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HYDROLYSIS OF ANGIOTENSIN I BY PEPTIDASES IN HOMOGENATES OF RAT LUNG AND AORTA

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Abstract—The hydrolytic cleavage of angiotensin I has been studied in homogenate preparations of rat lung and aorta using gradient elution HPLC to monitor the formation of peptide products. Fresh crude homogenate preparations produced a rapid breakdown of angiotensin I to largely unidentifiable fragment peptides. Neither His-Leu nor angiotensin II was observed in these preparations even in the presence of captopril (20 μM) and the amino-peptidase inhibitors, puromycin, amastatin and bestatin. However, in freeze-thawed homogenates, angiotensin II and His-Leu were detectable together with the tetrapeptide, angiotensin (1–4). The addition of captopril (20 μM) reduced the amount of angiotensin II produced but did not completely block its formation. Higher concentrations of captopril or the addition of enalaprilat or EDTA did not further reduce the amount of angiotensin II produced. In the presence of captopril a peptide corresponding to des-Leu(10)angiotensin I was formed in relatively large amounts (equivalent to 40% of angiotensin I catabolized). Homogenates purified by concanalavin A affinity chromatography gave a clean hydrolysis of angiotensin I to angiotensin II and His-Leu which was completely blocked by captopril. These results suggest an ACE-like activity in rat lung and aorta that is not sensitive to converting enzyme inhibitors.

The primary function of the renin–angiotensin system is the generation of angiotensin II (AII), a potent vasoconstrictor octapeptide from an inactive plasma precursor, angiotensin I (AI) by the action of a dipeptidyl carboxypeptidase, commonly known as angiotensin converting enzyme (ACE) (EC 3.4.15.1), which cleaves the dipeptide His-Leu from the carboxylterminus of AI to form AII [1]. ACE exists as a number of related molecular forms [2] and is found in plasma and endothelial cells of most mammalian tissues, including lungs, blood vessels and kidney [3–5].

Purified ACE has been shown *in vitro* to be able to hydrolyse a number of other physiologically active peptides, such as bradykinin [6, 7], the enkephalins [8, 9], substance P [10], substance K [10] and neurotensin [11]. More recently, alternate pathways of AII generation have been reported. These include the granulocyte–angiotensin system [12] and the characterization of other peptidases capable of producing AII directly from AI or angiotensinogen [13]. Enzyme activities have also been described in cultured bovine endothelial cells [14] which can transform AI to AII. These peptidases are not, however, inhibited by conventional converting enzyme inhibitors such as captopril and enalaprilat which potently inhibit ACE.

The biotransformation of AI *in vivo*, however, is most probably based on a competition between ACE and angiotensinases including aminopeptidase A and M, dipeptidyl aminopeptidase, carboxy-peptidase N, neutral endopeptidases and possibly other further, yet uncharacterized, peptidases [15]. The precise role of these peptidases in the physiological regulation of AI and AII is not properly understood particularly during treatment with ACE inhibitors.

In this paper, we describe the hydrolysis of AI and its transformation to AII by peptidase preparations obtained from rat lung and aorta and describe the effects of peptidase inhibitors on these processes.

MATERIALS AND METHODS

Chemicals. Angiotensin I, angiotensin II, angiotensin III, angiotensin (1–4) (Peninsula Laboratories Inc., USA), fast garnet green, tween-20, -naphthylamine, *O*-phthaldialdahyde, puromycin, amastatin, bestatin (Sigma Chemical Co., MO), acetonitrile (HPLC grade, Mallinckrodt Australia Pty. Ltd.), trifluoroacetic acid (TFA) (HPLC grade, Pierce Chemical Company, IL), concanalavin A (Con-A) sepharose (Pharmacia, Uppsala, Sweden), hippuryl-histidyl-leucine (HHL), histidyl-leucine (HL) (Peptide Institute Inc., Osaka, Japan), and 1-arginyl-β-naphthylamine (Cambridge Research Biochemicals Limited, U.K.) were obtained from the stated suppliers. Catopril and enalaprilat were generous gifts from E. R. Squibb & Sons Inc. (Melbourne, Australia) and Merck & Co. (Sydney, Australia), respectively. All other reagents were analytical reagent grade. Distilled de-ionized water (>18 megaohm.cm) was used for all solutions.

Enzyme preparations. Sprague–Dawley rats weighing 200–300 g were anaesthetized with halothane and their lungs perfused with ice-cold heparinized (75 IU/ml) saline to remove blood. The lungs and aorta were removed and homogenized in 20 vol. of ice-cold 50 mM Tris, pH 7.4 and centrifuged for 1000 *g*.min to sediment unlysed cells and tissue fragments. The resultant pellet, which was largely composed of incompletely homogenized cell debris and fibrous tissue, was discarded and the

supernatant (S1), was then used either fresh or frozen at −15°.

Semi-purified ACE was obtained by applying the supernatant to a con-A lectin column buffered to pH 7.4 with 50 mM Tris in 500 mM NaCl and eluting ACE activity (as determined by HHL hydrolysis) with 500 mM α-methyl-D-mannoside. The eluant was concentrated in a 50 ml ultrafiltration cell and desalted using Centricon-10 centrifugal ultrafiltration cells obtained from Amicon Scientific Australia. The specific activity of the lung preparation was 100 nmol HL formed/mg protein/min and for aorta 140 nmol HL formed/mg protein/min, being approximately a 6-fold and 20-fold purification for lung and aorta preparations, respectively.

Enzyme assays. Aminopeptidase activity was measured using 200 μM Arg-β-NA in 50 mM Tris pH 7.4 and 180 mM NaCl as the substrate solution. The amount of β-NAP liberated was determined by reacting it with fast garnet green and measuring the change in absorbance at 525 nm essentially as described by Hopsu *et al.* [16].

Angiotensin-converting enzyme activity was measured fluorometrically using Hipp-His-Leu as the substrate according to the method of Friedland and Silverstein [17].

Protein concentration was determined according to the method of Bradford [18] using bovine serum albumin as standard.

HPLC enzyme assays. Angiotensin I metabolism and the generation of angiotensin II were assessed by incubating a given volume of enzyme preparation, with and without inhibitors, with 100 μM AI in a total volume of 200 μl at 37°. The incubation mix was buffered at pH 7.4 using 50 mM Tris and contained 180 mM NaCl. Aliquots (50 μl) were usually taken at 0, 20 and 40 min and added to 25 μl of 3% (v/v) TFA in acetonitrile to stop the reaction and to precipitate protein. The tubes were then spun for 5000 *g*.min in 1.5 ml plastic centrifuge tubes and 30 μl of the supernatant was injected onto a 15 cm Partisil ODS-2 reverse-phase column using a sample processor (Waters WISP model 710b). The sample was eluted with a linear gradient of 1–50% acetonitrile in 10 mM $(NH_4)_3PO_4$, pH 4.25 over 25 min at a flow rate of 1 ml/min. At the end of each run a 5 min washout period using 50% acetonitrile was used. The peptides eluted were detected at 214 nm using a UV detector (Waters Associates model 441) and an integrator/plotter (Waters Data Module). The amounts of peptides present were calculated by comparing peak areas of the unknowns with those of authentic standards.

RESULTS

The 25 min HPLC gradient conditions described in Materials and Methods gave complete baseline separation of known angiotensin peptides. The retention times of HL, AI (1–4), AII, AIII and AI were 6.0, 9.5, 19.4, 21.9 and 22.5 min, respectively. Digestion of AI with semi-purified enzyme purified by lectin affinity chromatography gave only two significant peaks that corresponded to the formation of both AII and HL (Fig. 1). The addition of captopril (20 μM) totally inhibited the formation of both

Table 1. Breakdown of angiotensin I and generation of angiotensin II in rat aorta and lung preparations over a 40-min incubation

Preparation	AI breakdown[1] (nmol/mg protein/min)		AII generation[1] (nmol/mg protein/min)	
	No inhibitor	+20 μM captopril	No inhibitor	+20 μM captopril
Fresh aorta homogenate	6.90 ± 0.68	5.52 ± 0.74†	<0.10[2]	<0.10[2]
Freeze/thawed aorta homogenate	1.35 ± 0.13*	0.95 ± 0.12*‡	0.80 ± 0.07*	0.67 ± 0.08*†
Fresh lung homogenate	7.4 ± 0.10	6.4 ± 0.28‡	<0.10[2]	<0.10[2]
Freeze/thawed lung homogenate	3.5 ± 0.16*	2.1 ± 0.14*‡	1.24 ± 0.09*	0.41 ± 0.02*‡

Values are expressed as means ± SE, N = 4.
* Significant difference ($P < 0.005$) between freeze–thawed and fresh preparations.
† No significant difference ($P > 0.05$) between no inhibitor and 20 μM captopril rates.
‡ Significant difference ($P < 0.05$) between no inhibitor and 20 μM inhibitor rates.
[1] Average rate over 40 min incubation. [2] Detection limit.

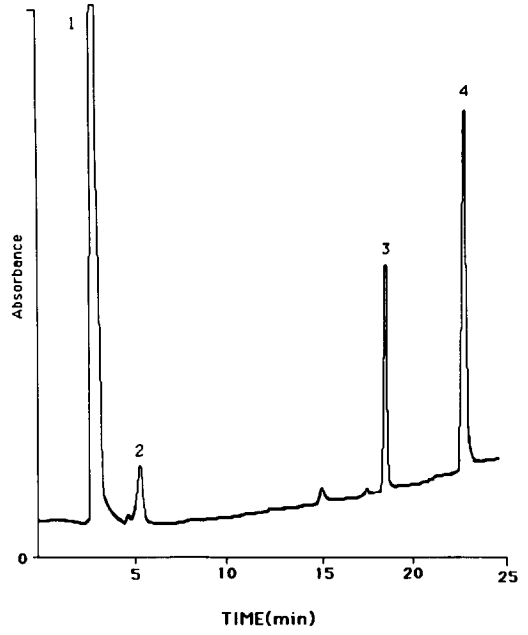

Fig. 1. HPLC profile of AI degradation by lung homogenate purified by lectin-affinity chromatography as described in Materials and Methods. Peak 1 is the solvent front, peak 2 is His-Leu, peak 3 is AII and peak 4 is AI. Incubation conditions are as described in Materials and Methods. Flow rate was 1.0 ml/min, and detection was by UV at 214 nm. Separation conditions were based on a 25 min linear gradient from 1 to 50% acetonitrile in 10 mM amonium phosphate, pH 4.25 using a Partisil ODS-2 column.

AII and HL with this preparation and prevented any significant loss of AI over the period of the incubation (8 hr).

Digestion of AI, however, with crude homogenates of both rat lung and aorta in the absence of any inhibitors resulted in the rapid breakdown of AI to largely unidentifiable fragments (Fig. 2). Neither AII, AI(1–4), AIII nor HL were produced in measurable amounts in the incubation after 40 min at which stage 80% of AI had already been digested. The addition of captopril (20 μM) only reduced the breakdown over 40 min of AI by 20% and 13.5% in aorta and lung, respectively (Table 1); however, only the lung data were significantly different ($P < 0.05$) due to the smaller standard errors. The addition of puromycin (50 μM), bestatin (10 μM) and amastatin (10 μM) also did not significantly reduce the rate of AI breakdown.

Freezing overnight at −15° followed by thawing of homogenates resulted in a marked change in patterns of peptide products produced after digestion of AI. The formation of AII and HL in both tissues were now both readily measurable (Figs 3a and c). The rate of formation of AII was 0.80 and 1.24 nmol/mg/min in aorta and lung, respectively (Table 2). This was accompanied by a reduction in the degradation rate of AI by 80% and 53% compared to fresh rat aorta and lung homogenates ($P < 0.05$) respectively (Table 1). The addition of captopril (20 μM) to the incubation medium only marginally (16%) reduced the formation of AII in aorta homogenates ($P > 0.05$) but significantly reduced (57%) the formation of AII in the lung preparation

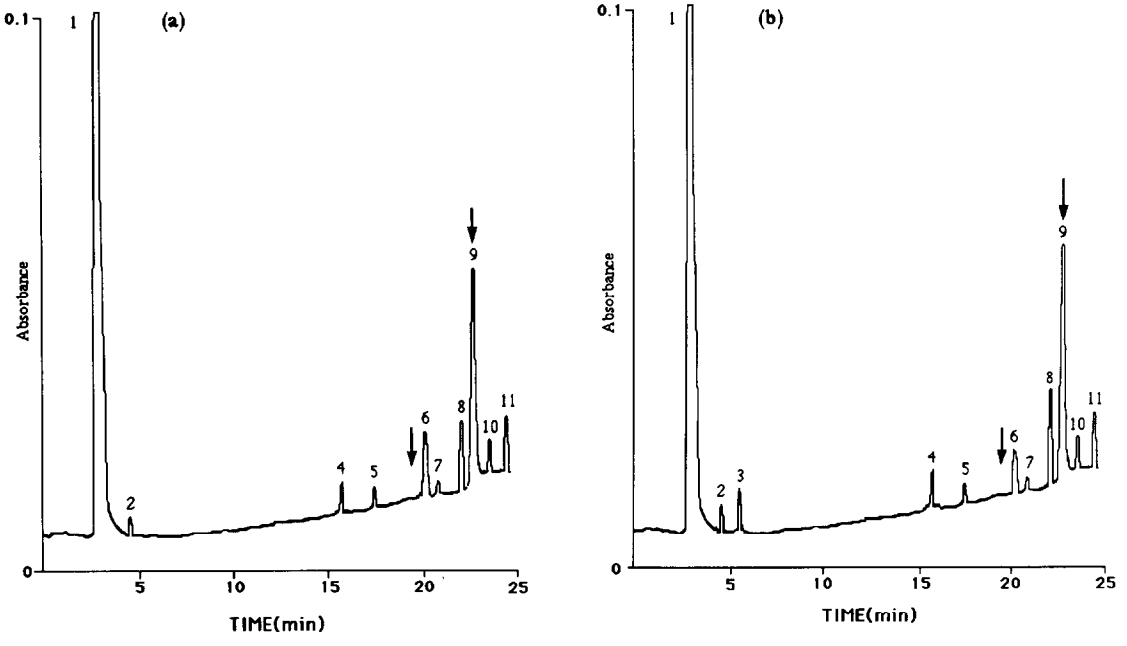

Fig. 2. HPLC profiles of enzyme digests of AI after 40 min incubation with (a) fresh aorta homogenate and (b) fresh lung homogenate. Peak 1 is the solvent front, peaks 2–8 and 10–11 are unidentified metabolites. Peak 9 corresponds to AI. AII elutes between peaks 6 and 7. Arrows mark the retention times of AI and AII. Incubation conditions are as described in Materials and Methods. Separation conditions are identical to those described in Fig. 1.

Table 2. Effect of captopril on AI breakdown and AII formation in lectin purified preparations

Preparation	AI breakdown (nmol/mg protein/min)		AII formation (nmol/mg protein/min)	
	No inhibitor	+10 μM captopril	No inhibitor	+10 μM captopril
Aorta[1]	14.3	<0.5[3]	11.3	N.D.[4]
Lung[2]	12.2	<0.5[3]	10.5	N.D.[4]

Results are from one experiment, AI breakdown and formation of AII were measured by HPLC as described in Materials and Methods.
[1] 8 hr incubation. [2] 4 hr incubation. [3] Detection limit. [4] Not detectable.

($P < 0.05$). Higher concentrations of captopril (200 μM), or the addition of enalaprilat (10 μM), or EDTA (10 μM) did not further attenuate the formation of AII in these freeze-thawed preparations.

The process of freeze-thawing of rat lung and aorta SI fractions, however, resulted in a substantial loss of aminopeptidase activity (87% and 80% respectively) ($P < 0.005$), but did not affect the angiotensin converting enzyme activity as assessed by HHL digestion (Table 3). ACE activity was undetectable in the presence of captopril (20 μM) when measured by fluorometric assay.

Digestion of AI by freeze-thawed homogenates also resulted in the formation of peptides other than AII. In particular, a clearly identifiable peptide co-eluting with authentic AI(1–4) was present in both lung and aorta preparations. The process of freeze-

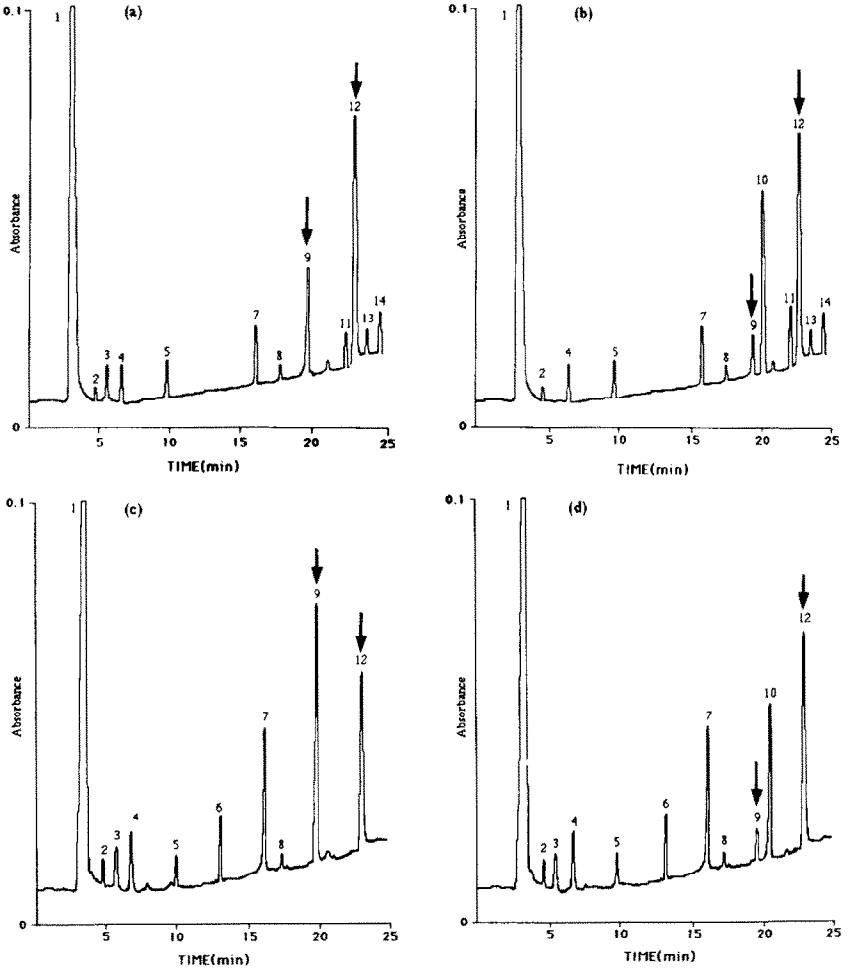

Fig. 3. HPLC profiles of freeze–thawed enzyme digests of AI with (a) aorta homogenate, (b) aorta homogenate in the presence of 20 μM captopril, (c) lung homogenate and (d) lung homogenate in the presence of 20 μM captopril. Peak 1 is the solvent front, peaks 2, 4, 6, 7, 8, 11, 13 and 14 are unidentified metabolites, peak 3 is His-Leu, peak 5 is AI (1–4), peak 9 is AII, peak 10 is most probably des-leu (10) AI and peak 12 is AI. Arrows mark the retention times of AI and AII. Separation conditions are identical to those described in Fig. 1.

Table 3. Specific activities of aminopeptidase and converting enzyme in aorta and lung preparations

Preparation	Aminopeptidase activity[1] (nmol/mg protein/min)	Converting enzyme activity[2] (nmol/mg protein/min)	
		Absence of captopril	+20 μM captopril
Fresh aorta homogenate	41 ± 3 (N = 3)	6.2 ± 0.6 (N = 3)	N.D.
Freeze/thawed aorta homogenate	8.2 ± 0.7 (N = 4)*	6.0 ± 0.1 (N = 3) N.S.	N.D.
Fresh lung homogenate	39 ± 2 (N = 4)	31.1 ± 2.1 (N = 3)	N.D.
Freeze/thawed lung homogenate	5.2 ± 0.6 (N = 4)*	38.8 ± 3.2 (N = 3) N.S.	N.D.

[1] Aminopeptidase activity expressed as nmol β-naphthylamine hydrolysed from substrate/mg protein/minute as described in Materials and Methods.

[2] ACE activity expressed as nmol His-Leu hydrolysed from substrate/mg protein/minute as described in Materials and Methods.

* $P < 0.005$ compared to fresh homogenate.

N.S. $P > 0.005$ compared to fresh homogenate.

N.D. Not detectable (<0.3 nmol/mg/min for aorta and <1 nmol/mg/min for lung).

thawing or the addition of the converting enzyme inhibitor captopril did not change the activity of endopeptidases producing this peptide.

Another peptide eluting after AII but before AIII and AI (peak No. 10) was only detectable in freeze-thawed preparations in the presence of 20 μM captopril (Fig. 3b and d). In fact, a substantial proportion (50%) of digested AI appeared as this peak in both aorta and lung. Its higher lipophilicity to AII and lower lipophilicity to AI suggest it is des-Leu (10) AI, which is formed from AI by the action of carboxy peptidases similar to kininase I.

Two further peptide products were identified in lung freeze-thawed preparations (peaks 6 and 7, Fig. 3a and c). Peak 7 was found in both aorta and lung preparations whilst peak 6 was only found in the lung preparation. These unidentified peaks are likely to correspond to peptides with approximately 6 amino acid residues and would be formed by the action of endopeptidases similar to that seen with the formation of AI(1–4). Peaks 13 and 14 (Fig. 3a and b) were only detected in the aorta preparation and due to their longer retention and therefore higher lipophilicity to AI, probably correspond to des-Asp(1)AI and des-Asp-(1)Arg(2)-AI, indicating aminopeptidase activity.

DISCUSSION

The use of HPLC is established as a reliable and powerful technique to monitor the formation of peptide fragments by digestion of larger peptides by enzyme preparations [19]. Digestion of angiotensin I by Con-A-purified lung homogenate resulted in the digestion of AI and the reciprocal formation of AII and the dipeptide HL. Over a period of 8 hr no other significant peaks were seen. Captopril (20 μM) totally abolished the formation of AII in this preparation leaving AI undegraded over the time course of experiments (8 hr). These glycoprotein-enriched preparations show properties which are typical of purified ACE. Although purification of ACE-like activity was only 6–20-fold with this form of affinity chromatography, peptidases other than carboxy dipeptidases appear to be selectively removed.

The use of crude homogenates of tissues rich in ACE directly in these digestion experiments such as lung and aorta show a very different profile of peptidase activity as assessed by monitoring the formation of peptide products on the HPLC. In fact, although substantial ACE activity was present in these tissues using biochemical assays no detectable formation of AII was seen on the HPLC despite the rapid digestion of AI. This is presumably related to substantial amino- and endo-peptidase activities in these preparations which mask the formation of AII. The addition of non-specific amino-peptidase inhibitors such as puromycin (50 μM), bestatin (10 μM) and amastatin (10 μM) did not appear to reduce significantly the rate of digestion of AI or increase the amount of AII produced. This implies that although aminopeptidase A and leucine aminopeptidase, which are inhibited by amastatin are capable of rapidly degrading AII [20] there are probably many other amino peptidases capable of hydrolysing angiotensins [21].

Freeze–thawing of crude homogenates of both rat lung and aorta, however, was fortuitously found to be a convenient technique to selectively destroy (more than 80%) this non-specific amino-peptidase activity whilst still retaining full ACE activity. Since ACE activity (as measured by HHL hydrolysis) was not affected by this treatment, it is unlikely that activation of a latent form of ACE could be responsible for the change in the hydrolysis patterns of AI. Formation of AII in freeze–thawed aorta and lung preparations now accounted for 60% and 40% of the loss in AI over 40 min, respectively, and was associated with the appearance of the carboxy dipeptide fragment His-Leu in the HPLC profile which is characteristic of the action of a peptidyl dipeptidase, such as ACE. Inhibition of the formation of AII by specific inhibitors of ACE, such as captopril and enalaprilat, although substantially reducing the formation of AII, surprisingly did not completely prevent the formation of AII even when present at high concentrations. The presence in most cases of His-Leu in these digests strongly suggests that this peak was indeed AII. Due to the small amounts of this peptide in these digests we were

unable to confirm the identity of this peptide; however, both des-leu-AI and des-Asp-AI eluted at different times to this peak which eluted at the identical time to AII. The inability to completely block the formation of AII was a consistent finding in freeze-thawed preparations of both rat lung and aorta homogenates. EDTA, a non-specific inhibitor of metallo-peptidases, was also unable to block formation of AII, suggesting that this enzyme activity was not related to ACE, or the related endo-peptidase 24.11, which is, in part, responsible for enkephalin degrading activity [22]. The ability of AII to be generated by enzymes independent of ACE has been previously shown to occur in some specific cell types, for example, a chymotrypsin-like activity in human lung mast cells [23], a cathepsin-G-like enzyme [25]. Our results in lung and aorta homogenates suggest that alternate pathways to ACE for the production of AII may also exist in aorta and lung. These biochemical studies are consistent with observations in intact tissues in which vasoconstrictor response of AI in isolated aortic strips [26] and in isolated perfused rat kidney [27] were not totally abolished by converting enzyme inhibitors and that plasma AII concentrations in humans are not completely abolished following oral treatment with ACE inhibitors such as enalapril even at doses which completely abolish plasma ACE for many hours [28]. The type of peptidyl dipeptidase and its relationship to other known peptidases is not known at this stage, but its ability to be inhibited by EDTA suggests that it may either be a serine protease similar to chymotrypsin or a thiol protease.

It is of interest in these freeze–thawed preparations when captopril (20 μM) is present in the incubation that the pathway of AI digestion changes to now involve the formation of des-Leu AI as a major product. This peptide is not seen in the digests when captopril is absent from the incubation. This redirection in the pathways of AI metabolism during chronic ACE inhibitor therapy may be important in the overall expression of activity of ACE inhibitors for two major reasons. Firstly, des-leu AI may itself express some physiological and pharmacological activity, such as acting as a competitive inhibitor of ACE or other peptidases [29, 30], or alternatively des-leu AI may be a substrate for the further action of carboxy peptidases related to kininase I leading to the formation of AII similar to that isolated from kidney tissue [31].

In conclusion, we have shown that the metabolism of AI to AII may involve not only ACE which is blocked by captopril and enalaprilat but also peptidyl dipeptidases insensitive to converting enzyme inhibitors. We have also shown that during inhibition of ACE by captopril the metabolism of AI is redirected to other pathways not prominent in the absence of captopril. These pathways may themselves lead to AII providing a protective mechanism for the renin–angiotensin–aldosterone system during chronic ACE inhibitor therapy. If this is the case, the traditional view that AII is formed from AI solely by the action of ACE will require serious review. The physiological role, if any, of these alternative pathways of angiotensin activation, such as in the pathogenesis of hypertension remains to be elucidated.

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REFERENCES

1. E. G. Erdos, *Am. J. Med.* **60**, 746 (1976).
2. M. Van Sande, S. L. Scharpe, N. M. Neels and Y. Kasahara, *J. clin. Chem. clin. biochem.* **23**, 381 (1985).
3. H. J. Wigger and S. A. Stalcup, *Lab. Invest.* **38**, 581 (1978).
4. M. E. Van Sande, S. L. Scharpe, H. M. A. N. Neels and K. O. Van Camp, *Clin. Chim. Acta* **147**, 255 (1985).
5. D. W. Cushman and H. S. Cheung, *Biochim. biophys. Acta* **250**, 261 (1971).
6. F. E. Dorer, J. R. Kahn, K. E. Lentz, M. Levine and L. T. Skeggs, *Circ. Res.* **31**, 356 (1972).
7. R. L. Soffer, R. Reza and P. R. B. Caldwell, *Proc. natn. Acad. Sci. U.S.A.* **71**, 1720 (1974).
8. R. Defendini, E. A. Zimmerman, J. A. Weare, F. Alhenc-Gelas and E. G. Erdos, *Adv. Biochem. Psychopharmac.* **33**, 271 (1982).
9. B. Malfroy and J. Schwartz, *Biochem. biophys. Res. Commun.* **130**, 372 (1985).
10. E. A. Thiele, S. M. Strittmatter and S. H. Snyder, *Biochem. biophys. Res. Commun.* **128**, 317 (1985).
11. R. A. Skidgel, S. Engelbrecht, A. R. Johnson and E. G. Erdos, *Peptides* **5**, 769 (1984).
12. B. U. Wintroub, L. B. Klickstein, V. J. Dzau and K. W. K. Watt, *Biochemistry* **23**, 227 (1984).
13. B. U. Winthroub, N. B. Schechter, G. S. Lazarus, C. E. Kaempfer and L. B. Schwartz, *J. Invest. Dermatol.* **83**, 336 (1984).
14. J. J. Lanzillo, Y. Dasanathy, J. Stevens and B. L. Fanburg, *Biochem. biophys. Res. Commun.* **134**, 770 (1986).
15. P. Bunning, W. Budek, R. Escher and E. Schonherr, *J. Cardiovasc. Pharmac.* **8** (s10), S52 (1986).
16. V. K. Hopsu, K. K. Makinen and G. G. Glenner, *Archs Biochem. Biophys.* **114**, 557 (1966).
17. J. Friedland and E. Silverstein, *Am. J. Clin. Pathol.* **66**, 416 (1976).
18. M. A. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
19. T. P. Davis and A. Culling-Berglund, *J. Chromatogr.* **327**, 279 (1985).
20. S. Miztani, H. Akiyama, O. Kurauchi, H. Taira, O. Narita and Y. Tumoda, *Acta Endocrinol.* **110**, 135 (1985).
21. A. J. Turner, R. Matsas and A. J. Kenny, *Biochem. Pharmac.* **34**, 1347 (1986).
22. M. Benuck and N. Marks, *Biochem. biophys. Res. Commun.* **95**, 822 (1980).
23. B. U. Wintroub, C. E. Kaempfer, N. M. Schechter and D. Proud, *J. clin. Invest.* **77**, 196 (1986).
24. J. C. Powers, T. Tanaka, J. W. Harper, Y. Minematsu, L. Barker, D. Lincoln, K. V. Crumley *et al.*, *Biochemistry* **24**, 2048 (1985).
25. R. Boucher, S. Demassieux, R. Garcia and J. Genest, *Circ. Res.* **4** (Suppl. 2), 26 (1977).
26. H. Okunishi, M. Miyazaki and N. Toda, *J. Hypertens.* **2**, 277 (1984).
27. M. Schmidt, E. Giesen-Crouse, J. Krieger, C. Welsch and J. Imbs, *J. Cardiovasc. Pharmac.* **8** (Suppl. 10), S100 (1986).
28. T. Unger, D. Hubner, B. Schull, T. Yukimura, W. Rascher, R. E. Lang and D. Ganten, *Am. J. Cardiol.* **49**, 1530 (1982).
29. R. A. Snyder, K. W. K. Watt and B. U. Wintroub, *J. biol. Chem.* **260**, 7857 (1985).
30. R. A. Snyder and B. U. Wintroub, *Biochim. biophys. Acta* **71**, 1 (1986).
31. D. G. Changarus, J. J. Miller and R. S. Levy, *Biochem. biophys. Res. Commun.* **138**, 573 (1986).