Symposium

The Influence of Chronic Antihypertensive Treatment on the Central Pressor Response in SHR

Yingzi LIN, Takuya TSUCHIHASHI, Shuntaro KAGIYAMA,
Kiyoshi MATSUMURA, and Isao ABE

We examined the influence of chronic antihypertensive treatment on the central pressor response in SHR. Adult male SHR were divided into 5 groups, *i.e.*, those receiving 1) enalapril (Enal: 25 mg/kg/day in drinking water, $n=12$); 2) losartan (Los: 40 mg/kg/day, $n=11$); 3) candesartan (Cand: 4 mg/kg/day, $n=12$); 4) hydralazine+hydrochlorothiazide (H&H: 50+7.5 mg/kg/day, $n=9$); 5) vehicle (Control: $n=9$). At 4 weeks of treatment, hypertonic saline (0.25, 0.5 M) was intracerebroventricularly (i.c.v.) injected into conscious rats. Plasma catecholamines were measured before and after i.c.v. injection. On completion of the experiment, heart weight was measured, and angiotensin-converting enzyme (ACE) activity of the cerebrum was determined. All antihypertensive drugs elicited comparable reductions in systolic blood pressure, while heart rate was significantly higher in the H&H group than in the other groups during treatment. Pressor response to i.c.v. hypertonic saline (0.5 M) was significantly smaller in the Enal (12 ± 3 mmHg) and Cand (11 ± 2 mmHg) groups than in the Los (22 ± 2 mmHg), H&H (16 ± 2 mmHg), and Control (29 ± 5 mmHg) groups. Plasma catecholamines did not differ among the groups. Heart weight was lowest in the Enal group, followed by the Los and Cand groups. ACE activity of the cerebrum was significantly decreased in the Enal group. The results suggest that chronic treatment with various antihypertensive drugs differentially alters the central pressor response in SHR, and enalapril and candesartan are effective in attenuating this response. (*Hypertens Res* 2001; 24: 173-178)

Key Words: angiotensin II antagonists, angiotensin-converting enzyme inhibitors, central nervous system, hypertonic saline

Introduction

It is well established that the brain renin-angiotensin system plays an important role in cardiovascular regulation (1). More specifically, angiotensin II (Ang II) acts at type 1 (AT_1) receptors in various regions within the central nervous system, including the hypothalamus and medulla oblongata, and elicits a variety of neurohormonal and cardiovascular responses (2-6). An augmented response to Ang II has also been reported in the hypothalamus and

ventrolateral medulla of experimental hypertensive rats (2, 7).

We have recently reported that chronic oral treatment with an angiotensin-converting enzyme (ACE) inhibitor decreases the ACE activity of the brain stem in spontaneously hypertensive rats (SHR), whereas treatment with an AT_1 antagonist increases it (8). Since the pressor stimuli within the central nervous system are mainly mediated through the activation of the brain renin-angiotensin system, chronic treatment with ACE inhibitors or AT_1 antagonists may differentially alter the cen-

From the Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. This study was presented at the Third China and Japan Joint Hypertension Symposium, Beijing, China, October 8, 2000.

A part of this study was performed in the Kyushu University Station for Collaborative Research.

Address for Reprints: Takuya Tsuchihashi, M.D., Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan.

Received December 4, 2000; Accepted in revised form December 12, 2000.

trally-mediated pressor responses.

Thus, the purpose of the present study was to investigate the influence of chronic oral treatment with various antihypertensive drugs on the centrally-mediated pressor responses in SHR.

Methods

Animal Preparation

Twelve-week-old male SHR (280 ± 2 g) were used. This experiment was carried out following the guidelines of the Committee of Ethics in Animal Experimentation of the Faculty of Medicine, Kyushu University. Rats were treated with the following drugs dissolved in drinking water for 4 weeks: 1) control: tap water ($n=9$); 2) hydralazine (50 mg/kg/day)+hydrochlorothiazide (HCTZ, 7.5 mg/kg/day, $n=9$); 3) enalapril (25 mg/kg/day, $n=12$); 4) losartan (40 mg/kg/day, $n=11$); and 5) candesartan (4 mg/kg/day, $n=12$). Systolic blood pressure (SBP) and heart rate (HR) were measured weekly by a tail-cuff method. Urine volume and water intake were also measured at 0, 2, and 4 weeks of treatment.

Experimental Protocol

At 4 weeks of treatment, the rats were anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally, and indwelling arterial and venous catheters (PE-10 connected to PE-50) were advanced through the right femoral artery and vein into the abdominal aorta and inferior vena cava, respectively. The catheters, filled with heparinized saline, were exteriorized and secured at the back of the rat's neck. A 22-gauge stainless steel guide cannula was placed on the right lateral ventricle of the brain 1.5 mm posterior from the bregma, 2 mm lateral from the midline, and 5 mm deep from the surface of the dura. The cannula was fixed to the skull with two screws and dental cement. After 24–48 h for recovery, the rats were placed in plastic boxes. The arterial catheter was connected to a pressure transducer (P50; Gould Statham Instruments, Hato Rey, Puerto Rico) for continuous recording of the mean arterial pressure (MAP) and HR. The venous line was used for injections of drugs. After an at least 30-min stabilization period, blood (0.5 ml) was drawn from the arterial line to determine plasma catecholamines. Then, artificial cerebrospinal fluid (aCSF, in mmol/l: NaCl 133.3, KCl 3.4, CaCl_2 1.3, MgCl_2 1.2, NaH_2PO_4 0.6, NaHCO_3 32.0, and glucose 3.4, pH 7.4) as a vehicle control and hypertonic saline (0.25 and 0.5 M) were injected intracerebroventricularly (i.c.v.) in a volume of 5 μl . The injections were done at intervals of at least 20 min. Five minutes after i.c.v. injection of 0.5 M of hypertonic saline, blood samples (2 ml) were drawn to determine plasma catecholamines, serum electrolytes, creatinine, and uric acid. At

the end of the experiments, dye was injected through the i.c.v. cannula to verify the correct placement of the cannula. The rats were deeply anesthetized with pentobarbital sodium (50 mg i.v.), and the brains were quickly removed by decapitation. After blood vessels were carefully stripped off, the cerebrum was dissected and stored at -80°C until the measurement of ACE activity. Finally, the heart was separated, cleaned of blood, and weighed.

Biochemical Measurements

Blood samples were centrifuged at 4°C . Plasma samples for the measurement of catecholamines were stored at -80° , whereas other samples were stored at -20°C until assayed. Plasma catecholamines were determined by high-performance liquid chromatography. Tissue ACE activity was determined using a fluorometric assay that was described by Cheung and Cushman (9), and was calculated as nanomoles His-Leu generated per mg tissue weight/h.

Drugs

Hydralazine, hydrochlorothiazide, and enalapril were purchased from Sigma Chemical Co. (St. Louis, USA). Losartan and candesartan were the generous gifts of Merck Research Laboratories (Rahway, USA) and Takeda Chemical Industries, Ltd. (Osaka, Japan), respectively.

Statistical Analysis

Data are expressed as the means $\pm$ SEM. Comparisons among the groups were done by ANOVA, followed by Scheffe's multiple comparison test. *P* values less than 0.05 were considered to indicate statistical significance.

Results

Figure 1 shows the changes in body weight during the antihypertensive treatment. The increase in body weight was significantly less in the rats treated with hydralazine+HCTZ than in the control rats or rats treated with other antihypertensive drugs. Water intake and urine volume at 2 and 4 weeks of treatment were also significantly less in the rats treated with hydralazine+HCTZ (Fig. 2). Figure 3 shows the changes in SBP and HR during treatment. The rats treated with antihypertensive drugs showed similar reductions in SBP, whereas the rats treated with hydralazine+HCTZ showed a significant increase in HR.

Cardiovascular responses to i.c.v. injection of hypertonic saline are shown in Fig. 4. Hypertonic saline at a concentration of 0.25 M did not elicit significant changes in MAP or HR. On the other hand, hypertonic saline at a concentration of 0.5 M induced a significant increase in MAP; MAP increased by 29 ± 5 mmHg in the untreated

SHR. This pressor response was significantly attenuated in the rats treated with either enalapril (12 ± 3 mmHg; $p < 0.05$ vs. control) or candesartan (11 ± 2 mmHg; $p < 0.05$). In contrast, the pressor response was not significantly different from the control value in the rats treated with either hydralazine+HCTZ (16 ± 2 mmHg; ns) or losartan (22 ± 2 mmHg; ns). HR did not change consistently by i.c.v. injection of hypertonic saline. Baseline plasma norepinephrine and epinephrine concentrations did not differ among the groups (Fig. 5). In addition, hypertonic saline did not alter these variables significant-

ly. Serum electrolytes, creatinine, and uric acid concentrations were not altered significantly by antihypertensive treatment (Table 1).

ACE activity of the cerebrum was significantly decreased in the rats treated with enalapril (Fig. 6, left). Heart weight was also significantly lower in the enalapril-treated rats (Fig. 6, right). Heart weight in the rats treated with either losartan or candesartan tended to be lower compared to that of control rats, but the difference was not statistically significant.

Discussion

In the present study, pressor responses to i.c.v. injection of hypertonic saline were attenuated in SHR treated with either the ACE inhibitor enalapril or the AT₁ antagonist candesartan. Since the pressor response to i.c.v. hypertonic saline is mainly mediated through the activation of the brain renin-angiotensin system (10), the effects of enalapril and candesartan to attenuate the pressor response to i.c.v. hypertonic saline are considered to be attributable to the inhibition of the brain renin-angiotensin system. Indeed, the ACE activity of the cerebrum was significantly inhibited in the rats treated with enalapril. In our previous study, chronic oral treatment with candesartan also inhibited the pressor response to Ang II microinjected into the rostral ventrolateral medulla (8). Thus, these two drugs may effectively inhibit the brain renin-angiotensin system, resulting in the attenuation of the centrally-mediated pressor response. In contrast to candesartan, another AT₁ antagonist, losartan, did not alter the pressor response elicited by i.c.v. hypertonic saline. This observation seems compatible with the previous findings that oral or intravenous administration of losartan has little effect

Fig. 1. Time course of body weight in control rats and rats treated with hydralazine+hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means $\pm$ SEM. * $p < 0.05$, † $p < 0.01$ vs. control.

Fig. 2. Time courses of water intake (left) and urine volume (right) in control rats and rats treated with hydralazine+hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means $\pm$ SEM. * $p < 0.05$, † $p < 0.01$ vs. control.

Fig. 3. Time courses of systolic blood pressure (left) and heart rate (right) in control rats and rats treated with hydralazine + hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means $\pm$ SEM. $\dagger p < 0.01$ vs. control.

Fig. 4. Changes in mean arterial pressure (left) and heart rate (right) elicited by intracerebroventricularly injected hypertonic saline (0.25, 0.5 M) or artificial cerebrospinal fluid (aCSF, vehicle control) in control rats and rats treated with hydralazine + hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means $\pm$ SEM. * $p < 0.05$ vs. control.

on the pressor response to i.c.v. Ang II (11, 12). The difference between losartan and candesartan with respect to influence on the brain renin-angiotensin system may be attributable to their pharmacological properties, particularly their water solubility; losartan is hydrophilic, while candesartan is hydrophobic. Further studies will be required to confirm the differential influence of these two drugs on the brain renin-angiotensin system.

Although we hypothesized that sympathetic nervous activity may be differentially altered by different kinds of antihypertensive drugs, the results showed that plasma catecholamines, determined before and after i.c.v. injection of hypertonic saline, did not differ among the groups.

In the rats treated with hydralazine + HCTZ, an increase in HR was observed, suggesting that the sympathetic nervous system was activated by both vasodilation and volume depletion. However, we were unable to detect evidence of sympathetic activation by the measurement of plasma catecholamines. The differential influence of ACE inhibitors and AT₁ antagonists on plasma norepinephrine concentration was also suggested in a large-scale clinical trial (13). In the present study, however, plasma catecholamines were comparable among the rats treated with either ACE inhibitors or AT₁ antagonists. Since plasma catecholamines determined 5 min after i.c.v. injection of hypertonic saline did not show consistent increases, the

Table 1. Comparison of Blood Variables among the Groups

	Control	Hydralazine +HCTZ	Enalapril	Losartan	Candesartan
Sodium (mEq/l)	147±1	147±1	143±1	145±1	148±1
Potassium (mEq/l)	4.8±0.2	4.2±0.1	4.4±0.2	4.8±0.2	4.5±0.2
Creatinine (mg/dl)	0.30±0.01	0.33±0.03	0.28±0.02	0.26±0.02	0.32±0.06
Uric acid (mg/dl)	0.17±0.03	0.16±0.02	0.12±0.02	0.28±0.08	0.14±0.02

Values are means±SEM. HCTZ: hydrochlorothiazide.

Fig. 5. Plasma norepinephrine (left) and epinephrine (right) concentrations determined before intracerebroventricular (i.c.v.) injection of artificial cerebrospinal fluid and 5 min after i.c.v. injection of hypertonic saline (0.5 M) in control rats and rats treated with hydralazine+hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means+SEM.

Fig. 6. Angiotensin-converting enzyme (ACE) activity of the cerebrum (left) and heart weight (right) in control rats and rats treated with hydralazine+hydrochlorothiazide (HCTZ), enalapril, losartan, or candesartan. Values are the means+SEM. * $p<0.05$, † $p<0.01$ vs. control.

measurement of plasma catecholamines may not be sensitive enough to evaluate the changes in sympathetic nervous activity. Further studies utilizing the direct measurement of sympathetic nerve activity may be required to investigate the influence of antihypertensive drugs on the sympathetic nervous system.

The present finding that heart weight was significantly lower in the rats treated with enalapril than in untreated controls seems intriguing. Since the heart weight of rats treated with either losartan or candesartan was not significantly lower than that of control rats, ACE inhibitors may be more effective than AT_1 antagonists in preventing

the development of cardiac hypertrophy. Previous studies investigating the effects of ACE inhibitors and AT₁ antagonists on the prevention of cardiac hypertrophy show inconsistent findings. One study found that captopril and losartan had comparable effects in SHR (14), while another study showed that ACE inhibitors were superior to AT₁ antagonists in preventing cardiac hypertrophy in experimental hypertension induced by aortic banding (15). The superiority of ACE inhibitors may suggest that the inhibition of the degradation of bradykinin is more important than the inhibition of Ang II generation. A recent study proposed that AT₂ receptors play a major role in the development of cardiac hypertrophy (16). If AT₂ receptors are involved in cardiac hypertrophy in SHR, AT₁ antagonists by which increased plasma Ang II may stimulate AT₂ receptors could be less effective in preventing cardiac hypertrophy than ACE inhibitors.

Finally, it has been reported that losartan reduces serum uric acid concentration in patients with essential hypertension (17). In the present study, however, losartan did not alter serum uric acid concentration. As far as we know, the uricosuric action of losartan has not been reported in rats. Thus, the effect of losartan on the metabolism of uric acid may differ between humans and rats.

In conclusion, chronic oral treatment with various anti-hypertensive drugs differentially altered the centrally-mediated pressor response independently of reducing the baseline blood pressure in SHR, and enalapril and candesartan were effective in attenuating this response. This observation may provide additional pharmacological properties of these drugs.

Acknowledgements

The authors thank Ms. Miki Mihara and Ms. Akemi Takushima for their technical assistance, and Merck Research Laboratories and Takeda Chemical Industries, Ltd. for supplying losartan and candesartan, respectively.

References

1. Phillips MI: Functions of angiotensin in the central nervous system. *Annu Rev Physiol* 1987; **49**: 413-435.
2. Kubo T, Yamaguchi H, Tsujimura M, Hagiwara Y, Fukumori R: Blockade of angiotensin receptors in the anterior hypothalamic preoptic area lowers blood pressure in DOCA-salt hypertensive rats. *Hypertens Res* 2000; **23**: 109-118.
3. Kagiya S, Tsuchihashi T, Abe I, Matsumura K, Fujishima M: Antisense inhibition of angiotensinogen attenuates vasopressin release in the paraventricular hypothalamic nucleus of spontaneously hypertensive rats. *Brain Res* 1999; **829**: 120-124.
4. Averill DB, Tsuchihashi T, Khosla MC, Ferrario CM: Losartan, nonpeptide angiotensin II-type 1 (AT₁) receptor antagonist, attenuates pressor and sympathoexcitatory responses evoked by angiotensin II and L-glutamate in rostral ventrolateral medulla. *Brain Res* 1994; **665**: 245-252.
5. Matsumura K, Averill DB, Ferrario CM: Angiotensin II acts at AT₁ receptors in the nucleus of the solitary tract to attenuate the baroreceptor reflex. *Am J Physiol* 1998; **275**: R1611-R1619.
6. Matsumura K, Averill DB, Ferrario CM: Role of AT₁ receptors in the area postrema on baroreceptor reflex in spontaneously hypertensive rats. *Brain Res* 1999; **850**: 166-172.
7. Tsuchihashi T, Kagiya S, Matsumura K, Lin Y, Abe I, Fujishima M: Cardiovascular responses to glutamate and angiotensin II in ventrolateral medulla of hypertension induced by chronic inhibition of nitric oxide. *Hypertens Res* 2000; **23**: 359-364.
8. Tsuchihashi T, Kagiya S, Matsumura K, Abe I, Fujishima M: Effects of chronic oral treatment with imidapril and TCV-116 on the responsiveness to angiotensin II in ventrolateral medulla of SHR. *J Hypertens* 1999; **17**: 917-922.
9. Cheung HS, Cushman DW: Inhibition of homogenous angiotensin-converting enzyme of rabbit lung by synthetic venom peptides of *Bothrops jararaca*. *Biochem Biophys Acta* 1973; **293**: 451-463.
10. Takata Y, Takishita S, Yamashita Y, Tsuchihashi T, Fujishima M: Pressor response to NaCl solution administered intracerebroventricularly or intracisternally to conscious normotensive and spontaneously hypertensive rats. *J Hypertens* 1986; **4**: 713-718.
11. Morita O, Kushida H, Kuniyama M: Effects of U-97018 on pressor responses to intracerebroventricularly administered angiotensin II in conscious rats. *J Cardiovasc Pharmacol* 1995; **25**: 880-887.
12. Culman J, von Heyer C, Piepenburg B, Rascher W, Unger T: Effects of systemic treatment with irbesartan and losartan on central responses to angiotensin II in conscious, normotensive rats. *Eur J Pharmacol* 1999; **367**: 255-265.
13. Hall C, Segal R, Sharma D, Klinger GH, Velivis M, Pitt B: Neurohormonal profile following long-term treatment of heart failure patients with losartan or captopril-ELITE study. *Circulation* 1999; **18** (Suppl I): I-783 (Abstract).
14. Morton JJ, Beattie EC, MacPherson F: Angiotensin II receptor antagonist losartan has persistent effects on blood pressure in the young spontaneously hypertensive rat: lack of relation to vascular structure. *J Vasc Res* 1992; **29**: 264-269.
15. Linz W, Scholkens BA: A specific B2-bradykinin receptor antagonist HOE 140 abolishes the antihypertrophic effect of ramipril. *Br J Pharmacol* 1992; **105**: 771-772.
16. Senbonmatsu T, Ichihara S, Price E, Gaffney FA, Inagami T: Evidence for angiotensin II type 2 receptor-mediated cardiac myocyte enlargement during *in vivo* pressure overload. *J Clin Invest* 2000; **105**: R9-R14.
17. Tikkanen I, Omvik P, Jensen HA: Comparison of the angiotensin II antagonist losartan with the angiotensin converting enzyme inhibitor enalapril in patients with essential hypertension. *J Hypertens* 1995; **13**: 1343-1351.