Pulmonary Pharmacology (1992) **5**, 103–109

PULMONARY PHARMACOLOGY

Rapid Reversal of Angiotensin Converting Enzyme Inhibition by Lisinopril in the Perfused Rabbit Lung

S. Rimar*†‡, C. N. Gillis†§

*Departments of *Anesthesiology, †Pediatrics and §Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA*

SUMMARY: Lisinopril is a potent competitive inhibitor of purified rabbit lung ACE (dissociation $t_{1/2} = 105$ min). To examine reversibility of binding and ACE functional activity in situ, the single-pass extraction (*E*) of an ^{125}I-lisinopril analogue (351A) and the hydrolysis of an ACE substrate, benz-phe-ala-pro (BPAP) were studied. Lungs were perfused at 50 ml/min with a Krebs–albumin (3%) solution. A bolus containing [^{14}C]dextran, [^{3}H]BPAP, and 351A was injected and (*E*)351A measured by multiple indicator dilution technique. BPAP metabolism (*M*) was reflected by the appearance of its hydrolysis product [^{3}H]benz-phe in lung effluent. Control (*E*)351A was 66 ± 5% (mean ± SD, n = 6) and (*M*)BPAP was 69 ± 9% (n = 6). Unlabeled lisinopril (30 nmol) in the bolus significantly reduced *E*(351A) and *M*(BPAP) to 16 ± 16% and 3 ± 3%, respectively. Ten minutes later *E*(351A) and *M*(BPAP) had returned to control values. Reduction of *E*(351A) was partially reversible and *M*(BPAP) completely reversible after 1 min. After recirculation with 0.25 mM lisinopril for 30 min, however, significant depression of *E*(351A) was evident for 60 min after exposure to lisinopril was discontinued. Thus, rapid as well as slowly reversible components of inhibition of ACE inhibitor binding can be demonstrated in the perfused rabbit lung.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors are used extensively in the treatment of hypertension and congestive heart failure. While they have been shown to reduce plasma angiotensin II levels and decrease peripheral vascular resistance, the duration of blood pressure reduction correlates better with inhibition of tissue rather than plasma ACE activity.[1–4] The in vivo disappearance of these drugs from blood reveals biphasic kinetics with a long terminal phase thought to be related to persistent binding to tissue rather than circulating ACE.[5,6] These findings suggest that binding of ACE inhibitors to vascular ACE and their effect on local ACE activity are important factors in determining the clinical response to ACE inhibitor therapy.

Determination of the kinetics of inhibition of vascular (endothelial) ACE hydrolytic activity in vivo is complicated by the presence of serum ACE, the variable flow rate of blood (and hence delivery of ACE substrate), vascular surface area, and the lack of knowledge about the precise concentration of enzyme and inhibitor.[7] It is possible, however, to compare the in vitro half-times of dissociation with the duration of inhibitor binding and activity in perfused organs. Lisinopril, a clinically available ACE inhibitor, binds very tightly to ACE with an in vitro dissociation half-time of 105 min.[8] It was therefore of interest to determine the kinetics of ACE inhibition by lisinopril in an intact vascular preparation, free of plasma ACE, and to study the reversibility of lisinopril binding. For this purpose we studied binding of an ^{125}I-lisinopril analogue (351A) to perfused lung and its simultaneous effect on activity as reflected by hydrolysis of benz-phe-ala-pro (BPAP), a synthetic ACE substrate.[7,9]

METHODS

Isolated perfused lung, in situ

New Zealand white rabbits (2–3 kg) were given heparin (300 U/kg) intravenously and anesthetized with allobarbital (400 mg/kg) and urethane (100 mg/kg). The thoracic cavity was exposed via a median sternotomy and the trachea, pulmonary artery and left atrium were cannulated. The lungs were statically inflated to a pressure equal to or slightly below that in the left atrium, and pulmonary artery, left atrial and airway pressures were continuously monitored. Lungs

‡ For correspondence.

were perfused in a non-recirculating manner via the pulmonary artery at 50 ml/min with a Krebs bicarbonate solution (37°C) aerated with 95% O_2/5% CO_2 and containing 3% bovine serum albumin as previously described.[10,11] After each experiment, the lungs were dried for at least 72 h and wet/dry weight ratios calculated and compared with those of unperfused rabbit lungs. Those which had significantly increased wet/dry weight ratios or which showed a spontaneous rise in pulmonary artery pressure were excluded from further consideration.

Indicator dilution techniques

Single-pass uptake of the ^{125}I-lisinopril analogue (351A) and hydrolysis of BPAP were determined using [^{14}C]dextran as the reference indicator as described previously.[10,11] Briefly, a radioisotope mixture [containing 0.07 μCi of [^{125}I]351A (specific activity 2200 Ci/nmol), 0.75 μCi of [^{3}H]benz-phe-ala-pro (BPAP) (specific activity 20 Ci/nmol) and 0.3 μCi of [^{14}C]dextran (specific activity 0.8 μCi/g) in 0.3 ml of 0.9% NaCl] was injected as a bolus into the pulmonary artery (control). Simultaneously the left atrial outflow was collected in tubes each containing 2 ml of 0.5 nM captopril, in a fraction collector at a rate of 1 tube/s. An aliquot of each sample was assayed for total ^{125}I-radioactivity (Gamma 4000 counter, Beckman Industries, Fullerton, CA, USA) and for total ^{3}H and ^{14}C radioactivity with liquid scintillation spectrometry (Tricarb 4550, Packard Industries, Downer's Grove, IL, USA). A second aliquot was acidified with 1 N HCl, extracted with an equal volume of toluene,[9,12] and radioactivity associated with [^{3}H]BPhe, the metabolite of BPAP, was measured. Total radioactivity in each injection was determined by placing aliquots of the injectate directly into tubes in the fraction collector before radioactive lung effluent appeared. Calibrated efficiencies were used to convert counts per minute (cpm) to disintegrations per minute (dpm). The percentage of metabolism of [^{3}H]BPAP to [^{3}H]BPhe was determined as previously reported.[9]

Instantaneous outflow, concentration, extraction, and metabolism versus time curves were plotted for each experiment as previously described.[10,11] Fractional concentrations of the respective isotopes ($FC_{isotope}$) were calculated by normalizing individual isotope concentrations to a measured amount of isotope injected [FC = (dpm/ml in each effluent sample)/total dpm injected]. Instantaneous extraction (E) of [^{125}I]351A was calculated as:

$$E\,(351A) = [(FC_{dex} - FC_{351A})/FC_{dex}] \times 100\%$$

where FC_{dex} is the fractional concentration of the intravascular marker [^{14}C]dextran, and FC_{351A} is the fractional concentration of [^{125}I]351A.

Instantaneous metabolism (M) of BPAP was calculated as:

$$M(BPAP) = \{[^{3}H]BPhe/([^{3}H]BPhe + [^{3}H]BPAP)\} \times 100\%$$

where [^{3}H]BPAP and [^{3}H]BPhe are concentrations of unchanged BPAP and BPhe, respectively. Measurements of E(351A) and M(BPAP) are taken at the peak of the [^{14}C]dextran outflow curve.

Effect of single-pass injection of lisinopril on 351A extraction and BPAP metabolism

The initial determination of E(351A) and M(BPAP) was made after perfusing the lungs for 10 min or until the outflow appeared blood-free. Subsequent measurements were made in five lungs in the presence of added lisinopril or non-radioactive 351A (3–30 nmol). The interval between injections was 10 min and a final control determination was made at the end of each experiment. This protocol was repeated in five additional lungs with 1 min intervals between injections.

Effect of recirculation of lisinopril on duration of inhibition of 351A extraction and BPAP metabolism

An initial determination of E(351A) and M(BPAP) was made after perfusing the lungs for 10 min. In order to determine the effect of a longer duration of lisinopril exposure to pulmonary ACE, the lungs were then perfused in a recirculating manner with 0.25 mM lisinopril added to the Krebs–albumin solution for 30 min at which time E(351A) and M(BPAP) were measured. The appropriate concentration and duration of lisinopril circulation was determined in preliminary experiments to be the minimal concentration which inhibited E(351A) and M(BPAP). The perfusate was then changed to a non-recirculating lisinopril-free Krebs–albumin solution and E(351A) and M(BPAP) measured at 1, 5, 10, and 60 min after the recirculation as described above.

Effect of albumin and hypothermia on reversible inhibition of E(351A) and M(BPAP)

In order to examine the potential role of albumin binding in the reversal of lisinopril inhibition of E(351A) and M(BPAP), measurements were made in five separate lungs with 3% dextran (to maintain the same osmotic pressure) instead of albumin in the perfusate. Similarly, determinations of E(351A) and M(BPAP) were made at 37°C and 4°C in the same lungs to examine the effects of hypothermia on the reversibility of inhibition.

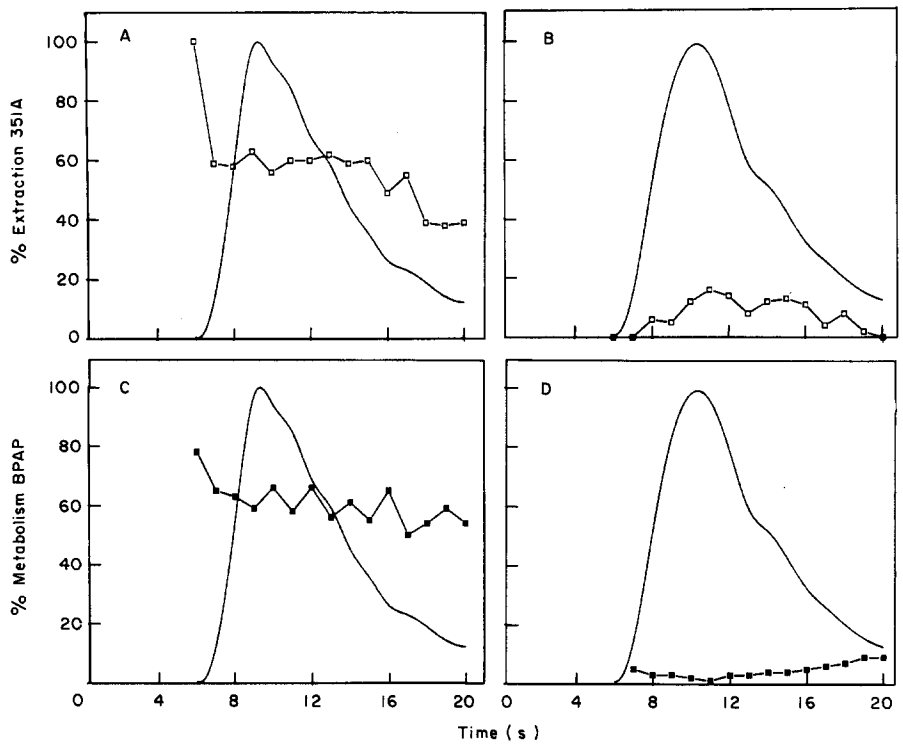

Fig. 1 Instantaneous extraction and metabolism curves from a representative experiment are shown for 351A and BPAP with reference outflow curves for comparison. Fractional concentrations of reference outflow curves are in arbitrary units. A and B, Extraction of 351A alone and in the presence of lisinopril. C and D, Metabolism of BPAP alone and in the presence of lisinopril. (A) and (C) are controls.

Statistical analysis

All data are presented as means ± SE. The effects of different concentrations of lisinopril, non-radioactive 351A, hypothermia and dextran versus albumin were determined by analysis of variance with the Newman-Keuls tests for multiple comparisons.[13] Differences are considered significant at $P < 0.01$.

RESULTS

Effect of single-pass injection of lisinopril

Figure 1 shows indicator dilution curves depicting instantaneous extraction-time curves for 351A and metabolism-time curves for BPAP in a typical experiment. At the peak of the outflow curve E(351A) was 63% and M(BPAP) was 59%. An injection containing 30 nmol lisinopril in the same experiment produced an outflow curve of similar shape but significant inhibition of [^{125}I]351A uptake and BPAP metabolism occurred. In fact, E(351A) and M(BPAP) were reduced to 12% and 2%, respectively.

Figure 2 summarizes the effect of increasing doses of lisinopril on E(351A) and M(BPAP). There was a dose-dependent decrease in E(351A) and M(BPAP) which was completely reversed in 10 min. Figure 3 shows a similar decrease in E(351A) and M(BPAP) with lisinopril but 1 min after the high-dose lisinopril injection, E(351A) and M(BPAP) were not different from control. When an additional lisinopril injection again reduced E and M significantly, 1 min later, E(351A) remained significantly reduced compared to controls but M(BPAP) did not.

Effect of recirculation of lisinopril

In order to compare the duration of E(351A) and M(BPAP) inhibition after a single-pass injection of lisinopril with that following a longer period of lisinopril exposure, E and M were determined before and after a 30-min recirculation of lisinopril. The results are summarized in Figure 4. Marked inhibition of E and M occurred in the presence of lisinopril but significant depression of E(351A) was still evident 60 min after the lisinopril infusion was discontinued. M(BPAP), however, returned to control values.

Effect of albumin and hypothermia on lisinopril reversibility

Neither the use of dextran instead of albumin nor hypothermia (4°C) significantly changed the ability of lisinopril to inhibit 351A uptake or BPAP metabolism, nor reversal of this effect 10 min later (data not shown). To determine whether non-iodinated 351A exhibited different effects on E(351A) and M(BPAP) than lisinopril, separate experiments were performed which showed that inhibition of E and M by non-

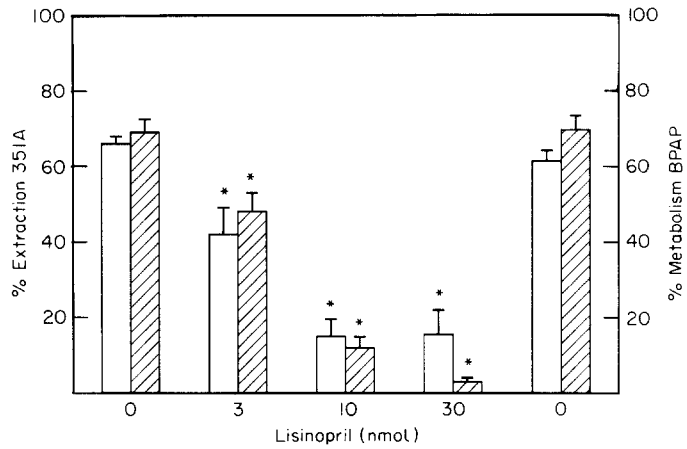

Fig. 2 Extraction of 351A (□) and metabolism of BPAP (▨) in the presence of increasing doses of lisinopril, co-injected at 10 min intervals. Bars indicate SE (n = 5); (*) $P < 0.01$.

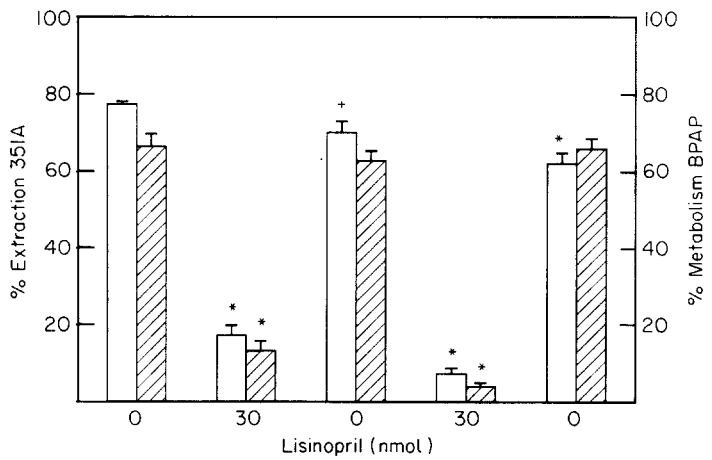

Fig. 3 Extraction of 351A (□) and metabolism of BPAP (▨) in the presence of increasing doses of lisinopril, co-injected at 1 min intervals. Bars indicate SE (n = 5); (†) $P < 0.05$; (*) $P < 0.01$.

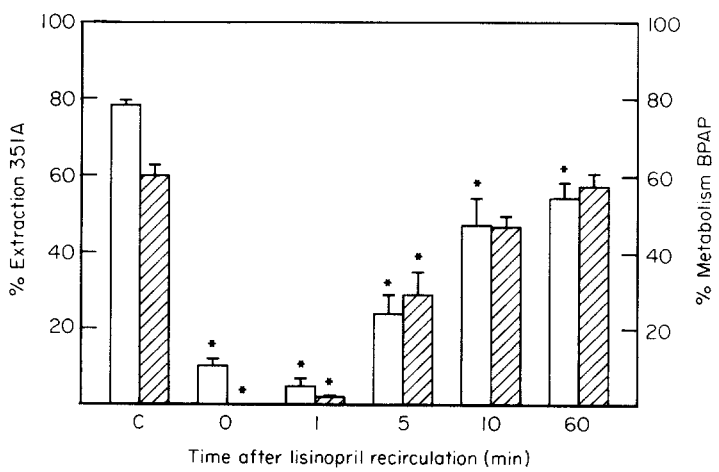

Fig. 4 Effect of a 30-min recirculation with lisinopril on extraction of 351A (□) and metabolism of BPAP (▨). Bars indicate SE (n = 5); (*) $P < 0.01$.

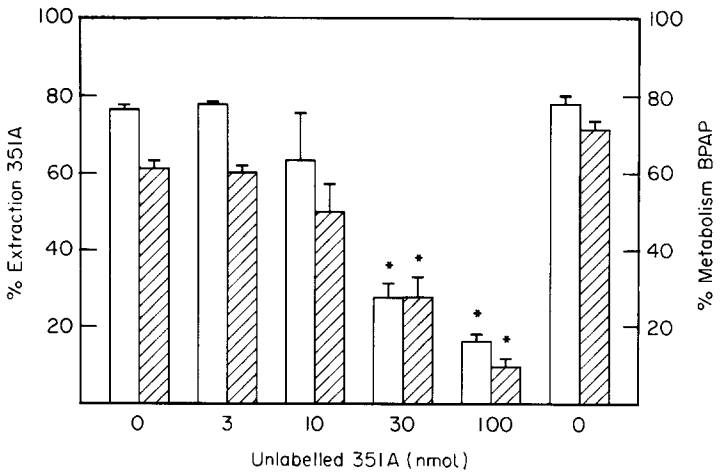

Fig. 5 Extraction of 351A (□) and metabolism of BPAP (▨) in the presence of increasing doses of non-radiolabeled 351A at 10 min intervals. Bars indicate SE (n = 5); (*) $P < 0.01$.

radioactive iodo-351A was similar to that of lisinopril with reversibility of effects seen after 10 min (Fig. 5).

There is a difference in control values of E(351A) between the first series of experiments (Fig. 2) and subsequent series (Figs 3–5). This difference most likely reflects changes in the purification procedure of 351A by the manufacturer (M. Hichens, personal communication). Control M(BPAP) remained consistent in all the experiments.

DISCUSSION

Lisinopril has been described as a slow, tight binding inhibitor on the basis of its biochemical behavior in vitro.[8] The rate constant for dissociation of the ACE-lisinopril complex using purified rabbit ACE in vitro corresponds to an average residence time of 165 min or a half-life of 105 min.[8] This study examined the reversibility of lisinopril binding and its effect on ACE functional activity in situ to gain insight into the dissociation kinetics of lisinopril in the perfused lung.

A single-pass injection of lisinopril which caused significant inhibition of E(351A) and M(BPAP) when co-injected, exerted no effect on E or M 10 min later. In fact, 1 min after the lisinopril injection M(BPAP) was completely unaffected and E(351A) nearly returned to control values. This rapid reversibility of lisinopril's inhibitory effect occurred despite multiple injections with increasing doses (Figs 2 and 3). When lisinopril was recirculated in the lung for 30 min, however, significant inhibition of E(351A) occurred 60 min after the recirculation was discontinued (Fig. 4).

The rapid reversibility of lisinopril in the intact rabbit lung suggests a mechanism of ACE inhibition and binding that occurs rapidly and is less sustained than the in vitro dissociation half-life of 105 min predicts. In order to obtain sustained inhibition of lisinopril binding in the lung [as measured by E(351A)] a much longer period of exposure or residence time was required.

Two mechanisms have been proposed for the formation and dissociation of the enzyme (ACE)-inhibitor complex (E–I), based on the use of purified rabbit ACE:[8,14]

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EI$$

Mechanism A

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EI \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EI^*$$

Mechanism B

Mechanism A postulates a slow, single-step formation of a tightly bound E–I complex ($k_1 >>> k_{-1}$). Mechanism B proposes that two E–I complexes exist, one rapidly formed and loosely bound ($k_1 > k_{-1}$) and the second slowly formed and very tightly bound ($k_2 >>> k_{-2}$).

Both of these mechanisms provide a satisfactory description of lisinopril binding in vitro.[8] Other ACE inhibitors, however, demonstrate more clearly a two-step mechanism of binding and dissociation from the enzyme (mechanism B).[14] Ryan has shown that the degree of ACE inhibition is enhanced as exposure time of inhibitor to purified ACE increases.[15] This suggests a progression of an initial enzyme–inhibitor complex into a more stable tightly bound form. Similarly our data in the intact lung suggests the presence of both a rapidly formed, rapidly reversible E–I complex and a more slowly formed and slowly reversible E–I complex.

The interaction of albumin with drugs, hormones and endothelial cells is well described.[16,17] In order to

assess the role of albumin in the rapid reversibility of the lisinopril effect, additional experiments were performed using dextrose instead of albumin in the Krebs perfusion medium. The reversibility of lisinopril was unchanged.

Several investigators have demonstrated a difference between the acute time course of plasma ACE inhibition and the hemodynamic effects of the drug suggesting that ACE inhibitors exert their principal action at local tissue sites rather than on circulating plasma ACE.[1,2,3] By examining the time course and degree of inhibition of ACE using 351A binding in rat tissues, Jackson et al found that a discrepancy between plasma, lung and aortic ACE also exists.[6] If ACE is located primarily on the surface of the vascular endothelium[18,19] the differences in time course of 351A displacement were surprising given that ACE in these organs should be in continuous contact with the circulating drug. Their interpretation was that tissue penetration of the ACE inhibitor is a major determinant of the drug effect on ACE.

The presence of two types of kinetic mechanisms may also explain this discrepancy between clinical onset and tissue binding. Perhaps the formation of rapidly formed, rapidly reversible E–I complexes plays a role in the early onset of the hypotensive effect of the ACE inhibitor and the sustained binding demonstrated by tissue binding studies is dependent on formation of more tightly bound E–I complexes as contact time with the enzyme increases. As the drug is cleared from the circulation only the tightly bound tissue ACE-inhibitor complexes persist.

Recent studies suggest an extra-endothelial location of ACE as well. Several investigators have shown in isolated vascular preparations that formation of angiotensin II and blockade of its vasoconstrictive properties by converting enzyme inhibition occurs in the vessel wall in the absence of endothelium.[20–22] It is possible that a rapidly reversible E–I complex occurs on the endothelial surface while the slowly formed, tightly bound complex exists on the non-endothelial arterial surface. Tissue penetration as well as binding kinetics might then play a role in the effect of ACE inhibitors on blood pressure. It is interesting to speculate that endothelial and non-endothelial ACE may themselves have different kinetic properties.

The mechanism responsible for the antihypertensive effects of ACE inhibitors is not completely known. Inhibition of bradykinin metabolism,[23] production of prostaglandins,[24] and reduction in catecholamine release,[25] have all been proposed as alternative mechanisms to inhibition of angiotensin II production. It is interesting to note that in our experiments, inhibition of metabolism of the ACE substrate (BPAP) was unaffected 1 h after recirculation with lisinopril, although evidence of lisinopril binding [reduced *E*(351A)] remained.

In summary, a rapidly reversible inhibition of ACE inhibitor binding and ACE activity as well as a slowly reversible effect in the intact rabbit lung has been demonstrated. This rapidly reversible effect is independent of temperature and exists in the absence of albumin.

Acknowledgements

The authors wish to thank Martin Hichens, Ph.D., Merck, Sharpe & Dohme Research Laboratories for generous supplies of 351A; Marilyn Merker, Ph.D. and Mary Beth Clark for their advice and technical assistance; and Donna Madigan for help in preparation of the manuscript. This work was supported in part by National Heart, Lung and Blood Institute grants HL13315, HL40863 and HL07410. Presented in part at the 61st Annual Scientific Assembly, American Heart Association, 1988.

References

1. Dzau V J. Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am J Cardiol 1987; 59: 59A–65A.
2. Under T, Ganten D, Lang R E, Scholkens B A. Is tissue converting enzyme inhibition a determinant of the anti-hypertensive efficacy of converting enzyme inhibitors? Studies with the two different compounds, Hoe498 and MK421, in spontaneously hypertensive rats. J Cardiovasc Pharmacol 1984; 6: 872–880.
3. Cohen M L, Kurz K D. Angiotensin converting enzyme inhibition in tissues from spontaneously hypertensive rats after treatment with captopril or MK421. J Pharmacol Exp Ther 1982; 220: 63–69.
4. Chen X, Pitt B R, Moalli R, Gillis C N. Correlation between lung and plasma angiotensin converting enzyme and the hypotensive effect of captopril in conscious rabbits. J Pharmacol Exp Ther 1984; 229: 649–653.
5. Todd P A, Heel R C. Enalapril: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs 1986; 31: 198–248.
6. Jackson B, Cubela R B, Johnston C I. Inhibition of tissue angiotensin converting enzyme by perindopril: in vivo assessment in the rat using radioinhibitor binding displacement. J Pharmacol Exp Ther 1988; 245: 950–955.
7. Ryan J W. Assay of peptidase and protease enzymes in vivo. Biochem Pharmacol 1983; 32: 2127–2137.
8. Bull H G, Thornberry N A, Cordes M H J, Patchett A A, Cordes E H. Inhibition of rabbit lung angiotensin converting enzyme by N^a-[(S)-1-carboxy-3-phenylpropyl]L alanyl-L-prolene and N^a-[(S)-1-carboxy-e-phenylpropul]L lysil-L-prolene. J Biol Chem 1985; 260: 2952–2962.
9. Dobuler K J, Catravas J D, Gillis C N. Early detection of oxygen induced lung injury in conscious rabbits. Am Rev Respir Dis 1982; 126: 534–539.
10. Moalli R, Howell R E, Gillis C N. Kinetics of captopril and enalapril-induced inhibition of pulmonary angiotensin converting enzyme in vivo. J Pharmacol Exp Ther 1985; 234: 372–377.
11. Turrin M, Pitt B R, Ryan J W, Chung A Y K, Clark M B, Gillis C N. Uptake of N-[1-(S)-carboxy-(4-OH-3-^{125}I-phenyl)-ethyl]-L-Ala-L-pro, an inhibitor of angiotensin converting enzyme by rabbit lungs in situ. J Pharmacol Exp Ther 1986; 238: 14–18.

12. Ryan J W, Chung A, Martin L C, Ryan U S. New substrates for the radioassay of angiotensin converting enzyme of endothelial cells in culture. Tissue Cell 1978; 10: 555–562.
13. Zar J H. Biostatistical analysis, 2nd edn. Englewood Cliffs, NJ: Prentice Hall Inc, 1984.
14. Goli U B, Galardy R E. Kinetics of slow, tight-binding inhibitors of angiotensin converting enzyme. Biochemistry 1986; 25: 7136–7142.
15. Ryan J W, Chung A Y K, Berryer P, Murray M A, Ryan J P A. Slow tight-binding inhibitors of angiotensin converting enzyme. Adv Exp Med Biol 1986; 198A: 19–25.
16. McMenany R H. Albumin binding sites. In: Rosenoer V M, Orata M, Rothschild M A, eds. Albumin structure, function and uses. New York: Pergamon Press, 1977: 143–158.
17. Schnitzer J E, Carley W W, Palade G E. Specific albumin binding to microvascular endothelium in culture. Am J Physiol 1988; 254: H425–437.
18. Caldwell P R B, Seegal B C, Hsu K C, Das M, Soffer R L. Angiotensin-converting enzyme: vascular endothelial localization. Science 1976; 191: 1050–1051.
19. Ryan U S, Ryan J W, Whitaker R, Chiu A. Localization of angiotensin converting enzyme (kininase II) II. Immunochemistry and immunofluorescence. Tissue Cell 1976; 8: 125–145.
20. Egleme C, Cressier F, Wood J M. Local formation of angiotensin II in the rat aorta; effect of endothelium. Br J Pharmacol 1990; 100: 237–240.
21. Juul B, Aalkjaer C, Mulvany M J. Responses of femoral resistance vessels to angiotensin in vitro. Eur J Pharmacol 1987; 135: 61–68.
22. Pipili-Synetos E, Sideri E, Catravas J D, Maragoudakis M E. Endothelium removal does not abolish angiotensin converting enzyme activity from the mesenteric arterial bed of the rat. Biochem Pharmacol 1990; 40: 1149–1151.
23. Carretero O A, Miyazaki S, Scicli A G. Role of kinins in the acute antihypertensive effect of the converting enzyme inhibitor, captopril. Hypertension 1981; 3: 18–22.
24. Moore T J, Crantz F R, Hollenberg N K, et al. Contribution of prostaglandins to the antihypertensive action of captopril in essential hypertension. Hypertension 1981; 3: 168–173.
25. Antonaccio M J, Kerwin L. Pre- and post-junctional inhibition of vascular sympathetic function of captopril in SHR: implication of vascular angiotensin II in hypertension and anti-hypertensive actions of captopril. Hypertension 1981; 3 (suppl.): I-54–62.

Date received: 15 October 1990
Date revised: 10 February 1991
Date accepted: 1 July 1991