Inhibitory Actions of Captopril on Norepinephrine Release from Adrenergic Nerve Endings in Spontaneously Hypertensive Rats

Kazushi TSUDA, M.D., Seiko TSUDA, M.D., Masako URA, M.D.,
Hiroki SHIMA, M.D., Ichiro NISHIO, M.D.,
and Yoshiaki MASUYAMA, M.D.

SUMMARY

The purpose of the present study was to investigate the mechanisms of the hypotensive actions of an angiotensin converting enzyme inhibitor (captopril) in hypertension. In perfused mesenteric vasculatures from spontaneously hypertensive rats (SHR, Okamoto and Aoki strain, 10–13 weeks of age) and age-matched normotensive Wistar Kyoto rats (WKY), the effects of captopril on vascular responsiveness and norepinephrine release from the adrenergic nerve endings were examined. The vasoconstrictor responses and norepinephrine release during the electrical nerve stimulation were significantly enhanced in SHR compared to those in age-matched WKY. Captopril reduced both vasoconstrictor responses and norepinephrine release during the electrical nerve stimulation, dose-dependently. These inhibitory effects of captopril were significantly greater in SHR than in WKY.

The results demonstrate that captopril affects presynaptic sites on the resistance vessels and causes a decrease in electrically-stimulated norepinephrine release from the adrenergic nerve endings. The marked reduction of both pressor responses and norepinephrine release by captopril in SHR suggests an enhanced renin-angiotensin system in the vascular walls of hypertension.

Additional Indexing Words:

Angiotensin converting enzyme inhibitor Captopril Norepinephrine release
Sympathetic nervous system Vascular responsiveness
Mesenteric vasculature Spontaneously hypertensive rats

CAPTOPRIL, an orally active inhibitor of the angiotensin converting enzyme, has an effective antihypertensive action in hypertensive animals and human hypertensive subjects.^{1),2)} This hypotensive action of captopril has been believed to depend on a reduction of the circulating angiotensin II level in the blood. However, there is some evidence that the effi-

From the Division of Cardiology, Department of Medicine, Wakayama Medical College, Wakayama, Japan.

Received for publication December 27, 1986.

Accepted September 16, 1987.

cacy of captopril is found not only in high renin models but also in normal renin models of hypertension.³⁾ Recently, it has been suggested that the renin-angiotensin system exists in a variety of tissues, such as brain, adrenal medulla and vascular tissues,^{4),5)} and that locally generated angiotensin II might play an important role in the development of hypertension. Schacht⁶⁾ reported that captopril acted on the central nervous system, which might be explained by a decrease in the central sympathetic nerve activity. Collis et al.⁷⁾ observed that captopril could depress the sympathetic vasoconstriction in rat mesenteric arteries. It was also demonstrated that captopril decreased the pressor responses evoked by electrical stimulation, suggesting that the drug could interfere with peripheral adrenergic neuro-effector mechanisms.⁸⁾ The present study was performed to investigate whether captopril has an inhibitory effect on adrenergic transmission in the resistance vessels of hypertensive rats.

MATERIALS AND METHODS

Male spontaneously hypertensive rats (SHR, Okamoto and Aoki strain, 10–13 weeks old) were studied in comparison with age- and sex-matched Wistar Kyoto rats (WKY) from Charles River Inc. (Japan). The systolic blood pressures, measured by the tail-cuff method (programmed electro-sphygmomanometer, Narco Biosystem Inc., Model PE-300), were 171.0 ± 5.6 mmHg (mean $\pm$ SEM, $n=5$) in SHR and 121.2 ± 9.7 mmHg ($n=6$) in WKY. Rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneal injection), and perfused mesenteric vasculatures were prepared according to the method of Castellucci et al.⁹⁾ The superior mesenteric artery was perfused through a cannula inserted at its origin from the aorta. In each preparation, only four main branches from the superior mesenteric artery trunk were used. All other branches were tied off. The isolated preparation was placed in a chamber maintained at 37°C and gently laid on gauze to avoid any traction on the cannulated artery. The preparation was perfused with a modified Ringer-Locke solution (mmol/l: NaCl 120.7, KCl 5.9, CaCl₂ 2.5, MgSO₄ 1.3, NaHCO₃ 15.5, NaH₂PO₄ 1.2 and glucose 11.5, pH 7.4, 37°C), and bubbled with a 95% O₂ and 5% CO₂ mixture. A constant flow rate was maintained with a peristaltic pump (Harvard apparatus, Model 1200) at 0.8 ml/min. Changes in the perfusion pressure were monitored with a pressure transducer connected to a polygraph (Nihon Kohden, Model CP-620G).

Periarterial nerve stimulation was performed with bipolar platinum electrodes around the proximal end of the mesenteric artery. The stimulation was applied at a supramaximal voltage (40 V), with rectangular pulses of 5 msec duration for 1 min at 5 and 15 Hz (electrical stimulator: Nihon

Kohden, Model SEN-3201). Pressor responses to the electrical nerve stimulation were determined as increases in the perfusion pressure. After perfusion of the mesenteric vascular beds was initiated, the basal perfusion pressure was allowed to stabilize for 30 min prior to onset of the experimental manipulations.

Measurement of norepinephrine release during nerve stimulation in mesenteric vasculatures:

For the measurement of norepinephrine release from sympathetic nerve endings, the perfusate through the mesenteric preparation was collected in a tube containing EGTA (90 mg/ml) and glutathione (60 mg/ml) in 20 μ l of the perfusate. The collecting periods were 3 min before nerve stimulation (A) and 2 min after cessation of 1 min-stimulation (total 3 min) (B). The norepinephrine release evoked by the electrical nerve stimulation was defined as the norepinephrine overflow, which was the difference of the norepinephrine contents between (A) and (B), and was normalized per gram of wet tissue weight for each preparation.

Norepinephrine in the perfusate was adsorbed on alumina, extracted in 200 μ l of perchloric acid (0.1 mol/l), and assayed by high performance liquid chromatography^{10),11)} with an electrochemical detector (Bioanalytical system, Model LC-4A, carbon electrode, 700 mV). The HPLC unit was equipped with a delivery system (Waters Assoc., Model 510, flow rate 1.0 ml/min), an automatic injector (Waters Assoc., Model 710B, injected volume 100 μ l) and Biophase ODS 5 μ m column. The solvent for the separation of norepinephrine was 0.1 mol/l of monochloric acid with 2 mmol/l of EDTA, 300 mg/l of sodium octyl sulfate and 9% (v/v) of acetonitrile (pH 3.02 at room temperature).

In the first series of experiments, the pressor responses and norepinephrine release during the electrical nerve stimulation were examined in SHR and WKY. Then, captopril was added to the perfusion medium to achieve final concentrations of 5.0×10^{-6} mol/l to 5.0×10^{-5} mol/l. After drug administration, a 9 min-period elapsed before the next electrical stimulation. For each preparation, the results were evaluated by comparing the responses before (control) and during the infusion of captopril (experimental), and then expressed as percentage ratios of the control response for each experiment. Thus, a value greater than 100% would indicate enhancement and that less than 100% would represent inhibition.¹²⁾

Values are expressed as mean $\pm$ SEM. Statistical significances were determined by Student's t-test, Mann-Whitney U-test and 2 way-analysis of variance (ANOVA).¹³⁾ The differences of $p < 0.05$ were considered signifi-

cant.

Captopril was kindly donated by Sankyo Co., Ltd. (Tokyo, Japan).

RESULTS

The pressor responses and norepinephrine overflow during the electrical nerve stimulation were completely blocked when guanethidine was added into the perfusion medium, reproducing a previous result.¹⁴⁾ This indicates that the effects of periarterial electrical stimulation are neurally mediated. The pressor responses and norepinephrine overflow during the nerve stimulation did not significantly change for at least 7 repeated stimuli in the same preparation, documenting reproducibility and stability.¹⁴⁾

The basal perfusion pressure and norepinephrine content in the perfusate during the unstimulated period (spontaneous norepinephrine efflux from the vascular beds) were not significantly different between SHR and WKY (basal perfusion pressure: SHR 22.6 ± 2.4 mmHg, $n=5$, WKY 22.3 ± 2.2 mmHg, $n=6$; norepinephrine content in the perfusate during unstimulated 3 min: SHR 0.32 ± 0.06 ng/g of wet tissue weight, $n=5$, WKY 0.30 ± 0.04 ng/g of wet tissue weight, $n=5$). As shown in Fig. 1, the pressor re-

Fig. 1. Pressor responses to electrical nerve stimulation in the mesenteric vasculature preparations from SHR and age-matched WKY. Electrical nerve stimulation was performed at 5 and 15 Hz frequencies (supramaximal voltage 40 V, 5 msec duration of rectangular pulses for 1 min). Pressor responses are represented as increases in the perfusion pressures of the preparations.

Fig. 2. Norepinephrine overflow during electrical nerve stimulation in the mesenteric vascular preparations from SHR and WKY. Norepinephrine overflow was expressed as nanogram/gram of wet tissue weight of each preparation.

sponses to the electrical nerve stimulation were significantly greater in SHR than in WKY (5 Hz: SHR 24.5 ± 1.3 mmHg, $n=5$, WKY 5.7 ± 0.3 mmHg, $n=6$, $p < 0.005$, 15 Hz: SHR 169.5 ± 3.5 mmHg, $n=5$, WKY 56.5 ± 10.7 mmHg, $n=6$, $p < 0.005$). The norepinephrine overflow during the electrical nerve stimulation was also enhanced in SHR (Fig. 2; 5 Hz: SHR 0.84 ± 0.07 ng/g of wet tissue weight, $n=5$, WKY 0.23 ± 0.08 ng/g of wet tissue weight, $n=5$, $p < 0.005$, 15 Hz: SHR 2.21 ± 0.14 ng/g of wet tissue weight, $n=5$, WKY 0.74 ± 0.12 ng/g of wet tissue weight, $n=5$, $p < 0.001$).

Effects of captopril on pressor responses and norepinephrine release in SHR:

When captopril (5.0×10^{-6} mol/l— 5.0×10^{-5} mol/l) was added into the perfusion medium, the basal perfusion pressure did not change in any preparations. Figure 3 and Table I show the effects of captopril on the pressor responses evoked by the electrical nerve stimulation. Captopril dose-dependently reduced the pressor responses both in SHR and WKY, and the suppressive magnitudes were significantly greater in SHR than in WKY. Further, Table I documents changes in the norepinephrine release in the presence of captopril in SHR and WKY. The norepinephrine overflow during the electrical nerve stimulation was also inhibited by captopril. This neurosuppressive action was more marked in SHR than in WKY.

Fig. 3. Typical changes in the stimulation-evoked pressor responses in SHR and WKY when captopril was added into the perfusion medium (SHR: upper half, WKY: lower half).

Table I. Comparison of the Effects of Captopril on Electrically-induced Pressor Responses (15 Hz) and Norepinephrine (NE) Overflow (5, 15 Hz) in the Mesenteric Vasculatures from SHR (10-13 weeks old) and Age-matched Normotensive WKY

Captopril	5.0×10^{-6} mol/l	1.0×10^{-5} mol/l	5.0×10^{-5} mol/l
Pressor responses (15 Hz)			
SHR	$77.4 \pm 1.8\% \uparrow$ (n=5) *	$67.6 \pm 2.5\% \uparrow$ (n=5) *	$39.2 \pm 2.9\% \uparrow$ (n=5) *
WKY	$100.7 \pm 2.1\%$ (n=6)	$85.3 \pm 4.6\%$ (n=6)	$71.5 \pm 4.0\% \uparrow$ (n=6)
NE overflow (5 Hz)			
SHR	$55.6 \pm 5.6\% \uparrow$ (n=4) *	$40.4 \pm 4.7\% \uparrow$ (n=4) *	$42.3 \pm 6.3\% \uparrow$ (n=4) *
WKY	$99.6 \pm 6.4\%$ (n=5)	$81.9 \pm 6.0\%$ (n=4)	$65.6 \pm 3.1\% \uparrow$ (n=4)
NE overflow (15 Hz)			
SHR	$57.7 \pm 3.8\% \uparrow$ (n=5) *	$41.9 \pm 3.5\% \uparrow$ (n=5) *	$37.6 \pm 2.7\% \uparrow$ (n=5) *
WKY	$98.3 \pm 5.8\%$ (n=5)	$70.5 \pm 3.4\% \uparrow$ (n=5)	$63.8 \pm 4.6\% \uparrow$ (n=5)

Values are expressed as percentage ratios of the control responses without captopril both in SHR and WKY, respectively (mean $\pm$ SEM, $\uparrow$ $p < 0.05$ vs control values in the absence of captopril in SHR and WKY, respectively; * $p < 0.05$, differences between SHR and WKY, determined by Mann-Whitney U-test).

The data of 2 way-ANOVA (analysis of variance) are as follows⁽¹³⁾:

	Pressor responses (15 Hz)	Norepinephrine overflow	
(Source of variation)		(5 Hz)	(15 Hz)
Captopril concentrations	$F = 21.4$ $p < 0.05$	$F = 4.6$	$F = 108.4$ $p < 0.05$
Strains (SHR vs WKY)	$F = 33.0$ $p < 0.05$	$F = 30.9$ $p < 0.05$	$F = 127.8$ $p < 0.05$

DISCUSSION

The present study demonstrated that captopril has a presynaptic effect on sympathetic vascular transmission, resulting in a decrease in the stimulation-induced norepinephrine release from the adrenergic nerve endings. It was also observed that these inhibitory actions were more prominent in SHR than in WKY. These results confirm the hypothesis that the hypotensive mechanisms of captopril are related to inhibition of the sympathetic nerve activity.^{7),8)} Captopril is a potent inhibitor of angiotensin converting enzyme. Thus, it is likely that the sympathetic inhibitory action of captopril might be due to the suppression of angiotensin II formation in the vascular beds. It has been suggested that the renin-angiotensin system is present in a variety of tissues, including vascular walls,^{4),5)} and that locally generated angiotensin II might facilitate the adrenergic transmission by enhancing the norepinephrine release from nerve endings.¹⁵⁾ Garst et al¹⁶⁾ reported that the vascular renin level was elevated in SHR whereas plasma renin activity was not high; this state could produce greater angiotensin II formation in the blood vessel walls of SHR. It is now clearly established that captopril is an effective antihypertensive drug in SHR but not in normotensive WKY, although plasma renin activity is not different between the 2 groups.^{1),16),17)} Therefore, the efficacy of captopril in SHR may reflect an action of captopril on the enhanced renin-angiotensin system in the vascular beds. This action would decrease angiotensin II formation, a concomitant reduction of norepinephrine release and an inhibition of pressor responses.

Collis et al⁷⁾ reported that captopril antagonized the vasoconstrictor responses evoked by sympathetic nerve stimulation. Antonaccio et al¹⁸⁾ found that oral administration of captopril into conscious SHR caused significant reduction in the pressor responses to sympathetic nerve stimulation, but had no effect on the positive chronotropic responses evoked by exogenous angiotensin II or norepinephrine in the same animals. These suggest that captopril presynaptically inhibits norepinephrine release in SHR. This study with direct measurement of endogenous norepinephrine release observed that captopril markedly reduced norepinephrine release during the electrical nerve stimulation at both low (5 Hz) and high (15 Hz) frequencies in SHR.

These neurosuppressive effects of captopril may reflect a direct action on the sympathetic neurons. This possibility cannot be excluded. However, it has been reported that the blood captopril concentration peaks at an order of 10^{-6} mol/l when 50 mg of the drug is orally administered to essential hypertensive patients.¹⁹⁾ Further, our data showed that a low concentration of captopril (5.0×10^{-6} mol/l) significantly affected the norepinephrine release in

SHR but not in WKY (Table I). This similarity in effective doses suggests a similar basis for these effects, which may reflect differences in the vascular renin-angiotensin system between SHR and WKY.

Antonaccio and Kerwin²⁰ reported that the effects of captopril on blood pressure were not prominent in DOCA-salt hypertension with very low plasma and vascular renin activities. This finding also provides evidence that the hypotensive action of captopril depends upon its accessibility to the vascular renin-angiotensin system. In summary, the effects of captopril on norepinephrine release from the sympathetic nerve endings are consistent with the hypothesis that the antihypertensive action of captopril might be due to interference with the peripheral adrenergic neuro-effector mechanisms in the resistance vessels.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aids for Scientific Research (60570404, 60440049, 62304041) from the Ministry of Education, Science and Culture (Japan), and by the Nanki Scholarship Fund.

REFERENCES

1. Antonaccio MJ, Rubin B, Horowitz B: Effects of captopril in animal models of hypertension. *Clin Exp Hypertens* **2**: 613, 1980
2. Case DB, Atlas SA, Laragh JH, Sealey JE, Sullivan PA, McKinstry DN: Clinical experience with blockade of the renin-angiotensin-aldosterone system by an oral converting enzyme inhibitor (SQ 14 225, captopril) in hypertensive patients. *Progr Cardiovasc Dis* **21**: 195, 1978
3. Cushman DW, Ondetti MA, Cheung HS, Antonaccio MJ, Murthy VS, Rubin B: Inhibitors of angiotensin converting enzyme. *in* The Renin Angiotensin System, ed by Johnson JA, Anderson RA, Plenum Publishing Corporation, New York, p 199-225, 1980
4. Re R, Fallon JT, Dzau V, Quay SC, Harber E: Renin synthesis by canine aortic smooth muscle cells in culture. *Life Sci* **30**: 99, 1982
5. Ganten D, Schelling P, Vecsei P, Ganten U: Iso-renin of extrarenal origin: the tissue angiotensinogenase system. *Am J Med* **60**: 760, 1976
6. Schacht U: Effects of angiotensin II and ACE inhibitors on electrically stimulated nor-adrenaline release from superfused rat brain slices. *Clin Exp Hypertens* **A6**: 1847, 1984
7. Collis MG, Keddle JR: Captopril attenuates adrenergic vasoconstriction in rat mesenteric arteries by angiotensin—dependent and independent mechanisms. *Clin Sci* **61**: 281, 1981
8. Clough DP, Collis MG, Hatton R, Keddle JR: Effect of angiotensin converting enzyme inhibitor on neurogenic vasoconstriction in the rat. *Blood Vessels* **17**: 147, 1980
9. Castellucci A, Bacciarelli C, Boni P, Fedi M: The rat mesenteric artery-intestinal loop preparation. *Arzneim Forsch Drug Res* **31**: 54, 1981
10. Hjemdahl P, Daleskog M, Kahan T: Determination of plasma catecholamines by high performance liquid chromatography with electrochemical detector. *Life Sci* **25**: 131, 1979
11. Hallman H, Farnebo LO, Hamberger B, Jonsson G: A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. *Life Sci* **23**: 1049, 1978
12. Campbell WB, Jackson ED: Modulation of adrenergic transmission by angiotensins in the

- perfused rat mesentery. *Am J Physiol* **236**: H211, 1979
13. Murphy EA: Analysis of variance. *in* Biostatistics in Medicine, ed by Murphy EA, The Johns Hopkins University Press, p 137-174, 1982
 14. Tsuda K, Kuchii M, Kusuyama Y, Hano T, Nishio I, Masuyama Y: Neurotransmitter release and vascular reactivity in spontaneously hypertensive rats. *Jpn Circ J* **48**: 1263, 1984
 15. Toda N, Miyazaki M, Okamura T: Vascular neuroeffector function in two-kidney, one-clip hypertensive dogs. *J Hypertens* **3**: 503, 1985
 16. Garst J, Koletsky S, Wisenbaugh PE, Haddy M, Matthews D: Arterial wall renin and renal venous renin in the hypertensive rat. *Clin Sci* **56**: 41, 1979
 17. Asaad MM, Antonaccio MJ: Vascular wall renin in spontaneously hypertensive rats. *Hypertension* **4**: 487, 1980
 18. Antonaccio MJ, Kerwin L: Pre- and postjunctional inhibition of vascular sympathetic function by captopril in SHR: Implication of vascular angiotensin II in hypertension and antihypertensive actions of captopril. *Hypertension* **3** (suppl I): 54, 1981
 19. Shimada K, Tanaka M, Nambara T, Imai Y, Abe K, Yoshinaga K: Determination of captopril in human blood by high-performance liquid chromatography with electrochemical detection. *J Chromatogr* **227**: 445, 1982
 20. Antonaccio MJ, Kerwin L: Evidence for prejunctional inhibition of norepinephrine release by captopril in spontaneously hypertensive rats. *Eur J Pharmacol* **68**: 209, 1980