Biochemical Pharmacology Vol. 27. pp. 2871–2877.

0006-2952/78/1215-2871 $02.00/0

IN VIVO AND *IN VITRO* CONVERSION OF DES-1-ASP ANGIOTENSIN I TO ANGIOTENSIN III*

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(*Received* 9 *December* 1977; *accepted* 7 *April* 1978)

Abstract—The conversion of exogenous des-Asp angiotensin I to des-Asp angiotensin II (angiotensin III) was studied *in vivo* in the pulmonary circulation of the intact pentobarbital-anesthetized dog and *in vitro* in whole blood and heparin-treated plasma by fractionation of ^{125}I-labeled peptides and by radioimmunoassay. Conversion and hydrolysis of [^{125}I]des-Asp angiotensin I and [^{125}I]angiotensin I were compared *in vitro* and *in vivo*. The time course of conversion of des-Asp angiotensin I to angiotensin III *in vitro* was more rapid than that of angiotensin I to II, but the peak concentration of angiotensin III generated was less than angiotensin II due to the more rapid hydrolysis of the des-Asp peptides. Injection of des-Asp angiotensin I into the right atrium was associated with a pressor response and with the appearance of a small amount of angiotensin III in aortic blood. SQ20881 (a nonapeptide converting enzyme inhibitor) abolished both the pressor response and the generation of angiotensin III. After injection of [^{125}I]des-Asp angiotensin I, only 10 per cent of the labeled material appearing in arterial blood was angiotensin III; 15 per cent was unchanged des-Asp angiotensin I; 15 per cent was an unidentified metabolite; and 60 per cent was tyrosine. This contrasts with the pattern of peptides recovered after injection of [^{125}I]angiotensin I: 70 per cent angiotensin II, 20 per cent intact angiotensin I and only 10 per cent peptide metabolites and tyrosine. Results indicate that conversion of des-Asp angiotensin I to angiotensin III does occur in the pulmonary circulation of the dog but that hydrolysis of the des-Asp peptides by angiotensinases is so rapid that little circulating angiotensin II and only a small pressor response appear. The data suggest that pulmonary conversion of des-Asp angiotensin I is not an important source of circulating angiotensin III in the dog.

Conversion of angiotensin I (AI) to angiotensin II (AII) occurs *in vivo* in the pulmonary capillary bed and *in vitro* in plasma and whole blood. Previous studies [1] have emphasized the importance of the amino acid sequence at the carboxyl-terminus of AI in the conversion process. The effects of structural changes at the amino-terminus of AI have yet to be determined fully. Recent studies in which partially purified pulmonary converting enzyme was used have shown that deletion of the amino terminal aspartic acid of AI enhances the conversion of the resultant nonapeptide (des-Asp AI) to des-Asp AII (angiotensin III or AIII) *in vitro* [2, 3]. This is a potentially important metabolic pathway, since exogenous AIII has a potent stimulatory effect on aldosterone synthesis and release *in vitro* and *in vivo* [4–10]. The present study was undertaken to determine whether des-Asp AI is converted to AIII *in vivo* in the pulmonary circulation of the intact anesthetized dog and *in vitro* in whole blood and in plasma. Our results indicate that conversion of des-Asp AI does occur both *in vivo* and *in vitro* but that hydrolysis of the des-Asp peptides by angiotensinases is so rapid that little circulating AIII and only a small pressor response appear.

* This work was supported in part by USPHS Grant HL 14982, the Medical Cardiology Graduate Training Grant HL 05673, a grant from the Biomedical Center for Population Research USPHS HD 07110, the Louis Block Fund of the University of Chicago and Grant 76-777 from the American Heart Association. Dr. Oparil is an Established Investigator of the American Heart Association.

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MATERIALS AND METHODS

Preparation and characterization of peptides

1-Asp-5-Ile-angiotensin I (AI), 1-Asp-5-Ile-angiotensin II (AII), des-1-Asp-5-Ile-angiotensin II (AIII), and their constituent peptides Val-Tyr-Ile-His-Pro-Phe-His-Leu (3–10), Val-Tyr-Ile-His-Pro-Phe (3–8) and Asp-Arg-Val-Tyr (1–4) were synthesized manually by the solid phase technique and purified as described previously [11, 12]. The nonapeptide des-1-Asp-5-Ile-angiotensin I (des-Asp-AI) was either synthesized by the solid phase technique, purchased from Bachem, Inc., Marina Del Ray, CA, or donated by Dr. M. C. Khosla, Research Division, Cleveland Clinic. All three preparations were used in all studies and consistent results were obtained. The nonapeptide converting enzyme inhibitor SQ20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) was a gift from the Squibb Institute for Medical Research, Princeton, NJ.

Peptides were labeled with ^{125}I by the chloramine-T method of Greenwood *et al.* [13] as modified by Nielsen *et al.* [14]. The details of the iodination procedure and the purification of the monoiodinated peptides on columns of DEAE Sephadex A-25 have been described previously [1]. Over 95 per cent of the labeled material eluted from the columns was monoiodinated peptide. Estimated specific activity of the monoiodinated species was 700 mCi/μmole. The monoiodinated peptides were either used immediately

or frozen with human serum albumin (1 mg/ml) and stored at −20°.

To develop a system for distinguishing generated [^{125}I]AIII and [^{125}I]AII from [^{125}I]des-Asp-AI and [^{125}I]AI and from the (3–10), (3–8) and (1–4) peptides and [^{125}I]Tyr, the probable products of circulating angiotensinases, labeled peptides and Tyr were subjected to high voltage paper electrophoresis and column chromatography on DEAE Sephadex A-25, as reported previously [1]. Peptides were spotted on Whatman 3MM paper and subjected to electrophoresis at 4000 V for 1 hr at pH 3.6 in pyridine acetate buffer. The papers were cut into thirty 1-cm strips and counted in a gamma counter. The remainder of the sample was passed over a column of DEAE Sephadex A-25. The 0.9 × 100 cm column was developed with 0.1 N sodium phosphate buffer, pH 7.4, at room temperature. One ml of a 3-ml column eluate was counted. Since all the conversion experiments were analyzed by both techniques, it was possible to distinguish [^{125}I]AIII and [^{125}I]AII from most of the probable peptide metabolites of [^{125}I]des-Asp-AI and [^{125}I]AI and from [^{125}I]Tyr. The positions of the model peptides are included for reference in Fig. 5.

In order to quantify unlabeled des-Asp AI, AII and AIII in plasma, the peptides were subjected to radioimmunoassay by standard procedures for AI [15] and AII [16]. The amount of peptide required to produce 50 per cent displacement of [^{125}I]AI from anti-AI or [^{125}I]AII from anti-AII was determined and compared with the amount of AI or AII required to do so. The ratio represents per cent cross-reactivity.

In vitro *studies*

Plasma. Mongrel dogs were anesthetized with pentobarbital (20 mg/kg, i.v.). Samples (20 ml) of blood were drawn from the femoral artery into plastic syringes containing 200 units (USP) of heparin, immediately placed on ice, and subsequently centrifuged at 4° and the plasma separated. [^{125}I]des-Asp AI, [^{125}I]AI, or [^{125}I]AIII was added to 8 ml plasma to a final concentration of 1.5 × 10^6 cpm/ml or 0.85 pmoles/ml and incubated in a water bath at 37°. Aliquots of 1.1 ml were removed at 0 time and at 0.5, 1, 2, 5, 10, 15 and 30 min and placed in tubes containing 0.15 ml of an inhibitor solution made up of ethylenediaminetetraacetate (2.6 mM, final concentration), dimercaptol (1.6 mM, final concentration) and 8-OH-quinoline (3.4 mM, final concentration). Fifty μl of each sample was immediately spotted on Whatman 3MM paper and subjected to high voltage paper electrophoresis as described previously. The remainder of the sample was passed over a column of DEAE Sephadex A-25 as described previously.

Whole blood. Samples (35 ml) of blood were drawn from the femoral artery of pentobarbital-anesthetized dogs into siliconized plastic syringes containing [^{125}I]des-Asp AI, [^{125}I]AI, or [^{125}I]AIII (7.0 × 10^6 cpm/ml or 3.5 pmoles/ml, final concentration). At 0 time and at 10, 20, 30, 45, 60 and 120 sec 5-ml samples of blood were placed in an iced tube containing 0.5 ml of the inhibitor solution described above. The samples were centrifuged at 4° and the plasma was separated. Fifty μl of each sample was immediately spotted on Whatman 3MM paper for electrophoresis and 1 ml of the remainder of each sample was passed over a column of DEAE Sephadex A-25 as described previously.

In vivo *studies*

Nine male or non-pregnant female mongrel dogs (18–22 kg) were anesthetized with sodium pentobarbital (20 mg/kg, i.v.). An endotracheal tube was placed in each dog and connected to a Harvard respirator. A large bore catheter was placed in the right femoral artery and advanced to the middle of the descending aorta for blood sampling. A smaller catheter was passed through the left femoral artery to the aorta and connected to a pressure-transducer (Hewlett–Packard, Loveland, CO, model 1280B). Mean blood pressure was recorded continuously on a Hewlett–Packard Sanborn System. A Swan–Ganz catheter was advanced through the right jugular vein into the right atrium under fluoroscopic monitoring.

The pressor response to various peptides was determined by injecting, in a single bolus, AI (10 or 20 nmoles), AII (5 nmoles), AIII (5, 10 or 20 nmoles), or des-Asp AI (20, 50 or 100 nmoles) into the right atrium while monitoring the mean blood pressure.

In order to assess intrapulmonary conversion of des-Asp AI to AIII and compare it with the conversion of AI to AII, peptides were injected into the right atrium and the products of conversion were identified and quantified in aortic blood. [^{125}I]des-Asp AI (nine dogs) or [^{125}I]AI (two dogs) was injected as a single bolus of 1 × 10^8 cpm or 60 pmoles into the right atrium. To assess the stability of AIII in the pulmonary circulation, [^{125}I]AIII (four dogs) was injected as a single bolus of 1 × 10^8 cpm or 60 pmoles into the right atrium. This dose of labeled peptide was subpressor but gave a convenient concentration of circulating radioactive material in aortic plasma (1 × 10^5 cpm/ml or 0.06 pmole/ml). Unlabeled des-Asp AI was injected in doses of 20, 50 and 100 nmoles and AI in doses of 20 nmoles into the right atrium of six dogs. The effect of the converting enzyme inhibitor SQ20881 on the pulmonary handling of des-Asp AI was examined in six dogs. Once baseline responses to des-Asp AI and AI had been established, SQ20881 (5 μmoles) was injected as a bolus into the right atrium, followed in 30 sec by a second bolus of [^{125}I]des-Asp AI (1 × 10^8 cpm) and shortly thereafter one of unlabeled AI (20 nmoles). AI was then injected in 20-nmoles boluses 30 min post-SQ20881 and at 15-min intervals until the pressor response returned. At that time the [^{125}I]des-Asp AI injection was repeated. After each injection blood was collected in six sequential 10-ml samples from the descending aorta at 5-sec intervals. A series of plastic syringes connected via a manifold system and containing 2.6 mM ethylenediaminetetraacetate (EDTA), 1.6 mM dimercaptol, and 3.4 mM 8-OH-quinoline (final concentration) was used for collection. Samples which contained labeled peptides were processed and analyzed as described previously for plasma. Products were identified by comparison with standard peptides and [^{125}I]Tyr as described previously [1]. Samples which contained unlabeled material were subjected to radioimmunoassay for AI [15] and AII [16]. The second and third sequential samples obtained 5–15 sec after injection represented one circulation through the lung as determined by the appearance time of

radioactive material. The sample containing the largest amount of labeled material (always either the second or third sample in these dogs) was used to assess the extent of pulmonary conversion. Since the systemic circulation times of the dogs averaged 20 ± 2 sec, significant recirculation was unlikely, so the results were not distorted by the effects of conversion in the systemic capillary beds. At least 10 min were allowed to elapse between injections of unlabeled material to permit resolution of the pressor response and to allow for metabolism and clearance of immunoreactive material; 30 min were allowed to elapse between injections of labeled peptides to avoid interference with residual labeled material in the plasma.

RESULTS

Peptide characterization

The peptides synthesized in our laboratory were subjected to amino acid analyses on a Durrum analyzer after hydrolysis with 5.7 N HCl for 24 hr at 110°. Acid hydrolysis yielded the amino acids in the expected ratio.

Each unlabeled peptide (1 mg) or each iodinated peptide (1×10^5 cpm) was subjected to descending paper chromatography followed by electrophoresis in a second dimension as described previously [1]. Each peptide produced a single spot with ninhydrin-collidine stain or a single radioactive spot.

The concentration of each peptide at 50 per cent displacement and the per cent cross-reactivity are summarized in Table 1. Des-Asp AI showed nearly 100 per cent cross-reactivity with anti-AI. The cross-reactivity of AIII with anti-AII was 20 per cent. This was sufficient to allow detection of pressor doses of AIII in aortic blood by radioimmunoassay using anti-AII and [^{125}I]AII.

In vitro *studies*

Plasma. Figure 1 summarizes the metabolism of [^{125}I]des-Asp AI and [^{125}I]AI in previously frozen dog plasma treated with heparin. The half-life of [^{125}I]des-Asp AI was 45 sec; of [^{125}I] AI, 5.5 min. Over 80 per cent of the [^{125}I]des-Asp AI was hydrolyzed within the first 2 min of incubation. Measurable amounts of AIII appeared in plasma within the first 30 sec of incubation, but the per cent of total counts present as AIII was never > 20. In contrast, the time course of conversion of AI to AII was slower and the absolute amount of AII generated was greater. Figure 2 shows that AIII, like des-Asp AI, was hydrolyzed rapidly. The half-life of [^{125}I]AIII was 60 sec.

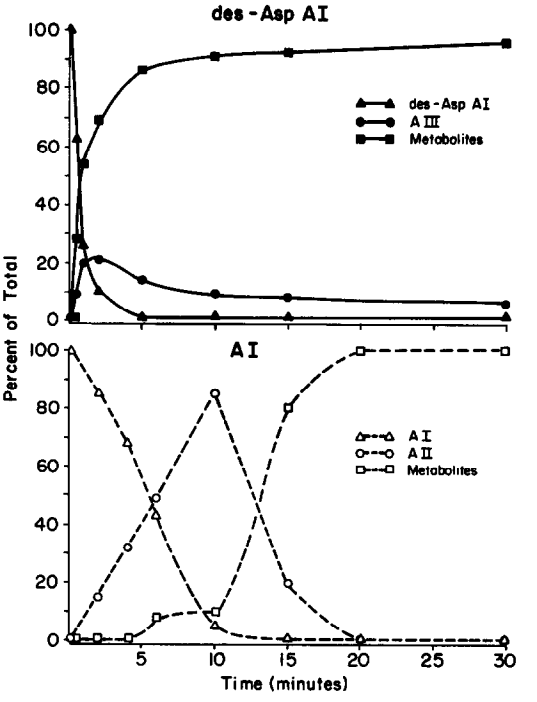

Fig. 1. *In vitro* metabolism of [^{125}I]des-Asp AI (upper panel) and [^{125}I]AI (lower panel) in heparin-treated dog plasma. The disappearance of des-Asp AI and AI and appearance of AIII, AII and metabolites are plotted against time. Each labeled species is quantified as a per cent of the total labeled material.

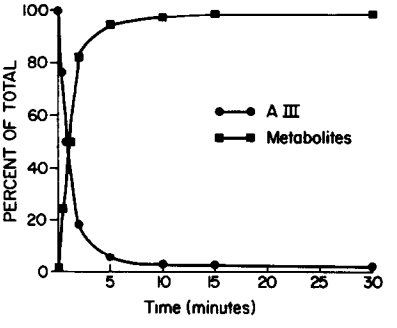

Fig. 2. *In vitro* metabolism of [^{125}I]AIII in heparin-treated dog plasma. The disappearance of AIII and appearance of metabolites are plotted against time. Each labeled species is quantified as a per cent of the total labeled material.

Table 1. Cross-reactivity studies—Comparative displacement by various peptides of [^{125}I]AI from anti-AI and of [^{125}I]AII from anti-AII

	Anti-AI		Anti-AII	
Peptide	Concn at 50% displacement (pmoles/ml)	% Cross-reactivity	Concn at 50% displacement (pmoles/ml)	% Cross-reactivity
AI	0.05	100	5	0.10
des-Asp AI	0.05	100	5	0.10
AII	300	0.016	0.005	100
AIII	> 10,000	< 0.001	0.025	20

Whole blood. Figures 3 and 4 summarize the time course of metabolism of [^{125}I]des-Asp AI, of [^{125}I]AI and of [^{125}I]AIII in whole dog blood with no anticoagulant. The half-life of [^{125}I]des-Asp AI and of [^{125}I]AIII was approximately 25–30 sec, or less than two pulmonary circulation times. In contrast, [^{125}I]AI had a half-life of 3 min and disappearance (summation of breakdown and conversion) occurred to the extent of only 20 per cent in 25 sec. As in plasma, measurable amounts of AIII appeared before AII, but AIII

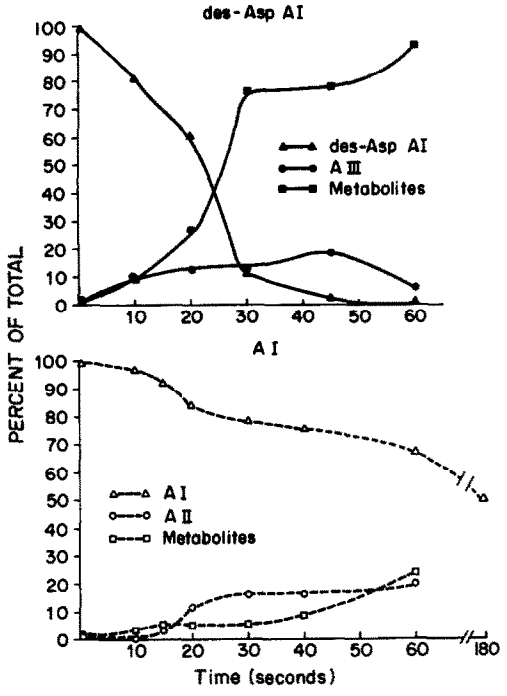

Fig. 3. *In vitro* metabolism of [^{125}I]des-Asp AI (upper panel) and [^{125}I]AI (lower panel) in dog blood without added anticoagulant. The disappearance of des-Asp AI and AI and appearance of AIII, AII and metabolites are plotted against time. Each labeled species is quantified as a per cent of the total labeled material.

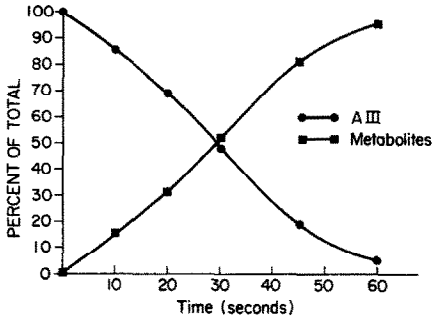

Fig. 4. *In vitro* metabolism of [^{125}I]AIII in dog blood without added anticoagulant. The disappearance of AIII and the appearance of metabolites are plotted against time. Each labeled species is quantified as a per cent of total labeled material.

generated from [^{125}I]des-Asp never accounted for > 20 per cent of total counts present.

The data suggest that in whole blood, as in heparin-treated plasma, conversion of des-Asp AI to AIII was more rapid than conversion of AI to AII but that degradation of the des-Asp peptides was so rapid that the net generation of AIII was small.

In vivo *studies*

Table 2 summarizes the mean (± 1 S. E. M.) pressor responses to various peptides in a series of

Table 2. Pressor responses to infused peptides*

Peptide	Dose (nmoles)	Increase in mean arterial pressure (mm Hg)
AI	10	42 ± 5
	20	63 ± 4
Immediately post-SQ20881	20	No pressor response
30 min post-SQ20881	20	36 ± 6
des-Asp AI	20	14 ± 1
	50	20 ± 1
	100	21 ± 1
Immediately post-SQ20881	100	No pressor response
30 min post-SQ20881	100	16 ± 2
AII	5	34 ± 2
AIII	5	21 ± 1
	10	27 ± 2
	20	36 ± 4

* Values given = mean ± S. E. M. (N = 8).

eight pentobarbital-anesthetized dogs. The data indicate that des-Asp AI had one-fifth the pressor activity of an equimolar amount of AI and that AIII had one-fourth the pressor activity of AII in this preparation. Administration of SQ20881 blocked, for a brief period, the pressor response to both des-Asp AI and AI but did not lower baseline blood pressure. The pressor effects of des-Asp AI and AI reappeared within 30 min of injecting SQ20881. SQ20881 did not modify the pressor response to exogenous AII or AIII (not shown).

The apparent concentrations of immunoreactive AI and AII in aortic blood after injection of AI or des-Asp AI into the right atrium alone and after SQ20881 are summarized in Table 3. The values for AII after injection of either AII or AIII are included for comparison. The apparent concentration of AII represents the value calculated for AII concentration using the standard radioimmunoassay for AII and assuming that AII is the peptide being measured. After injection of des-Asp AI or AIII, the principal peptide measured by the radioimmunoassay for AII

Table 3. Pulmonary conversion of unlabeled peptides *in vivo**

Peptide Injected	Dose (nmoles)	Apparent concn of peptide in aortic blood (pmoles/ml)	
		AI	AII
Pre-SQ20881			
AI	20	39 ± 6	9 ± 2
des-Asp AI	100	58 ± 18	5 ± 1
AII	5		5 ± 2
AIII	20		2 ± 1
Post-SQ20881			
AI	20	46 ± 11	0.6 ± 0.1
des-Asp AI	100	61 ± 13	0.3 ± 0.1

* Immunoreactive peptides in aortic blood after injection of AI or des-Asp AI into the right ventricle. AII and AIII were injected as controls.

Values given = mean ± S. E. M. (N = 6). Apparent concentrations of peptides represent direct measurements of each peptide using the radioimmunoassays for AI or AII.

was really AIII, so values for apparent concentration of AII should be corrected for the 20 per cent cross-reactivity between AIII and anti-AII. Peptides containing the (3–8) and (4–8) sequence of AII showed a smaller per cent cross-reactivity with anti-AII. The experiments with labeled peptides did not give evidence of generation of the (3–8) peptide *in vivo*, so no attempt was made to estimate its contribution to the apparent concentration of AII here. The (4–8) peptide could not be identified and quantified by the methods used. The presence of the (3–8) and (4–8) peptides in arterial blood would cause an overestimation of the concentrations of AII and AIII by the radioimmunoassay for AII. The apparent concentration of AI after injection of des-Asp AI represents the value calculated for AI concentration using the standard radioimmunoassay for AI and assuming that AI is the peptide being measured. Since there was 100 per cent cross-reactivity between des-Asp AI and anti-AI, no correction is necessary. After injection of 100 nmoles des-Asp AI, the concentration of injected peptide and the total concentration of immunoreactive material appearing in aortic blood were only 1.5 times the corresponding values when 20 nmoles AI was administered. This indicates that the des-Asp peptides were hydrolyzed to a greater extent in the pulmonary circulation than were AI and AII. The concentration of AIII in aortic blood as assessed by the radioimmunoassay for AII reflects a detectable conversion of des-Asp AI to AIII. There was no measurable AII or AIII in aortic blood after injection of AI or des-Asp AI, respectively, in animals pretreated with SQ20881. This indicates that SQ20881 blocked conversion of both AI and des-Asp AI in the pulmonary circulation.

Table 4 summarizes the results of six experiments in which [^{125}I]des-Asp AI was injected. After administration of [^{125}I]des-Asp AI alone, only 10 per cent of labeled material appearing in arterial blood was [^{125}I]AIII, 15 per cent was unchanged [^{125}I]des-Asp AI, 15 per cent was an unidentified metabolite, and 60 per cent was [^{125}I]tyrosine. This contrasts with the pattern of metabolites recovered after injection of [^{125}I]AI alone in this and previous studies [1]: 70 per cent [^{125}I]AII; 20 per cent intact [^{125}I]AI, and only 10 per cent peptide metabolites and [^{125}I]tyrosine. Immediately after administration of SQ20881, no [^{125}I]des-Asp AI was converted to [^{125}I]AIII, but the hydrolysis of the des-Asp peptide was otherwise unaffected. Recovery of AIII generation was demonstrated at 30 min post-SQ20881. SQ20881 in comparable doses completely blocked conversion of [^{125}I]AI to [^{125}I]AII, and after administration of SQ20881 90 per cent of the labeled material passing through the lung was intact [^{125}I]AI. Results from the six experiments were reproducible within 10 per cent.

Table 4 also summarizes the results from four experiments in which [^{125}I]AIII was injected. Approximately 75 per cent of the [^{125}I]AIII administered into the right atrium was hydrolyzed to labeled tyrosine and an unidentified metabolite in a single passage through the pulmonary circulation. After administration of [^{125}I]AIII, 25 per cent of labeled material appearing in arterial blood was intact [^{125}I]AIII, 20 per cent was an unidentified metabolite, and 55 per cent was [^{125}I]tyrosine. Results from four experiments in which [^{125}I]AIII was injected were reproducible within 10 per cent.

Figure 5 demonstrates the results of a typical experiment in which [^{125}I]des-Asp AI was injected. The [^{125}I]des-Asp AI was largely hydrolyzed to smaller peptides and [^{125}I]tyrosine. Fifteen per cent of the material recovered was an unidentified metabolite. No (3–10), (3–8) or (1–4) peptide was detected in the pulmonary venous effluent, as indicated in the figure.

DISCUSSION

Recent studies have shown that des-Asp AI is a better substrate than AI for partially purified angiotensin converting enzyme from porcine lung *in vitro* [2, 3]. The K_m for conversion of des-Asp AI as estimated from Lineweaver–Burk plots was one-third that of AI [2]. Conversion of des-Asp AI was less dependent on the chloride concentration of the media and less easily inhibited by other peptides, such as bradykinin, SQ20881, AII and AIII than was the conversion of AI. Further, Lys-bradykinin and Met-Lys-bradykinin have been shown to be hydrolyzed more slowly than bradykinin and the tetradecapeptide renin substrate more slowly than its C-terminal nonapeptide by the dipeptidylcarboxypeptidase converting enzyme [17, 18]. Thus, the selectivity of angiotensin converting enzyme, which is determined primarily by the C-terminal sequence of its substrate [19], appears to be modified by the sequence of N-terminal residues distant from the site of hydrolysis [17, 18]. Taken together, these data suggest that the specificity of

Table 4. *In vivo* metabolism of [^{125}I]des-Asp AI and [^{125}I]AIII in the pulmonary circulation of the dog*

	N	des-Asp AI	AIII	Metabolite	Tyrosine
		After injection of [^{125}I]des-Asp AI			
No SQ20881	6	15.6 ± 3.6	10.4 ± 1·1	14.4 ± 3.2	59.6 ± 7.8
Immediately post-SQ20881	6	22.4 ± 2.9	0	28.4 ± 5.2	49.2 ± 6.0
Thirty min post-SQ20881	6	18.9 ± 1.5	7.1 ± 1.3	27.0 ± 5.0	47.0 ± 7.5
		After injection of [^{125}I]AIII			
No SQ20881	4		24.6 ± 5.3	20.2 ± 3.8	45.2 ± 8.3

* Per cent distribution of labeled material in aortic blood after injection into the right ventricle. Values given = mean ± S. E. M.

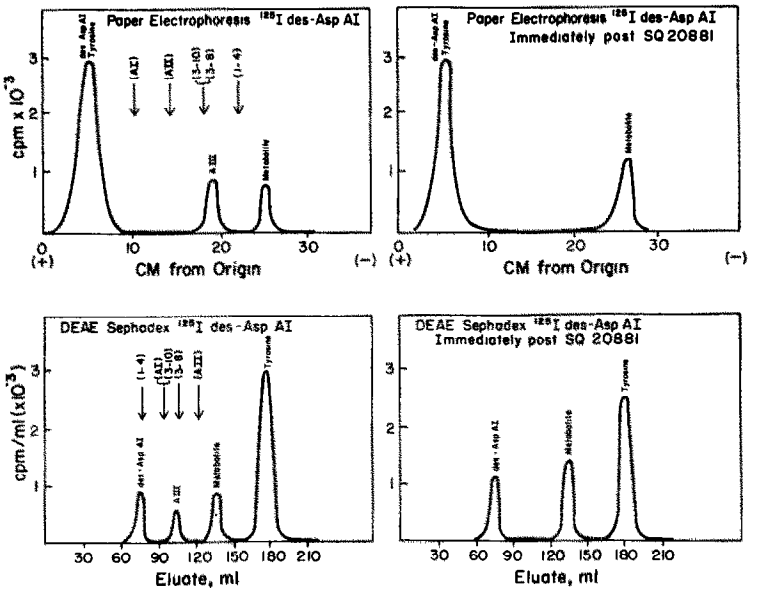

Fig. 5. Pulmonary handling of [^{125}I]des-Asp AI *in vivo*. Paper electrophoretic separation (above) and column chromatography (below) of ^{125}I-labeled peptides collected in aortic blood 10–15 sec after a bolus injection into the right ventricle. The positions of standard [^{125}I]AI, [^{125}I]AII, [^{125}I]AIII, [^{125}I]des-Asp AI, [^{125}I](1–4), [^{125}I](3–8), [^{125}I](3–10) and [^{125}I]Tyr are included in parentheses in the left panel for reference.

angiotensin converting enzyme is such that conversion of des-Asp AI to AIII could take place *in vivo* in the pulmonary capillary bed and *in vitro* in systems containing plasma converting enzyme.

The current study has given evidence that exogenous des-Asp AI is converted to AIII *in vitro* in dog blood and in heparin-treated dog plasma and *in vivo* in the pulmonary circulation of the intact dog. [^{125}I]AIII was generated *in vitro* from [^{125}I]des-Asp AI more rapidly than [^{125}I]AII from [^{125}I]AI. Further, [^{125}I]AIII appeared in aortic blood within a single circulation time after injection of [^{125}I]des-Asp AI into the right atrium in doses which gave rise to near physiologic concentrations of labeled peptide. Similarly, AIII was detected by radioimmunoassay with anti-AII after injection of unlabeled des-Asp AI at several thousand times the physiologic concentration. Des-Asp AI had a pressor effect which was similar in timing but lesser in magnitude than that of AI. The converting enzyme inhibitor SQ20881 abolished both the pressor response and the generation of AIII that followed the administration of des-Asp AI. The data indicate that there is a mechanism for intrapulmonary conversion of des-Asp AI to AIII in the pentobarbital-anesthetized dog which is of high capacity and is susceptible to inhibition by SQ20881. Further, the early appearance time of [^{125}I]AIII *in vitro* suggests that des-Asp AI may be a better substrate than AI for plasma converting enzyme.

Exogenous des-Asp AI has been shown to have steroidogenic and pressor activities in the conscious rat that can be blocked using a converting enzyme inhibitor [20, 21]. Des-Asp AI was found to be less potent than AI or AII in stimulating aldosterone release or raising blood pressure. These observations suggest the possibility that conversion of des-Asp AI to AIII may represent a metabolic pathway for the generation of AIII *in vivo* and that the AIII so formed has steroidogenic and pressor effects. Since des-Asp AI and its peptide metabolites were not specifically identified and quantified, however, the biochemical events responsible for these observations and the biological importance of pulmonary conversion of des-Asp AI in the rat remain uncertain.

The role of intrapulmonary conversion of des-Asp AI to AIII in mediating circulatory homeostasis depends on: (1) the availability of circulating des-Asp AI as substrate for converting enzyme, (2) the survival of the des-Asp peptides in the circulation, and (3) the pressor and aldosterone-stimulating potency of generated AIII. Aminopeptidases capable of removing the N-terminal aspartic acid from AI or AII have been demonstrated in plasma, red blood cells and tissue homogenates [22–24]. These enzymes have not been highly purified, and their specificities and selectivities are incompletely understood. Thus, a combination of these enzymes circulating in blood may not only cause generation of des-Asp AI from AI but may also hydrolyze the entire molecule by stepwise removal of N-terminal residues. Generation of a peptide that resembled des-Asp AI from exogenous AI in rat lung and cat adrenal has been reported [25–27]. The sequence of the peptide was not established with certainty, however. Direct evidence that endogenous des-Asp AI is present in the circulation is lacking.

The current study demonstrated rapid hydrolysis of both des-Asp AI and AIII in the pulmonary circulation *in vivo* and in blood and plasma *in vitro*. After injection of either [^{125}I]des-Asp AI or [^{125}I]AIII into the right atrium, 75 per cent of the labeled material in aortic blood after a single passage through the lung was in the form of hydrolysis products, either [^{125}I]-tyrosine or an unidentified metabolite. Experiments in which total immunoreactive material was measured by radioimmunoassay using anti-AI and anti-AII after injection of unlabeled des-Asp AI generally con-

firmed these observations. The radioimmunoassay experiments yielded less precise results, however, because of the need to correct for the cross-reactivity of AIII with anti-AII and because some of the unidentified metabolites of AII cross-react with anti-AII. In contrast, after injection of [^{125}I]AI, 90 per cent of the labeled material in aortic blood was either intact AI or AII. This is consistent with a number of studies which have shown that angiotensin II survives the pulmonary circulation intact [28–30].

The rapid ($t_{1/2}$ = 25 sec, less than two circulation times in our anesthetized dog preparation) destruction of [^{125}I]des-Asp AI and of [^{125}I]AIII in whole dog blood *in vitro* is compatible with the notion that circulating angiotensinases contribute to their hydrolysis in the pulmonary circulation *in vivo*. In contrast, the $t_{1/2}$ of [^{125}I]AI in dog blood was prolonged (3 min), suggesting that circulating angiotensinases were not important determinants of its metabolism *in vivo*. The predominant angiotensinase in plasma under physiological conditions is an α-aspartyl aminopeptidase frequently referred to as angiotensinase A [31] which has the same specificity as aminopeptidase A [32]. Accordingly, one would expect peptide fragments of AII from which the amino terminal sequence of AII has been deleted to have shorter half-lives than the parent octapeptide [9]. Our data and those of Semple *et al.* [33], who observed that the half-lives of AIII and the (3–8) and (4–8) peptides in human blood *in vitro* are shorter (2.0, 1.0 and 2.4 min, respectively) than that of AII (4.4 min), support this hypothesis.

The lability of des-Asp AI and AIII *in vitro* in dog blood and *in vivo* in the intact dog results in low circulating concentrations of AIII. In the current study, AIII never accounted for >20 per cent of the total labeled or immunoreactive material *in vivo* or *in vitro*. This is consistent with the observations of Caravaggi *et al.* [34], who found that endogenous AIII and the (3–8) and (4–8) fragments of AII circulate in very low concentrations (< 20 per cent of the simultaneous AII concentration) in the dog. Stimulation of endogenous AII generation by renal artery constriction and infusion of exogenous AII did not change the proportions of the peptides. Similar data were obtained in man: AIII accounted for only 10 per cent of the immunoreactive (with anti-AII) material in arterial plasma [33]. Of species studied to date only the rat has a higher concentration of AIII than AII in arterial and venous plasma [35].

AIII and AII are equipotent on a molar basis as stimulators of aldosterone release in the dog [34], and AIII is a less potent pressor agent than AII [3] in that species. These data, together with the enhanced susceptibility of the des-Asp angiotensins to hydrolysis by circulating angiotensinases and the low plasma concentration of AIII achieved after administration of des-Asp AI observed in the current study, suggest that the pulmonary conversion of des-Asp AI to AIII is not an important source of circulating vasoactive hormone in the dog.

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