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FORMATION OF ANGIOTENSIN II AND OTHER ANGIOTENSIN PEPTIDES FROM DES-LEU 10-ANGIOTENSIN I IN RAT LUNG AND KIDNEY

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Abstract—The formation of AII from a metabolite of AI, des-leu^{10}-angiotensin I [A(1–9)] has been studied in centrifugal fractions of rat lung and kidney using gradient elution HPLC to monitor the formation of peptide products. AII-forming activity was present in kidney S2 (22.3 nmol/mg protein/min) but not in kidney P2 centrifugal fractions. Lung S2 fractions showed relatively weak AII-forming activity (0.34 nmole/mg protein/min) whilst no activity was observed in lung P2. Carboxypeptidase N-like activity measured using both Hipp-Arg and Hipp-Lys as synthetic substrates did not parallel AII-forming activity, since this activity was highest in the P2 fractions of both lung and kidney, as were ACE and aminopeptidase activities.

Whilst the major peptide produced in kidney S2 was AII (71%) significant amounts of both AIII (23%) and A(2–9) (6%) were also observed. In lung the amounts of these peptides produced as a percentage of the A(1–9) degrading activity were 2.9%, 2.4% and 21% respectively.

The AII-forming activity in kidney S2 was not inhibited by enalaprilat, bestatin, amastatin, phosphoramidon or Pro-Phe but was inhibited (31%) by 1 mM cobalt (II). 1,10-Phenanthroline, iodoacetic acid, EDTA and puromycin significantly enhanced the formation of AII and increased the rate of degradation of the substrate, A(1–9). These results support the concept of a sequential carboxypeptidase pathway operating, particularly in kidney, to produce AII from AI. These results provide further evidence of an alternative metabolic pathway for the formation of AII not involving angiotensin converting enzyme.

Angiotensin II [AII, A(1–8)] is a potent vasopressor octapeptide involved in the regulation of fluid and electrolyte balance [1]. The formation of this peptide is generally believed to occur by the action of angiotensin converting enzyme (ACE) [EC 3.4.15.1] on the decapeptide angiotensin I (AI, A(1–10)] by removal of His-Leu from the carboxyl terminus.

There is now increasing evidence to suggest that, in tissues, other pathways are present, capable of producing AII from AI independent of ACE [2]. One such pathway originally proposed by Ng and Vane [3] is through the action of carboxypeptidases such as carboxypeptidase N (or kininase I) on AI in which the carboxyl terminal amino acids are removed sequentially, hence forming the intermediary peptide des-leu^{10}-angiotensin I [A(1–9)]. Carboxypeptidase activities such as these have recently been described in human kidney extracts [4] and in platelets [5]. However, the physiological role of this pathway in the generation of AII is not known. Recently, the formation of the intermediate nonapeptide A(1–9) was described when homogenates of rat lung and aorta were used to hydrolyse AI in the presence of captopril, an inhibitor of ACE [2].

It is not clear, however, whether this nonapeptide can act as a substrate for the formation of AII, particularly in lung and kidneys which are known to be important in expressing the action of ACE inhibitors. Consequently we have investigated the possibility that A(1–9) can be converted to AII by peptidase preparations obtained from rat lung and kidney.

MATERIALS AND METHODS

Chemicals. Angiotensin I [A(1–10)], angiotensin II [(1–8)] (Peninsula Laboratories Inc., U.S.A.), fast garnet green, β-naphthylamine, hippuryl-arginine (Hipp-Arg), hippuryl-lysine (Hipp-Lys), iodoacetic acid, bestatin, amastatin, prolyl-phenylalanine (Pro-Phe), puromycin, (Sigma Chemical Co., St Louis, MO), angiotensin III [A(2–8)], des-leu-angiotensin I [A(1–9)] (Auspep Pty Ltd, Melbourne, Australia), hippuryl-histidyl-leucine (Hipp-His-Leu), histidyl-leucine (His-Leu) (Peptide Institute Inc., Osaka, Japan), 1-arginyl-β-naphthylamine (Arg-β-NA) (Cambridge Research Biochemicals Ltd, U.K.), acetonitrile (HPLC grade, Mallinckrodt Australia Pty Ltd), trifluoroacetic acid (TFA) (HPLC grade, Pierce Chemical Co., IL) were obtained from the stated suppliers. Captopril and enalaprilat (MK-422) were generous gifts from E. R. Squibb & Sons Inc. (Melbourne, Australia) and Merck & Co. (Sydney, Australia). All other chemicals and reagents were analytical reagent grade. Distilled deionized water (>18 megaohm.cm) was used for all solutions.

Enzyme preparations. Sprague–Dawley rats

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weighing 200–300 g were anaesthetized with halothane and their lungs perfused with ice-cold heparinized saline (75 IU/ml) to remove blood. Lungs and kidneys were removed and homogenized in 20 vol. of ice-cold 50 mM Tris buffer, pH 7.4 containing 180 mM NaCl 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 further centrifuged at 10,000 *g*. min at 4°. The pellet (P2) was resuspended in 10 ml of ice-cold buffer. The supernatant was labelled as the S2 fraction. All fractions were either used fresh or were frozen at −15° until use.

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-NA 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.* [6].

Angiotensin-converting enzyme activity was measured fluorometrically using Hipp-His-Leu as the substrate according to the method of Friedland and Silverstein [7].

Protein concentration was determined according to the method of Bradford using bovine serum albumin as standard [8].

Carboxypeptidase N (CP-N) activity was measured using either Hipp-Lys (CP-N_2) or Hipp-Arg (CP-N_1) as substrate essentially as described by Schweisfurth [9] except that the hippuric acid produced in the reaction was measured by HPLC by evaporating the ethyl acetate extract under a stream of air at 60°. The residue was reconstituted with 100 μl of HPLC mobile phase and 10 μl aliquots were injected. HPLC conditions were as follows: column, Waters NOVAPAK-C18; flow rate, 0.8 ml/min; mobile phase, 15% acetonitrile in 0.1% orthophosphoric acid; detector, Waters variable wavelength M450; wavelength was 228 nm; sensitivity was 0.10 aufs.

HPLC enzyme assays. Des-leu angiotensin I [A(1–9)] metabolism and the generation of AII were assessed by incubating a given volume of enzyme preparation, either with or without inhibitors, with 100 μM A(1–9) in a total volume of 300 μl at 37°. The amount of enzyme added was adjusted to give approximately 50% digestion of A(1–9) in 20 min. 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, 10, 20 and 30 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 15 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 22.5% to 35% acetonitrile in 10 mM $(NH_4)_3$ PO_4, pH 4.25 over 20 min at a flow rate of 1 ml/min. 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 unknown with those of authentic standards.

For inhibitor studies, a 10 min preincubation with the enzyme preparation at 37° was used, except for cobalt, for which a 30 min preincubation was employed. For all studies, paired digestion experiments using the same enzyme preparation were carried out to reduce variability from one preparation to another.

Statistical evaluation of data. Comparison of means were performed using the Student's *t*-test when one tissue activity was compared to another and the paired *t*-statistic when paired experiments were conducted in the same tissue preparation.

RESULTS

Chromatography

Complete resolution of angiotensin peptides (seven or more amino acid residues) was obtained using a 20 min gradient of 22.5% to 35% acetonitrile in ammonium phosphate pH 4.25 buffer (Fig. 1). In particular the peptides A(1–9) (retention time, t_R = 3.7 min), AII (t_R = 4.2 min) and AIII (t_R = 7.7 min) which are often poorly resolved were well separated from each other in this chromatography system. In

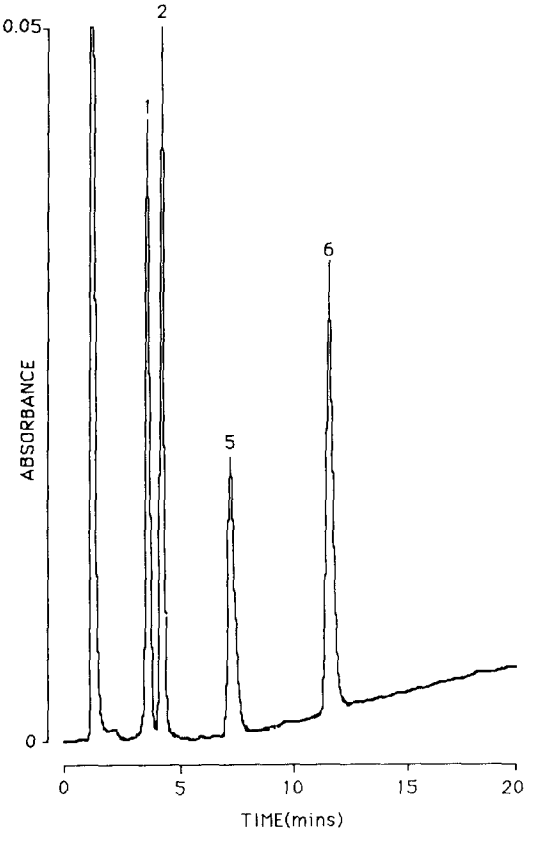


Fig. 1. Chromatogram showing elution of A(1–9) (peak 1), A(1–8) (peak 2), A(2–8) (peak 5) and A(1–10) (peak 6), using a linear program from 22.5% acetonitrile to 35% acetonitrile in 10 mM ammonium phosphate, pH 4.25 buffer. Each peak represents 0.5 nmole injected.

Table 1. ACE, AP and CP-N activities in lung and kidney sub-cellular preparations

Tissue	Subcellular fraction	Peptidase activity*			
		ACE†	AP‡	CP-N_1§	CP-N_2‖
Lung	S1	19.2 ± 2.2	26 ± 6.5	2.27 ± 0.33	3.05 ± 0.07
	S2	2.92 ± 0.47	46 ± 13	1.15 ± 0.22	1.80 ± 0.18
	P2	124 ± 31	140 ± 49	5.75 ± 1.0	17.8 ± 5.3
Kidney	S1	0.33 ± 0.03	91 ± 11	2.40 ± 1.38	3.28 ± 0.87
	S2	0.28 ± 0.05	27 ± 8	1.92 ± 0.22	1.82 ± 0.27
	P2	2.26 ± 0.37	551 ± 126	10.6 ± 4.0	5.05 ± 0.87

* Expressed as nmole substrate consumed/mg protein/min using freeze-thawed tissue fractions. Mean ± SEM of activities obtained from 6 separate tissues.
† Hipp-His-Leu as substrate.
‡ Arg-β-naphthylamine as substrate.
§ Hipp-Arg as substrate.
‖ Hipp-Lys as substrate.

addition two other peptides which were resolved from the other peptides were seen in some digestions eluting between AII and AIII and have been tentatively identified based on their retention times as A(3–9) and A(2–9), respectively.

Angiotensin converting enzyme activities

Angiotensin coverting enzyme (ACE) activity was highest in P2 fractions compared to the corresponding S1 and S2 fractions from lung and kidney (Table 1). Activities in lung P2 (124 ± 31 nmole/mg protein/min) were almost 50-fold higher than in lung S2 ($P < 0.01$) whilst activities in kidney P2 (2.26 ± 0.37 nmole/mg protein/min), although lower than lung P2 ($P < 0.01$), were over 100-fold higher than kidney S2 ($P < 0.005$).

Aminopeptidase activities

The aminopeptidase (AP) activity of rat lung, and kidney sub-cellular fractions assessed using Arg-β-naphthylamine as substrate are also shown in Table 1. AP activities in freeze-thawed preparations were 4-fold higher in kidney P2 compared to lung P2 ($P < 0.025$), but AP activities in the kidney soluble fraction (S2) were slightly lower than lung S2 although this was not statistically significant ($P > 0.05$). In contrast to ACE activity there was a loss of AP activity in both kidney and lung centrifugal fractions associated with freeze-thawing. This loss generally amounted to 50%.

Carboxypeptidase activities

The carboxypeptidase N-like (CP-N) activities were also measured in the same tissue fractions as for AP and ACE activity using both Hipp-Arg (CP-N_1) and Hipp-Lys (CP-N_2) as synthetic substrates [9] (Table 1).

In contrast to AP activity CP-N_1 activity in lung

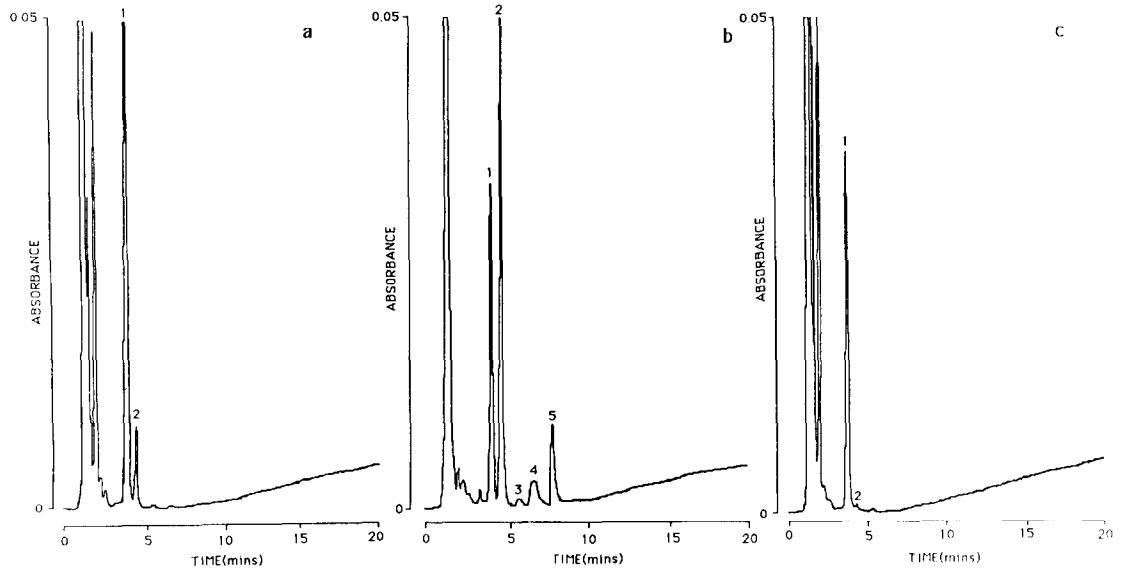


Fig. 2. Chromatogram of 20 min enzyme digest from (a) rat kidney S1, (b) rat kidney S2 and (c) rat kidney P2 freeze-thawed centrifugal fractions using A(1–9) as substrate. Conditions are as described in Fig. 1 and in Materials and Methods. Numbered peaks correspond to A(1–9) (1), A(1–8) (2), A(3–9) (3), A(2–9) (4) and A(2–8) (5).

Table 2. Angiotensin-fragment-forming activities* using des-leu-angiotensin I [A(1–9)] as substrate in rat lung and kidney enzyme preparations

Tissue	Sub-cellular fraction	A(1–8) [AII]	A(2–8) [AIII]	A(2–9)
Lung	S1	0.14 ± 0.02	N.D.	1.00 ± 0.29
	S2	0.340 ± 0.009	0.275 ± 0.065	2.50 ± 0.38
	P2	N.D.	N.D.	N.D.
Kidney	S1	11.6 ± 2.8	N.D.	N.D.
	S2	20.3 ± 2.6	6.5 ± 2.5	1.7 ± 0.25
	P2	N.D.	N.D.	N.D.

* nmole peptide produced/mg protein/min using freeze-thawed centrifugal preparations.
Means ± SEM of 4–7 experiments.
N.D. = not detected.

and kidney fractions were not affected by the freeze-thawing ($P > 0.05$). CP-N_1 activity was again highest in the P2 sub-cellular fractions. Lung and kidney P2 activities (5.75 and 10.6 nmole/mg protein/min respectively) were not statistically different to each other ($P > 0.05$). Activities in the S1 and S2 fractions of both lung and kidney (1.15 and 1.92 nmole/mg protein/min respectively) were over 5-fold lower than the activity in the P2 fractions, however, where there was a significant difference between the activities of the lung and kidney S2 fractions ($P < 0.025$).

CP-N_2 activity showed a similar tissue and sub-cellular distribution to CP-N_1 activity. The highest activity was again in the lung and kidney P2 fractions. The activity in lung and kidney S2 fractions were 1.80 and 1.82 nmole/mg protein/min, respectively ($P > 0.05$).

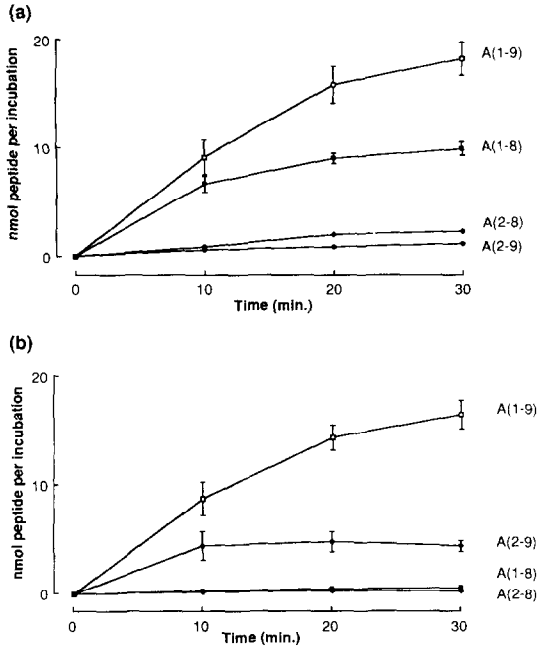


Fig. 3. Time course for (a) rat kidney S2 and (b) rat lung S2 freeze thawed enzyme digests showing the formation of peptide fragments A(1–8) and A(2–9) and consumption of substrate, A(1–9). Each point is the mean of 4 experiments ± SEM.

Peptide digestion experiments

Kidney. Incubation of A(1–9) with crude S1 homogenates of rat kidney resulted in rapid breakdown of A(1–9) and the resultant formation of angiotensin II [A(1–8)] (Fig. 2a). In the S1 fraction, only 16.4% of the breakdown products were identified as AII. However, in the S2 fraction, the rate of formation of AII was double that of the S1 fracton (expressed as nmole/mg/min), with over 60% of the hydrolysis products being identified as AII (Fig. 2b, see also Table 2).

In the kidney S2 fraction additional peptide peaks were also seen (peaks 3, 4 and 5 of Fig. 2b). Peptide 5 co-eluted with AIII whilst peptides 4 and 3 are most probably A(2–9) and A(3–9), respectively (Fig. 2). The time course for the formation of these peptides in kidney S2 is shown in Fig. 3a. The formation of those additional peptides A(2–9) (peak 4) and AIII but not AII were enhanced by the use of fresh enzyme preparations instead of freeze-thawed preparations. In the fresh kidney S2 fraction the enhancement of AIII and A(2–9) production was 55% ($P < 0.05$) and 112% ($P < 0.025$), respectively, whilst in kidney S1 AIII and A(2–9) were only detectable in the fresh preparation.

In contrast, the P2 fraction of kidney did not show the formation of either AII or any of the other peptide peaks seen in the S2 digestions (Fig. 2c and Table 2). Instead numerous smaller peptides (2, 3 and 5 amino acid residues) including A(1–4) were seen eluting near the solvent front indicating that this particulate fraction contained mainly endopeptidase activity toward A(1–9).

Table 3. Rate of des-Leu-Angiotensin I degradation in rat lung and kidney freeze-thawed centrifugal fractions

Tissue	Fraction	A(1–9) degrading activity (nmole/mg protein/min)	
Lung	S1	15.9 ± 2.2	(N = 6)
	S2	11.7 ± 0.49	(N = 4)
	P2	47.5 ± 15	(N = 3)
Kidney	S1	126 ± 12	(N = 6)
	S2	28.5 ± 1.9	(N = 6)
	P2	937 ± 196	(N = 3)

A(1–9) degrading activity was highest in the P2 fraction (937 ± 196 nmole/mg/min) and lowest in the S2 fraction (28.5 ± 1.9 nmole/mg/min) (Table 3).

Lung. The rate of digestion of A(1–9) was significantly less in lung subcellular fraction than the corresponding kidney fractions ($P < 0.05$). The greatest difference was observed between the kidney P2 and lung P2 fractions, the kidney P2 activity being 20-fold higher ($P < 0.01$) (Table 3). Formation of AII by lung sub-cellular fractions, although detectable, was substantially less than the corresponding kidney fractions (Table 2 and Fig. 4). Again the S2 preparation showed the highest activity in the lung at 0.34 nmole AII generated/mg protein/min, over 50-fold lower than the kidney S2 activity ($P < 0.001$). Again no AII forming activity was seen in the P2 fraction (Table 2) despite the high A(1–9) degrading activity (Table 3) and the high ACE activity (Table 1) in this fraction.

The formation of AIII was also seen in lung S2 (5.5 nmole generated/mg protein/min) but not in P2 fractions (Table 2 and Fig. 4). AIII was also observed in lung S1 but only in fresh enzyme preparations.

A(2–9) was clearly present in both lung S1 and S2 preparations but not in the P2 preparation (Table 2 and Fig. 4). Highest activity was in the S2 fraction at 2.5 nmole generated/mg protein/min. The time course for the formation of these peptides is shown in Fig. 3b.

Effect of inhibitors

A number of known peptidase inhibitors were tested for activity to inhibit the formation of AII from A(1–9) in the kidney freeze-thawed S2 preparation (Table 4). Inhibitors of ACE such as enalaprilat had no significant inhibitory activity nor did compounds such as phosphoramidon, bestatin, amastatin and Pro-Phe. Surprisingly, EDTA, 1,10-phenanthroline and iodoacetic acid caused an enhancement of AII formation from A(1–9) as well as accelerating the rate of breakdown of A(1–9). Cobalt (II) chloride, an activator of carboxypeptidase N activity caused a 69% reduction of AII formation ($P < 0.005$) (Table 4).

Table 4. Effect of inhibitors on the AII-forming activity from A(1–9) in rat kidney S2

Inhibitor	Concentration	% of control
1,10-Phenanthroline	1 mM	162 ± 13** (N = 7)
Iodoacetic acid	3 mM	242 ± 15*** (N = 7)
Puromycin	1 mM	135 ± 9* (N = 7)
EDTA	1 mM	170 ± 13* (N = 5)
Bestatin	20 μM	116 ± 5 (N = 3)
Amastatin	20 μM	85 ± 12 (N = 3)
Phosphoramidon	20 μM	90 ± 10 (N = 7)
Pro-Phe	0.27 mM	97 ± 2 (N = 3)
Enalaprilat	1 μM	97 ± 10 (N = 7)
Cobalt (II)	0.1 mM	31 ± 3** (N = 4)

* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.001$.

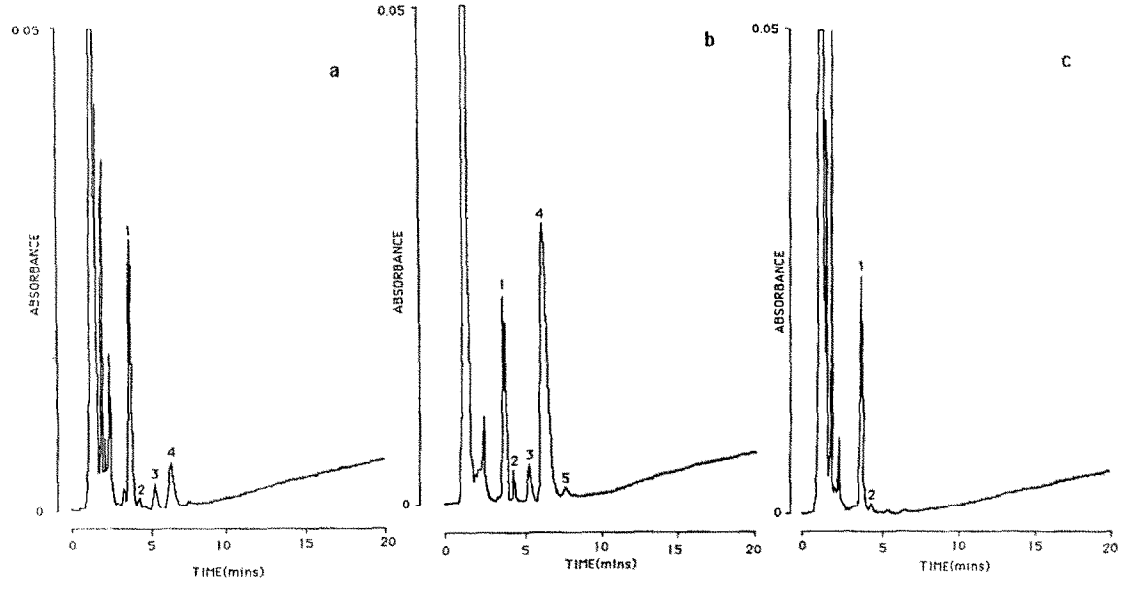


Fig. 4. Chromatograms of 20 min enzyme digests from (a) rat lung S1, (b) rat lung S2 and (c) rat lung P2 freeze-thawed centrifugal fractions using A(1–9) as substrate. Conditions are as described in Fig. 1 and in Materials and Methods. Numbered peaks correspond to those in Fig. 2.

DISCUSSION

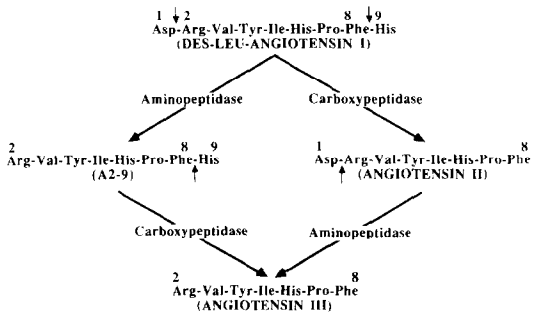
The use of HPLC is established as a reliable and powerful technique to monitor the formation of fragment peptides by enzyme preparations [10] and has proven to be of particular value in understanding the specificities of enzymes involved in the cleavage of angiotensin [2].

Crude homogenates (S1) of rat lung and kidney and the mainly cytosolic centrifugal fraction (S2) were capable of converting A(1–9) to AII. This activity was highest in kidney (S2) fractions which has an activity over 50-fold higher than lung S2. It is of interest that in kidney S2 digestions 57% of the substrate [A(1–9)] that was hydrolysed over 20 min was converted to AII, indicating that the major enzymes in this fraction were carboxypeptidases. The high activity in the kidney and lung S2 fractions compared to the S1 fractions are presumably related to the removal of other peptidases present in the P2 fractions which digest this peptide.

Small amounts of AIII [A(2–8)] and an intermediate peptide [A(2–9)] were also formed in the S2 preparations. These probably resulted from the action of aminopeptidases on AII and A(1–9), respectively (see schema below). In kidney, AIII was produced in larger amounts than A(2–9), whilst in lung S2 the predominant peptide was A(2–9). Only small or trace amounts of AII and AIII were seen in the lung S2. Freshly prepared tissue fractions produced greater amounts of AIII and A(2–9) suggesting that the process of freeze-thawing reduces aminopeptidase activity. This phenomenon has also been previously reported in aorta and lung preparations [2].

These digestion studies show that the kidney S2 but not the particulate fraction (P2) is a rich source of carboxypeptidase activity capable of acting on A(1–9) to produce AII. However, in lung very little carboxypeptidase activity is present capable of producing AII, but relatively high concentrations of aminopeptidase activity are present capable of converting A(1–9) to A(2–9).

The possible pathways of des-leu AI [A(1–9)] degradation leading to these observed peptides is shown in the following schema. In addition, other pathways, present mainly in the particulate fraction, are present which digest these angiotensin peptides to smaller fragments which elute well before the peptides shown in the schema. It is this fraction that also contains most of the ACE activity responsible for the direct activation of AI to AII and endopeptidase activity responsible for cleavage of angiotensin at the Tyr^4-Ile^5 and Pro^7-Phe^6 positions [14].



Inhibitor studies suggest that this carboxypeptidase activity does not resemble carboxypeptidase N [EC 3.4.12.7.] since it was not inhibited by EDTA and PMSF [11] and was not activated by 0.1 mM cobalt [11]. Similar considerations also rule out the related enzymes, carboxypeptidase A [EC 3,4,17,1] [12], carboxypeptidase B [EC 3,4,12,3] and carboxypeptidase E [EC 3,4,17,10] [13]. Carboxypeptidase activity observed recently in a human renal extract [4] was inhibited by Pro-Phe and iodoacetamide but the same concentrations did not inhibit formation of AII from A(1–9) in our studies.

These results suggest that either the carboxypeptidase activity observed acting on A(1–9) is not carboxypeptidase N but some other enzyme showing carboxypeptidase activity, or that the synthetic substrates Hipp-Lys and Hipp-Arg show poor specificity for carboxypeptidase N-like activity.

Initial studies indicate that the synthetic substrates are specific for the carboxypeptidase N group of peptidases, as the effects of cobalt, PMSF and EDTA on activity measured using the synthetic substrates are in accordance with those reported. Therefore, one of the conclusions that can be drawn from the data is that carboxypeptidase N plays a relatively insignificant role in the catabolism of angiotensin peptides by these tissue preparations. It is possible, however, that activity in the P2 fractions is masked by the rapid action of endopeptidases cleaving AI and A(1–9) into smaller peptide fragments. Studies to determine the inhibitor specificities of the soluble carboxypeptidase activity and that of the particulate peptidases are proceeding.

An interesting observation was the potentiation of AII-forming activity in kidney S2 by phenanthroline, EDTA and iodoacetic acid. These nonspecific enzyme inhibitors also accelerated the rate of breakdown of A(1–9). This is not consistent with the hypothesis that they inhibit other enzymes which compete for A(1–9) thus allowing a greater expression of carboxypeptidase activity. A possible explanation is that these inhibitors alter the local environment of the enzyme (pH, ionic strength etc.) in the incubation thus allowing greater expression of enzyme activity or that they inactivate an endogenous inhibitor of these angiotensinases.

In conclusion, these studies provide evidence that enzymatic activities are present in kidney, and to a lesser extent lung, which are capable of producing AII from A(1–9), a peptide which has previously been shown to be a metabolite of AI and whose concentration may rise during inhibition of ACE by inhibitors such as captopril and enalapril [2, 4, 5]. Moreover, A (1–9) has also recently been shown to be a relatively potent inhibitor of ACE *in vitro* [15]. These studies therefore raise the possibility that A(1–9) and the enzymes producing it may play an important role in the overall regulation of the renin-angiotensin system as well as providing a source of AII which is not sensitive to inhibitors of ACE.

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REFERENCES

1. Erdos EG, Conversion of angiotensin I to angiotensin II. *Am J Med* **60**: 749–759, 1976.
2. Johnson H and Drummer OH, Hydrolysis of angiotensin I by peptidases in homogenates of rat lung and aorta. *Biochem Pharmacol* **37**: 1131–1136, 1988.
3. Ng KKF and Vane JR, Fate of angiotensin I in the circulation. *Nature (Lond)* **218**: 144–150, 1968.
4. Changaris DG, Miller JJ and Levy RS, Angiotensin II generated by a human renal carboxypeptidase. *Biochem Biophys Res Commun* **31**: 573–579, 1986.
5. Snyder RA, Watt KW and Wintroub BU, A human platelet angiotensin I-processing system. Identification of components and inhibition of angiotensin-converting enzyme by product. *J Biol Chem* **260**: 7857–7860, 1985.
6. Hopsu UK, Makinen KK and Glenner GG, Purification of a mammalian peptidase selective for N-terminal arginine and lysine residues: aminopeptidase B. *Arch Biochem Biophys* **114**: 557–566, 1966.
7. Friedland J and Silverstein E, A sensitive fluorimetric assay for serum angiotensin converting enzyme. *Am J Clin Pathol* **66**: 416–424, 1976.
8. Bradford MA, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
9. Schweisfurth H, Reinhart E, Heinrich J and Brugger E, A simple spectrophotometric assay of carboxypeptidase N (kininase I) in human serum. *J Clin Chem Clin Biochem* **21**: 605–609, 1983.
10. Davis TP and Culling-Berglund A, High-performance liquid chromatographic analysis of in vitro central neuropeptide processing. *J Chromatogr* **327**: 279–292, 1985.
11. Plummer TH Jr and Erdos EG, Human plasma carboxypeptidase N. *Methods Enzymol* **80**(pt.C): 442–449, 1981.
12. Vallee BL and Galdes A, The metallo biochemistry of zinc enzymes. *Adv Enzymol* **56**: 283–430, 1984.
13. Fricker LD, Neuropeptide biosynthesis: focus on the carboxypeptidase processing enzyme. *Trends Neurosci* **8**: 210–214, 1985.
14. Stephenson SL and Kenny AJ, Metabolism of neuropeptides. Hydrolysis of the angiotensins, bradykinin, substance P and oxytocin by pig kidney microvillar membranes. *Biochem J* **241**: 237–247, 1987.
15. Snyder RA and Wintroub BU, Inhibition of angiotensin-converting enzyme by des-Leu 10-angiotensin I: a potential mechanism of endogenous angiotensin-converting enzyme regulation. *Biochim Biophys Acta* **871**: 1–5, 1986.