SHRs, the maximal increase in MAP after intracerebroventricular injection of DuP 753 was 19±2 mm Hg (n=8) and 22±3 mm Hg (n=6) for the 10-μg and 100-μg doses, respectively. These responses

| Table 1. Effects of Ang II on Pressor Response to Intracerebroventricular and Intravenous Angiotensin II in Spontaneously Hypertensive Rats |
|-----------------|-----------------|-----------------|-----------------|
| Ang II treatment | Dose Route | MAP (mm Hg) | MAP (mm Hg) |
| Without DuP 753 | ΔMAP | +10 min | +24 hr |
| DuP 753 (10 μg i.c.v.) | ΔMAP | ΔMAP |
| 100 ng i.c.v. | 37±7 | 7±2* | ND |
| 300 ng/kg i.v. | 49±4 | 48±3 | 47±3 |

Values are mean±SEM and represent the maximal change in mean arterial pressure (MAP) in response to angiotensin II (Ang II) with and without pretreatment with intracerebroventricular DuP 753. Note: MAP had returned to baseline control by 10 minutes after intracerebroventricular DuP 753 injection, at which time rats received intracerebroventricular or intravenous challenge with Ang II.

*Denotes intracerebroventricular DuP 753 significantly inhibited pressor response to intracerebroventricular Ang II (n=5) (p<0.05) but not response to intravenous Ang II (n=4). ND, not done; i.c.v., intracerebroventricular; i.v., intravenous.
were significantly greater ($p<0.05$) than the vehicle control (8±1 mm Hg, n=14). In furosemide-treated SHRs, the pressor response to intracerebroventricular DuP 753 at 10 µg (n=5) and again was significantly different ($p<0.05$) from the vehicle control group (6±2 mm Hg, n=6). The 100-µg intracerebroventricular group in the furosemide-treated SHRs exhibited a pressor response of 10±4 mm Hg (n=6); this was significantly less ($p<0.05$) than the response of normal SHRs at the same dose and was not different from the response to vehicle. The pressor responses to intracerebroventricular DuP 753 occurred between 3 and 7 minutes after injection and were transient; they do not necessarily show up when blood pressure is graphed at 5-minute intervals. Hence, although they appear absent in the bottom panel of Figure 2, they actually occurred between time points.

There was no pressor effect when DuP 753 was administered intravenously at 100 µg (data not shown) or orally (Figure 1), suggesting that the pressor effect of intracerebroventricular DuP 753 was not due to DuP 753 leaking out of the brain and acting at some peripheral site.

**Discussion**

In the present study, centrally administered Ang II appeared to increase blood pressure by stimulating AT1 receptors; however, blockade of these receptors with intracerebroventricular DuP 753 had little or no effect on blood pressure in SHRs. In contrast, oral administration of DuP 753 was extremely efficacious and lowered blood pressure with a very long duration, as described by Timmermans et al. These results suggest that 1) oral DuP 753 lowers blood pressure by blocking the action of Ang II receptors outside the blood–brain barrier; 2) because hypertension was maintained in the presence of central angiotensin receptor blockade, endogenous angiotensin inside the blood–brain barrier may not be essential for the maintenance of hypertension in SHRs; and 3) the existence of a pressor response to central injection of Ang II is not necessarily evidence of a role for endogenous brain angiotensin in the maintenance of hypertension. It remains possible that chronic intracerebroventricular infusion of DuP 753 or injection of DuP 753 during the development of hypertension would reduce MAP, possibly by getting more DuP 753 to critical brain targets inaccessible to acute DuP 753.

Our results are consistent with a large amount of data amassed by DuPont investigators showing blockade of a variety of responses by cardiovascular effector organs in the periphery. For the present study, two observations of the DuPont group are most relevant: 1) lowering of blood pressure after oral administration of DuP 753 (10 mg/kg) was not accompanied by any demonstrable blockade of central Ang II receptors, and 2) nephrectomy abolishes the antihypertensive effect of DuP 753 in SHRs. Together with our own data, these results are consistent with a site of antihypertensive action for DuP 753 that is outside of the blood–brain barrier.

The doses of DuP 753 in the present study were carefully chosen to maximize the blockade of central Ang II receptors. Estimating brain weight at approximately 1.8 g and extracellular space at approximately 20%, we arrived at a brain extracellular fluid estimate of 360 µl. The DuP 753 doses of 10 and 100 µg dissolved in this volume work out to concentrations of 60 and 600 µM, respectively. These concentrations are approximately 10³ to 10⁴ times the IC₅₀ of DuP 753 for inhibition of Ang II binding in brain (approximately 25 nM) and demonstrably were high enough to block the pressor response produced by intracerebroventricular injection of 100 ng Ang II, a dose that raises brain Ang II levels far higher than those normally present.

An interesting observation of the present study was that a brief pressor response occurred immediately after central injection of DuP 753. Although it is possible that this pressor response masked a depressor effect of DuP 753, we don’t think so; in our hands, acute intracerebroventricular captopril also fails to lower MAP in SHRs (unpublished observations). The nature of the
pressor response (e.g., whether saralasin pretreatment would block it) remains unknown. Although a nonspecific effect cannot be ruled out, this agonist-type effect was significantly larger than the response to vehicle injection and appeared dose dependent. This would not be the first instance wherein an Ang II antagonist caused pressor responses after central injection; Bruner et al. observed an increase in blood pressure when they centrally infused [Sar\(^1\),Thr\(^8\)]Ang II into SHRs.

Many studies have suggested that, in SHRs, an overactive brain RAS underlies the hypertension. Yet several studies, including the present one, indicate that blockade of the brain RAS in SHRs does not lower blood pressure. We used AT\(_1\) receptor blockade, but others have used combined AT\(_1\)/AT\(_2\) blockade with the same result. Saralasin and [Sar\(^1\),Thr\(^8\)]Ang II are examples of combined AT\(_1\)/AT\(_2\) antagonists. Bruner et al. and Berecek et al. found no lowering of blood pressure when these antagonists were given intracerebroventricularly to SHRs. Inhibition of converting enzyme is, in effect, a second method of combined antagonism, because it is a way of depriving both AT\(_1\) and AT\(_2\) receptors of Ang II. Here again, there are examples of studies in which angiotensin converting enzyme inhibition failed to lower blood pressure in SHRs.

Finally, a study by Gordon et al. showed that, when the anteroventral third ventricle (AV3V) region of the brain was destroyed in SHRs, responses to intracerebroventricular injection of Ang II were blocked, yet there was no decrease in blood pressure. Thus, AT\(_1\) antagonism, AT\(_1\)/AT\(_2\) antagonism, captopril, and lesion studies have all shown that central angiotensin responses can be blocked without lowering blood pressure in the SHR. Clearly, a number of other studies have shown that saralasin injection or captopril infusion did lower blood pressure in SHRs. Our point is simply that the literature is equivocal on the results of inhibition of the brain RAS in SHRs.

In our attempts to understand why intracerebroventricular DuP 753 did not lower blood pressure, we considered the possibility that its metabolite, EXP 3174, may not be generated in brain. This metabolite appears critical for the antihypertensive action of DuP 753. It is therefore possible that the reason we did not observe blood pressure lowering is that the metabolite is not generated after intracerebroventricular injection of DuP 753. This explanation does not account, however, for the failure of intracerebroventricular [Sar\(^1\),Thr\(^8\)]Ang II to lower blood pressure in the studies of Bruner et al. and Berecek et al. or for the failure of captopril to lower blood pressure in the study of Mann et al. and in the present study, we demonstrated blockade of the pressor response to intracerebroventricular Ang II with intracerebroventricular DuP 753. In our opinion, the absence of the metabolite does not explain why clearly demonstrable blockade of brain Ang II did not lower blood pressure in these studies.

In summary, we did not detect significant lowering of blood pressure in SHRs after intracerebroventricular administration of DuP 753. This finding suggests a reappraisal of the necessity of endogenous brain angiotensin for the maintenance of hypertension in SHRs.

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**References**

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