Effects of Captopril and Prostaglandin I₂ on Vasoconstrictor Responses to Norepinephrine and Potassium Ions in Rat Mesenteric Artery

Kazuoki KONDO, M.D., Tetsuji OKUNO, M.D., Hiromichi SUZUKI, M.D.,
Michicho HANDA, M.D., and Takao SARUTA, M.D.

SUMMARY

In the perfused rat mesenteric vascular bed, the effects of captopril and prostaglandin I₂ (PGI₂) on the vasoconstrictor responses to norepinephrine or potassium chloride were studied. Captopril or PGI₂ in the perfusate attenuated the vascular responses to norepinephrine in a dose-related manner, while these substances had no effect on the vascular contractions induced by potassium chloride. In preparations treated with indomethacin, the inhibitory effect of captopril on the vascular response to norepinephrine was similar to that found in the untreated preparations. These results suggest that, although the effect of captopril on the vascular reactivity is similar to that of PGI₂, the direct vascular action of captopril is not mediated by the synthesis of prostaglandins in the vascular bed.

Additional Indexing Words:

Indomethacin

CAPTOPRIL (SQ 14,225: D-3-mercapto-2-methylpropanoyl-L-proline) is an orally active inhibitor of angiotensin-converting enzyme and has potent antihypertensive effects in man and in animals.¹⁾⁻³⁾ The mechanism of the antihypertensive action of captopril was originally considered to be due to the prevention of angiotensin II formation and/or of degradation of bradykinin.⁴⁾⁻⁶⁾ Recent studies, however, have suggested that blockade of angiotensin conversion and reduction of bradykinin degradation can not completely account for the fall in blood pressure observed with captopril.^{7),8)} Recently we observed that, in the perfused rat mesenteric artery, captopril attenuated the vascular contraction induced by norepinephrine, suggesting that this substance has additional antihypertensive effects other than the inhibition of angiotensin-converting enzyme.⁹⁾ Using the same vascular bed, Dusting et al¹⁰⁾ postulated a possibility that hypotensive action of captopril might be at-

From the Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan.

Address for reprint: Takao Saruta, M.D., Department of Internal Medicine, School of Medicine, Keio University, 35, Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.

Received for publication August 22, 1980.

tributed to the increased synthesis of prostaglandin I_2 (PGI_2) in the vascular wall. PGI_2 has been considered a major product of prostaglandin synthesis in the vascular tissue, and it has been suggested that this prostaglandin modulates the vascular reactivity to vasoactive substances.¹¹⁾ In this study, therefore, we examined the effects of captopril on the vascular responses to norepinephrine and potassium ions, and compared its effects with those of PGI_2 . Furthermore, using the preparation treated with indomethacin, we tried to elucidate whether the vascular action of captopril is mediated by the synthesis of prostaglandins in the vascular wall.

METHODS

Male rats of the Wistar strain, weighing 250–300 Gm were used. The isolated superior mesenteric vascular bed was prepared as described by MacGregor.¹²⁾ The superior mesenteric artery was cannulated under ether anesthesia and the vascular bed was dissected out and was perfused by a peristaltic flow inducer (Master flex pump, Cole-Parmer) with the Krebs bicarbonate buffer (150 mM sodium, 4.3 mM potassium, 1 mM magnesium, 2.5 mM calcium, 1.7 mM phosphate, 25 mM bicarbonate, remaining anion chloride, and 11.1 mM glucose) at 37°C bubbled with 5% carbon dioxide in oxygen. The pH of the solution was 7.4. The perfusion pressure was recorded via a side arm of the arterial cannula by Nihon Koden MPU-0.5 transducer, the flow rate being adjusted to give a steady perfusion pressure of 25–35 mmHg. The actual flow rate was around 4 ml/min, which is not far from the expected superior mesenteric artery flow rate in an adult rat. The flow rate was constant during each experiment. We confirmed in our previous study¹³⁾ that, under the conditions described here, the preparation remained remarkably stable with no significant changes in baseline pressure for at least 2 h.

When injected into the arterial cannula, norepinephrine (L-arterenol bitartrate, Sigma) or potassium chloride produced dose-related rises in pressure. The maximum pressure rise which could be achieved by these substances was usually in the region of 80–100 mmHg. For each substance we determined a dose which gave an approximately 50% maximal response and used that in all subsequent experiments. The doses actually used were 20 ng of norepinephrine and 2 mg of potassium chloride; each substance was dissolved in 0.05 ml of buffer and injected at 5 min intervals into the cannula. Each injection produced a transient rise in pressure, which returned to base line within about 1 min. Once 3 pressor responses of constant amplitude ($\pm 5\%$) to injected norepinephrine or to potassium chloride had been obtained, the experiments were started, results being expressed as the percentages of this mean initial response. The vasoconstrictor responses to norepinephrine or to potassium ions remained constant ($\pm 10\%$) for at least 2 h, when no drugs were added to the perfusate.

(a) Effects of captopril and PGI_2 on the responses to either norepinephrine or potassium chloride

Norepinephrine and potassium were tested on different preparations. Progressively increasing concentrations of captopril (Squibb; 1–81 $\mu\text{g/ml}$) or PGI_2 (Hoechst; 0.1–100 ng/ml) were added to the perfusate. For each concentration,

perfusion was continued for 12 min, at the end of which the amplitude of the pressor response to norepinephrine or to potassium ions was determined. In preliminary experiments we observed that the effects of captopril and PGI₂ were rapid, and that they reached a plateau within 7 min. Thereafter the effect of captopril remained unchanged ($\pm 10\%$) and that of PGI₂ began to fade at about 17 min, probably indicating degradation of this substance. Therefore we examined the effects of these substances on the vascular contractions 12 min after adding these drugs in the perfusate.

(b) Effect of captopril on the response to norepinephrine in preparations treated with indomethacin

Indomethacin (Nippon Merck-Banyu), in a concentration of 10 $\mu\text{g/ml}$ was added to the perfusate. After the steady response to norepinephrine had been obtained (which was taken as 100%), captopril was added to the perfusate in progressively increasing concentrations (1–81 $\mu\text{g/ml}$) and its effect on the response to norepinephrine was noted.

(c) Effect of captopril on the response to norepinephrine in preparations treated with phentolamine

Phentolamine (Ciba) in a concentration of 15 ng/ml was added to the perfusate. After the steady response to norepinephrine had been obtained (which was taken as 100%), captopril was added to the perfusate in progressively increasing concentrations (1–81 $\mu\text{g/ml}$) and its effect on the response to norepinephrine was

Fig. 1. The effects of captopril on vascular responses to norepinephrine (open circles) or to potassium chloride (closed circles). The pressor responses to norepinephrine or to potassium chloride are expressed as a percentage of the mean amplitude of 3 control responses obtained prior to adding captopril in the perfusate. The bars indicate SD.

noted.

Six experiments were performed in each study. Results were expressed as mean $\pm$ SD. Comparisons were made by Student's *t*-test.

RESULTS

Captopril or PGI₂ in the perfusate did not change the basal pressure, but these substances inhibited the vascular response to norepinephrine in a dose-related manner (Figs. 1 and 2). Three μ g/ml of captopril or 0.1 ng/ml of PGI₂ significantly attenuated the norepinephrine response ($p < 0.01$). Both captopril and PGI₂, however, failed to alter the vascular contractions induced by potassium ions. Even in the presence of 81 μ g/ml of captopril or 100 ng/ml of PGI₂, potassium chloride produced contractions which were not significantly different from the control responses ($p > 0.05$).

Indomethacin by itself, when added to the perfusate in a concentration of 10 μ g/ml, attenuated the vascular response to norepinephrine to $52 \pm 10\%$ of the control response. In preparations treated with indomethacin, the effect of captopril on the norepinephrine response was indistinguishable from that found in the untreated preparations (Fig. 3).

Fig. 2. The effects of PGI₂ on vascular responses to norepinephrine (open circles) or to potassium chloride (closed circles). The pressor responses to norepinephrine or to potassium chloride are expressed as a percentage of the mean amplitude of 3 control responses obtained prior to adding PGI₂ in the perfusate. The bars indicate SD.

Fig. 3. The effect of captopril in the perfusate on the vascular response to norepinephrine in preparations perfused with buffer (open circles), with buffer containing 10 μ g/ml of indomethacin (open triangles) and with buffer containing 15 μ g/ml of phentolamine (closed triangles). The pressor responses to norepinephrine are expressed as a percentage of the mean amplitude of 3 control responses obtained prior to adding captopril in the perfusate. The bars indicate SD.

Phentolamine, when added to the perfusate in a concentration of 15 ng/ml, attenuated the norepinephrine response to $48 \pm 8\%$ of the control response. In preparations treated with phentolamine, the inhibitory effect of captopril on the norepinephrine response was quite similar to that found in the untreated preparations (Fig. 3).

DISCUSSION

This study confirmed our previous results⁹⁾ that, in the perfused rat mesenteric artery, captopril attenuated the vascular contraction induced by norepinephrine. In the same vascular bed, we observed that PGI₂, major product of prostaglandin synthesis in vascular tissue, similarly attenuated the norepinephrine response. On the contrary to their effects on the norepinephrine-induced contractions, neither PGI₂ nor captopril affected the vascular contraction induced by potassium chloride. Although the effects of PGI₂ and captopril on the responses to other vasoactive substances were not ex-

amined in this study, our results showed that the effects of captopril on the vascular reactivity to norepinephrine and to potassium ions are, at least in the rat mesenteric artery, similar to those of PGI_2 .

The mechanism of antihypertensive action of captopril was originally felt to be solely due to a decrease in angiotensin II formation.⁴⁾ However, captopril has been reported to be also effective on the patients with low plasma renin activity,^{2),14)} anephric patients,¹⁵⁾ and deoxycorticosterone-induced hypertensive rats,¹⁶⁾ indicating that this substance lowers blood pressure by an action additional to angiotensin blockade. Since angiotensin-converting enzyme is identical to the enzyme (kininase) that inactivates bradykinin, bradykinin has been proposed to be responsible for the hypotensive response to captopril.^{5),6)} However, inferential data by de Freitas et al¹⁷⁾ and by Streeten et al¹⁸⁾ were against a significant role for bradykinin. Swartz et al⁸⁾ demonstrated recently that the increments in bradykinin induced by captopril were small, not dose-related and did not correlate with changes in blood pressure. These results suggest a possibility that captopril possesses some additional, still undefined pharmacological actions to reduce blood pressure.

The search for other mediators of the vascular response to captopril has recently focused on the prostaglandins, since pretreatment with indomethacin, a prostaglandin synthesis inhibitor, significantly diminishes depressor response to captopril.^{19),20)} Although it is still unclear whether captopril's effect on prostaglandin production is direct or secondary to a change induced in the renin-angiotensin system or the kinin system, Swartz et al⁸⁾ reported that captopril significantly increased the plasma levels of PGE_2 . Mullane et al²¹⁾ also reported that captopril enhanced release of PGI_2 -like substance by the kidney. Dusting et al¹⁰⁾ postulated a possibility that hypotensive action of captopril might be attributed to the increased synthesis of PGI_2 in the vascular wall. These results, as well as the similarity of the effects of captopril to those of PGI_2 in the rat mesenteric artery, seem to indicate a possibility that the direct vascular effects of captopril might be mediated by the synthesis of prostaglandins, especially that of PGI_2 , in the vascular wall. In this experiment, however, the effects of captopril in preparations treated with $10\text{ }\mu\text{g/ml}$ of indomethacin were indistinguishable from its effects found in untreated preparations. Although it is still controversial whether this concentration of indomethacin completely inhibits the synthesis of prostaglandins in the vascular tissue,²²⁾ Malik et al²³⁾ reported in the same rat mesenteric artery that $5\text{ }\mu\text{g/ml}$ of indomethacin was enough to inhibit the endogenous synthesis of prostaglandins. Our results therefore indicate that the effects of captopril were not dependent on the synthesis of prostaglandins in the vascular wall.

From the present study, the mechanism by which captopril attenuates

the norepinephrine response is unclear. The effects of captopril were unaltered by phentolamine, a specific alpha-blocker, suggesting that the action of captopril is different from that of an alpha-blocker. In the rat mesenteric artery we used in this experiment, the vasoconstrictor action of potassium ions is immediately abolished in a calcium-free medium, whereas in such a medium norepinephrine still produces contractions which are 70–80% maximal.^{22), 24)} This suggests that potassium acts primarily to promote entry of calcium from extracellular fluid, whereas norepinephrine primarily releases calcium from intracellular stores. Manku et al²⁵⁾ has postulated the hypothesis that PGI₂ modulates the intracellular calcium release in the vascular tissue. It could thus be possible that captopril inhibits intracellular calcium release, thereby interfering with norepinephrine-induced contraction. Further studies on the direct vascular effects of captopril are necessary to elucidate the mechanism of its action.

Our previous study⁹⁾ revealed that teprotide (SQ 20,881), another converting enzyme inhibitor, failed to alter the vascular response to norepinephrine, suggesting that the inhibitory effect of captopril on the norepinephrine response is not dependent on the inhibition of angiotensin-converting enzyme. Larochelle et al²⁶⁾ and Waeber et al²⁷⁾ reported recently that, when measured 12 h after the administration of captopril to the hypertensive patients, no reduction in the angiotensin-converting enzyme activity was observed, even though the blood pressure remained reduced. Kokubu et al²⁸⁾ also reported that, on the contrary to the expectation, angiotensin-converting enzyme activities in the rat plasma, lung and kidney were elevated significantly on the 30th days of captopril administration. These results indicate that, although blockade of angiotensin II formation and reduction of plasma kinin degradation are important factors contributing to the hypotensive response to captopril, the another possibility, such as inhibiting the norepinephrine response as observed in our study, should also be considered when this substance is used in the clinical and experimental studies.

REFERENCES

1. Ondetti MA, Rubin B, Cushman DW: Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* **196**: 441, 1977
2. Gavras H, Brunner HR, Turini GA, Kershaw GR, Tift CP, Cuttlog S, Gavras I, Vukovich RA, Mckinstry DN: Antihypertensive effect of the oral angiotensin converting-enzyme inhibitor SQ 14225 in man. *New Engl J Med* **298**: 991, 1978
3. Rubin B, Antanaccio MJ, Horovitz ZP: Captopril (SQ 14,225) (D-3-mercapto-2-methylpropanoyl-L-proline). A novel orally active inhibitor of angiotensin-converting enzyme and antihypertensive agent. *Prog Cardiovasc Dis* **21**: 183, 1978
4. 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. *Prog Cardiovasc Dis* **21**: 195, 1978
5. Murthy VS, Waldron TL, Goldberg ME: The mechanism of bradykinin potentiation after inhibition of angiotensin converting enzyme by SQ 14, 225 in conscious rabbits. *Circulat Res* **43** (Suppl I): I-40 1978
 6. Thurston H, Swales JD: Converting enzyme inhibitor and saralasin infusion in rats. *Circulat Res* **42**: 588, 1978
 7. Vollmer RR, Boccagno JA, Harris DN, Murthy VS: Hypotension induced by inhibitor of angiotensin-converting enzyme in pentobarbital-anesthetized dogs. *Eur J Pharmacol* **51**: 39, 1978
 8. Swartz SL, Williams GH, Hollenberg NK, Levine L, Dluhy RG, Moore TJ: Captopril-induced changes in prostaglandin production. *J Clin Invest* **65**: 1257, 1980
 9. Okuno T, Kondo K, Konishi K, Saruta T, Kato E: SQ 14,225 attenuates the vascular response to norepinephrine in the rat mesenteric arteries. *Life Sci* **25**: 1343, 1979
 10. Dusting GJ, Mullins M, Doyle AE: Angiotensin-induced prostacyclin release may contribute to the hypotensive action of converting enzyme inhibitors. *Adv Prostaglandin Thromboxane Res* **7**: 815, 1980
 11. Armstrong JM, Dusting GJ, Moncada S, Vane JR: Cardiovascular action of prostacyclin (PGI_2), a metabolite of arachidonic acid which is synthesized by blood vessels. *Circulat Res* **43** (Suppl I): I-112, 1978
 12. McGregor DD: The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J Physiol (London)* **177**: 21, 1965
 13. Kondo K, Okuno T, Konishi K, Saruta T, Kato E: Effects of potassium and ouabain on the vascular reactivity to norepinephrine in the rat mesenteric artery. *Can J Physiol Pharmacol* **57**: 908, 1979
 14. Cody RJ, Tarazi RC, Bravo EL, Fouad FM: Haemodynamics of orally-active converting enzyme inhibitor (SQ 14225) in hypertensive patients. *Clin Sci Mol Med* **55**: 453, 1978
 15. Veld AJM, Schicht IM, Derckx FHM, DeBruyn JHB, Schalekamp MADH: Effects of an angiotensin-converting enzyme inhibitor (captopril) on blood pressure in anephric subjects. *Brit Med J* **280**: 288, 1980
 16. Marks ES, Bing RF, Thurston H, Swales JD: Vasodepressor property of the converting enzyme inhibitor captopril (SQ 14,225): the role of factors other than renin-angiotensin blockade in the rat. *Clin Sci* **58**: 1, 1980
 17. DeFreitas FM, Faraco EZ, DeAzevedo DF: General circulatory alterations induced by intravenous infusion of synthetic bradykinin in man. *Circulation* **29**: 66, 1964
 18. Streeten DHP, Kerr LP, Kerr CB, Prior JC, Dalakos TG: Hyperbradykininism. A new orthostatic syndrome. *Lancet* **2**: 1048, 1972
 19. Crantz FR, Swartz SL, Hollenberg NK, Moore TJ, Dluhy RG, Levine L, Williams GH: Role of prostaglandins in the hypotensive response to captopril in essential hypertension. *Clin Res* **27**: 529A, 1979
 20. Abe K, Itoh T, Imai Y, Sato M, Haruyama T, Sakurai Y, Goto T, Otsuka Y, Yoshinaga K: Implication of endogenous prostaglandin system in the antihypertensive effect of captopril SQ 14, 225, in low renin hypertension. *Jpn Circulat J* **44**: 422, 1980
 21. Mullane KM, Moncada S, Vane JR: Prostacyclin release induced by bradykinin may contribute to the antihypertensive action of angiotensin-converting enzyme inhibitors. *Adv Prostaglandin Thromboxane Res* **7**: 1159, 1980
 22. Manku MS, Horrobin DF: Indomethacin inhibits responses to all vasoconstrictors in the rat mesenteric vascular bed: restoration of responses by prostaglandin E_2 : Prostaglandins **12**: 369, 1976
 23. Malik KU, Ryan P, McGiff JC: Modification by prostaglandins E_2 and E_3 , indomethacin and arachidonic acid of the vasoconstrictor responses of the isolated perfused rabbit and rat mesenteric arteries to adrenergic stimuli. *Circulat Res* **39**: 163, 1976
 24. Kondo K, Manku MS, Horrobin DF, Boucher R, Genest J: Potentiation of pressor effects

- of noradrenaline and potassium ions in the rat mesenteric arteries by physiological concentrations of angiotensin II. *Clin Sci Mol Med* **53**: 233, 1977
25. Manku MS, Horrobin DF, Cunnane SC, Ally AI, Karmazyn M, Morgan RO, Nicolaou KC, Barnette WE: Prostaglandins E₁, E₂ and I₂: evidence for three distinct actions in vascular smooth muscle. *Biochem Biophys Res Commun* **83**: 295, 1978
 26. Larochelle P, Genest J, Kuchel O, Boucher R, Gutkowska Y, McKinstry D: Effect of captopril (SQ 14,225) on blood pressure, plasma renin activity and angiotensin I converting enzyme activity. *Can Med Assoc J* **121**: 309, 1979
 27. Waeber B, Brunner HR, Brunner DB, Curtet A, Turini GA, Gavras H: Discrepancy between antihypertensive effect and angiotensin converting enzyme inhibition by captopril. *Hypertension* **2**: 236, 1980
 28. Kokubu T, Ueda E, Ono M, Kawabe T, Hayashi Y, Kan T: Effects of captopril (SQ 14,225) on the renin-angiotensin-aldosterone system in normal rats. *Eur J Pharmacol* **62**: 269, 1980