Angiotensin II in Arterial and Renal Venous Plasma and Renal Lymph in the Dog

MICHAEL D. BAILIE, FLOYD C. RECTOR, JR., and DONALD W. SELDIN

From the Department of Internal Medicine, University of Texas (Southwestern) Medical School, Dallas, Texas 75235

ABSTRACT Angiotensin II was determined by radioimmunoassay in systemic arterial, pulmonary arterial, and renal venous plasma and in renal hilar lymph in dogs. Levels of the peptide were determined prior to and during progressive graded hemorrhage or reduction in renal perfusion pressure. Levels of angiotensin II in plasma consistently rose during transit through the lung indicating pulmonary conversion of angiotensin I to angiotensin II. On the other hand, angiotensin II in the renal vein plasma was less than that in arterial plasma indicating renal extraction of the peptide from plasma. When renal hilar lymph was sampled under similar conditions, angiotensin II in lymph was consistently higher than that in arterial or renal venous plasma. Furthermore, in some experiments angiotensin II in lymph increased at a time when the concentration in plasma was undetectable. No evidence was found to indicate that angiotensin II in plasma entered renal lymph. It was concluded that angiotensin II levels in lymph reflected the concentration of angiotensin II in renal tissue. The data further suggested that angiotensin II is partially removed from arterial plasma by hydrolysis during transit through the kidney.

INTRODUCTION

Several investigators have proposed models in which angiotensin II (AII) is a critical mediator for the control of glomerular filtration rate (1), renal autoregulation (2), and tubular sodium reabsorption (3, 4). It is not clear whether the AII postulated to mediate these control mechanisms is produced within the kidney or reaches the kidney from the systemic circulation.

This work was presented in part at the meeting of the American Federation for Clinical Research, Atlantic City, N. J., 3 May 1970 (*Clin. Res.* **18**: 493).

Dr. Bailie's present address is Department of Human Development, Michigan State University College of Human Medicine, East Lansing, Mich.

Received for publication 22 June 1970 and in revised form 14 September 1970.

The production of AII requires angiotensinogen and renin for the formation of angiotensin I (AI) and converting enzyme(s) for generation of AII from AI. Ng and Vane have demonstrataed that AII in the systemic circulation is mainly derived from the conversion of AI in the pulmonary capillary bed (5). If AII is produced locally within the kidney, then the appropriate enzyme systems must be present in renal tissue. Bakhle, Reynard, and Van were unable to demonstrate conversion of AI to AII in the kidney (6). Recently however, Gocke, Gerten, Sherwood, and Laragh, by use of a radioimmunoassay for AII, found increased concentrations of the peptide in the renal venous plasma of affected kidneys in patients with unilateral renal artery obstruction and hypertension (7). This finding would suggest that generation of AII can take place in the kidney.

In order to investigate further the metabolism of AII by both lung and kidney, a sensitive radioimmunoassay has been used to determine the concentration of AII in systemic arterial, renal venous, and pulmonary arterial plasma, and renal lymph during stimuli usually associated with increased renin secretion by the kidney. The results support the concept that the lung is a major site of conversion of AI to AII and that AII is extracted from plasma during transit through the kidney. However, high levels of AII in renal lymph when plasma levels are undetectable suggest the generation of AII within the kidney and the presence of the enzyme(s) needed for conversion of AI to AII.

METHODS

Preparation of animals. Experiments were carried out on mongrel dogs anesthetized with pentobarbital sodium (30 mg/kg). A peripheral vein was catheterized for infusion of solutions and additional anesthetic agent. Both femoral arteries were catheterized to record blood pressure by strain-gauge transducer, (Statham P23 AA), obtain arterial blood samples, and remove blood for the purpose of inducing hypotension. In all experiments the left kidney was exposed through an extraperitoneal flank incision and a catheter placed in the left renal vein.

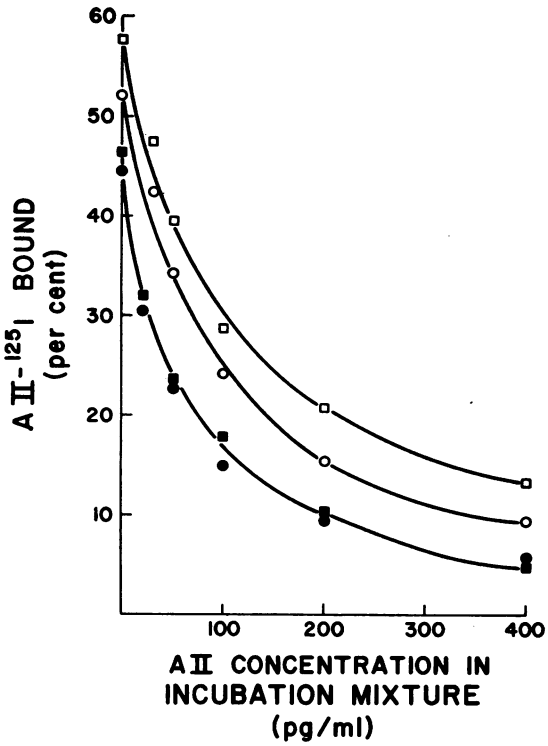

FIGURE 1 Effect of changing protein concentration in the incubation mixture at pH 7.5 and 8.6 on the radioimmunoassay of AII. ○——○ with protein pH 7.5; □——□ without protein pH 7.5; ●——● with protein pH 8.6; ■——■ without protein pH 8.6.

In those animals in which hypotension was induced by bleeding, the right kidney was removed through a flank incision; this procedure eliminated a source of renin or AII other than that from the kidney being studied. In some experiments, in order to sample pulmonary arterial blood, a catheter was passed into the pulmonary artery via the external jugular vein using pressure as a guide to placement. In seven experiments a hilar lymph vessel was isolated and cannulated with polyethylene tubing (PE 12). In three animals reduction in renal arterial perfusion pressure was accomplished by tightening a snare placed around the aorta above the renal artery. In those experiments involving intrarenal arterial infusions, a curved 23 gauge needle attached to a polyethylene catheter was placed directly into the renal artery.

A minimum of 1 hr was allowed for the recovery of the animal after the completion of surgery, and all animals were heparinized prior to the beginning of the experiment. All blood samples were drawn into plastic syringes and immediately transferred to chilled tubes containing disodium ethylenediaminetetraacetic acid (EDTA). Renal lymph was collected in similarly chilled tubes. After centrifugation at 4°C, the plasma was removed and both plasma and lymph stored at −20°C until assayed for angiotensin.

Radioimmunoassay of angiotensin II. The radioimmunoassay used was a modification of the method previously described by Gocke and coworkers (7). Antisera were produced in rabbits after immunization with aspartyl-*l*-valyl-5 angiotensin II (Ciba) conjugated to rabbit serum albumin (Pentex), as described by Goodfriend, Levine, and Fasman (8). Radioiodinated angiotensin II (AII-^{125}I) was obtained from a commercial source (Cambridge Nuclear Corp.).

To prepare the incubation mixture, standard AII or unknown plasma was added to 0.1 M Tris buffer (pH 8.6) containing 1 mg/ml lysozyme and 2 mg/ml neomycin sulfate. To this solution 50 μl of antiserum (dilution 1:3000) and 50 μl of labeled hormone containing 5 μμg AII (1500–2000 cpm) were added. To keep the protein content of the standards similar to that of the unknowns, 100 μl of AII-free plasma was added to the standard tubes. This plasma was prepared by dialyzing plasma for 24 hr against distilled water and 24 hr against 0.003 M disodium EDTA at 4°C. The final volume of the incubation mixture was brought to 0.5 ml with Tris buffer. The tubes were incubated for 24 hr at 4°C.

Separation of antibody-bound and free AII was accomplished with dextran-coated charcoal (9); 100 μl of dextran-coated charcoal was added to the incubation mixture and the tubes centrifuged at 10,000 *g* for 15 min at 4°C. 400 μl of supernatant was removed after which the charcoal was washed once with cold buffer. Both supernatant and charcoal were counted in a well type scintillation counter (Tracer Lab), and the per cent labeled hormone bound to antibody was calculated. Concentration of AII in unknowns was expressed as picograms per milliliter of plasma or lymph.

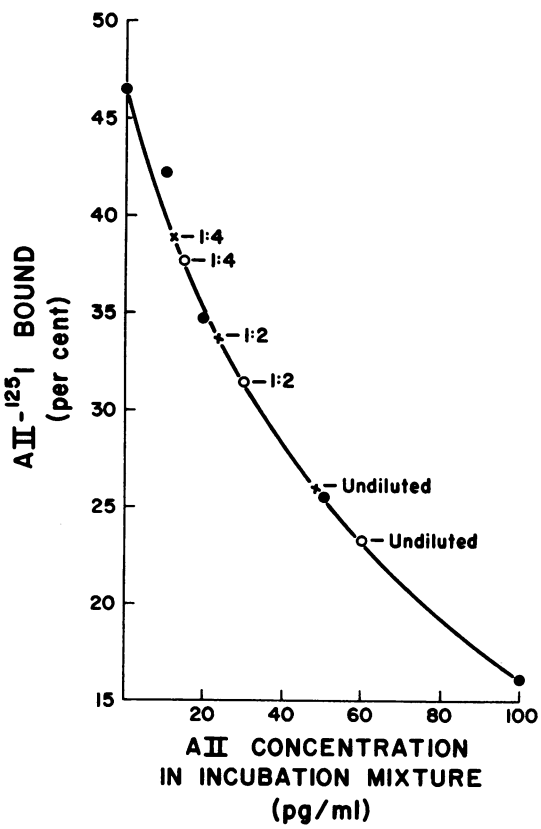

FIGURE 2 Effect of serial dilution of standard AII added to AII-free plasma (○) and AII in an unknown plasma (X). The diluted samples fall at the expected points on the standard curve (●——●).

^{14}C was determined by liquid scintillation counting (Beckman LS-100) using a cocktail containing toluene, PPO, and Triton X-100 (Packard).

RESULTS

Characterization of the radioimmunoassay. Gocke et al., utilizing Tris buffer at pH 7.5, found that replacement of plasma in the incubation mixture with buffer resulted in a shift in the standard curve, most likely owing to the change in protein concentration (7). This is demonstrated in Fig. 1 where the percentage of labeled antigen bound to antibody is plotted against the concentration of standard AII in the incubation mixture. At pH 7.5 the curve is shifted upward when plasma is replaced with buffer. On the other hand, at pH 8.6 the curve is not affected by removal of plasma. This modification is significant because the protein content of renal lymph is less than that of plasma (10).

Fig. 2 demonstrates that the naturally occurring AII in unknown plasma and the standard AII added to plasma react in a similar manner with the antibody. Serial dilution of the unknown or the standard result in a decrease of binding, and the points fall at the expected intervals on the curve.

The addition of ^{125}I-labeled AII in increasing 50-μl increments from 50 to 350 μl results in a uniform depression of binding as shown in Fig. 3. This finding indicates that the labeled hormone has a reactivity with the antibody similar to that of the standard material.

The specificity of the antibody for AII in comparison to AI was determined. It was found that there was less than 1% cross-reactivity with AI up to concentrations of 3000 pg/ml in the incubation mixture. The recovery of standard AII added to AII-free plasma is essentially 100% as indicated by Fig. 4.

Table I shows the results of analysis of six different plasma samples on 4 separate days. The variation in the result is greater at the low concentrations. However, duplicate determination of the per cent binding of AII-^{125}I to antibody were consistently reproducable to within 10%.

The system is, therefore, a sensitive, specific, and reproducible method for the determination of AII. It has the advantage over the previously described method in that it is not sensitive to protein concentration in the incubation mixture.

Plasma concentration of AII during hemorrhage. In five experiments aortic, renal venous, and pulmonary ar-

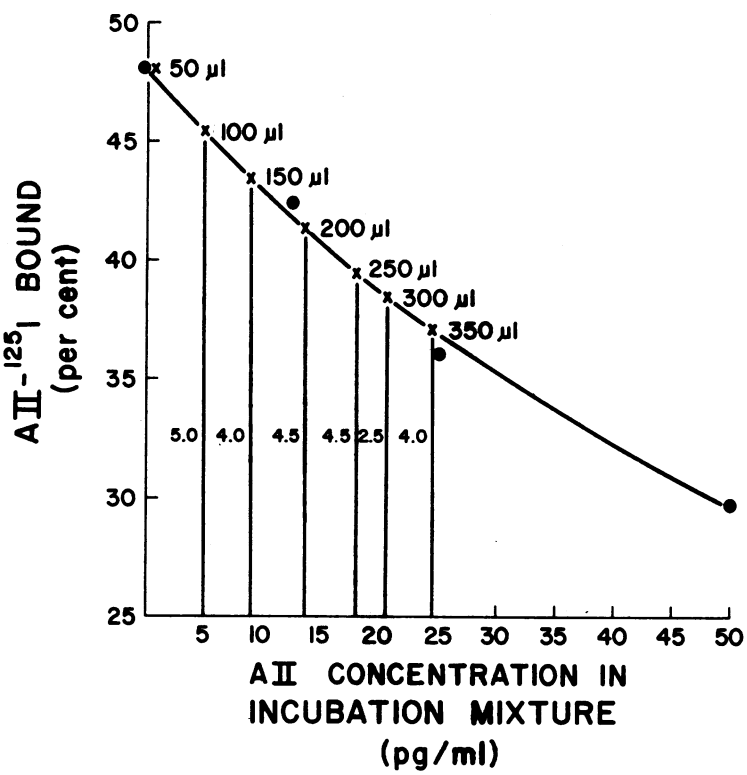

FIGURE 3 Effect of addition of 50 μl increments of ^{125}I-labeled AII to the incubation mixture (X). The binding falls along the standard curve (●——●) at regular intervals indicating that ^{125}I-AII and standard AII react in a similar manner with the antibody.

TABLE I

Reproducibility of Radioimmunoassay; Results of Assay of Six Different Plasma Samples (Columns A–F) on 4 Days

Date	AII concentration					
	A	B	C	D	E	F
	pg/ml					
11–6	150	380	47	110	76	260
11–19	132	370	40	105	65	225
11–21	107	362	30	117	42	250
12–4	117	420	25	115	65	230
Mean	127	383	36	112	61	246
±SD	±18	±26	±10	±6	±14	±16

terial blood were sampled during progressive hemorrhage. Hemorrhage was accomplished by continuous removal of arterial blood at 2.5 ml/min using an infusion pump (The Holter Co., Bridgeport, Pa.). The results of one of these experiments are shown in Table II. The concentration of AII in arterial plasma rose as hemorrhage progressed. There was a consistent increase in the concentration during transit through the lung as demonstrated by the difference in the aortic and pulmonary artery concentrations: pulmonary generation averaged 92%. By contrast, the concentration in the renal vein was consisently less than that in the aorta: renal extraction averaged 64%. Similar results were obtained in all five experiments (Table III). The level of AII in the renal vein was less than that in the aorta in all 45 simultaneously collected samples in the five experiments. Extraction by the kidney was greater than 40% in 39 samples.

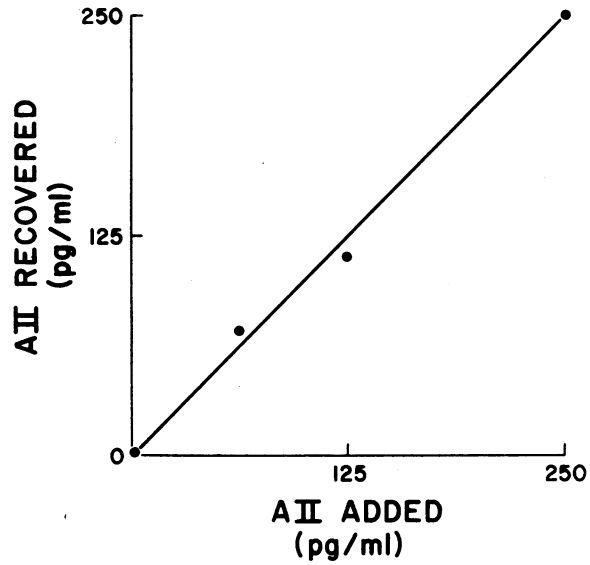

FIGURE 4 Recovery of AII added to AII-free plasma.

AII concentration in renal lymph. In four additional experiments renal lymph was obtained prior to and during hemorrhage. The results of these experiments are summarized in Table IV. Levels of AII in renal lymph were consistently greater than the concentration in either aorta or renal vein. Hemorrhage resulted in increased lymph AII concentrations in all experiments, and in two animals this rise in lymph level was unassociated with any significant change in plasma levels (Table IV, experiments 3 and 4). The increase in lymph concentrations was seen prior to any changes in blood pressure;

TABLE II

AII Concentrations in Aorta, Pulmonary Artery, and Renal Vein during Progressive Hemorrhage

Time	Mean BP	Aorta (A)	Pulmonary artery (PA)	Difference (A–PA)	Generation*	Renal vein (RV)	Difference A–RV	Extraction‡
min	*mm Hg*	*pg/ml*	*pg/ml*	*pg/ml*	%	*pg/ml*	*pg/ml*	%
Control								
−30	100	70	50	20	40	40	30	45
0	100	80	40	40	100	37	43	54
Start hemorrhage, 2.5 ml/min								
15	115	105	47	58	123	35	70	67
30	120	122	52	70	135	47	75	61
60	110	150	70	80	114	47	103	69
90	80	165	82	83	101	52	113	68
120	75	242	115	127	110	65	177	73
150	70	317	200	117	58	87	230	73
Mean					92			64
±SEM					±12			±3.3

* Per cent generation = (A–PA)/A × 100.

‡ Per cent extraction = (A–RV)/A × 100.

TABLE III

Mean Values of Renal Extraction and Pulmonary Generation of AII in Five Experiments

Experiment	Renal extraction	Pulmonary generation
	%*	%*
1	60 ±3.1	158 ±20
2	49 ±8.9	78 ±15
3	64 ±3.3	92 ±12
4	39 ±8.8	112 ±31
5	46 ±6.2	31 ±7

* Mean ±SEM number of periods (n = 9) in each experiment.

this is most notable in experiment 3. Furthermore, in the first two experiments in Table IV, lymph concentrations of AII rose in the face of continuing renal extraction.

In three experiments renal lymph was collected during reduction in renal perfusion pressure (Table V). Renal lymph concentration of AII increased when the perfusion pressure was reduced and fell when the pressure returned to control levels. Lymph levels of AII were again consistently greater than plasma. In experiments 2 and 3 (Table V) lymph flow from the single cannulated vessel was determined; flow increased in experiment 2 and in experiment 3 fell by less than 50% while AII concentration increased more than 13 times.

Origin of AII in renal lymph. In order to determine whether the AII in renal lymph represents extraction of the peptide from arterial plasma or is produced *de novo* in the renal interstitium, the following studies were carried out in three dogs. After obtaining a control collection of renal lymph, 5-L-isoleucine-^{14}C angiotensin II (New England Nuclear) was infused directly into the renal artery at the rate of approximately 30,000 cpm/min (35,000 pg/min). A period of 15 min was allowed for equilibrium and then the renal perfusion pressure was reduced to 90–100 mm Hg by an aortic snare. 15 min after the pressure was reduced a second collection of lymph was begun and continued for 1 hr. The specific activity of the infusate, renal arterial plasma, and renal lymph were compared. The amounts of ^{14}C and AII in the infused solution, plasma, and renal lymph were determined by liquid scintillation counting and radioimmunoassay respectively. The specific activity of the renal arterial plasma was estimated from the infusion rate of labeled AII, concentration of AII in arterial plasma, and the renal plasma flow estimated from the clearance of sodium *p*-aminohippurate.

TABLE IV

AII Concentrations in Renal Lymph and Aortic and Renal Venous Plasma during Progressive Hemorrhage in Four Dogs

Experiment	Time	Mean BP	Lymph	Aorta	Renal vein
	min	*mm Hg*	*pg/ml*	*pg/ml*	*pg/ml*
1	0	140	87	49	35
	100	140	120	42	32
	200	75	2000	125	87
2	0	105	1660	82	50
	100	115	>2000	122	107
	200	50	≫2000	1440	545
3	0	125	72	<20	<20
	100	130	122	<20	<20
	200	115	1130	<20	<20
4	0	120	1335	<20	<20
	100	120	>2000	<20	<20
	200	100	>2000	45	32

TABLE V

AII Concentration in Renal Lymph and Aortic and Renal Venous Plasma during Reduced Renal Perfusion Pressure

Experiment	Time	Angiotensin II				
		Mean BP	Lymph	Aorta	Renal vein	Lymph flow
	min	*mm Hg*	*pg/ml*	*pg/ml*	*pg/ml*	*µl/min*
1	0–30	125	292	<20	<20	27
	35–75	80–95	2000	55	25	17
	80–110	140	245	30	25	17
2	0–30	129	325	50	<20	17
	35–75	80–90	820	80	55	33
	80–110	137	345	75	70	25
3	0–30	125	72	<20	<20	—
	35–75	75	≫2000	<20	<20	—
	90–110	125	1800	<20	<20	—

TABLE VI

AII Concentration and Specific Activity of ^{14}C-Labeled AII in Renal, Arterial Plasma and Renal Lymph

Experiment	Mean BP	Angiotensin II		Specific activity	
		Renal arterial	Renal lymph	Renal arterial	Renal lymph
	mm Hg	*pg/ml*	*pg/ml*	*cpm/pg*	*cpm/pg*
1	140	60	485	—	—
	100	80	1035	0.375	0.087
2	115	<20	<20	—	—
	100	<20	760	0.878	0.263
3	140	<20	290	—	—
	100	30	1200	0.795	0.140

As can be seen from Table VI the reduction of renal perfusion pressure was associated with a rise in the concentration of AII in renal lymph as previously observed. In each case the specific activity of the AII in the renal lymph was significantly less than that in the renal arterial plasma. Since we are unable to determine whether the ^{14}C in lymph is actually AII or hydrolyzed fragments, the values for specific activity in lymph are maximal. It is important to emphasize that the infusion of AII-^{14}C was begun 15 min before aortic constriction while renal lymph concentrations of AII were low. Therefore, the low specific activity in renal lymph cannot be attributed to failure of labeled AII to equilibrate with a large pool of intrarenal AII, but instead must reflect the *de novo* synthesis of new unlabeled AII.

Mechanism of removal of AII from renal plasma. In order to define the mechanism of removal of AII from plasma during transit through the kidney, 5-L-isoleucine-^{14}C angiotensin II was infused directly into the renal artery. The ^{14}C-labeled peptide was first diluted in AII-free plasma, and then 1 ml of this plasma infused over a 30 sec period into the artery. To reduce circulating endogenous AII, renin secretion was suppressed by expanding the animal with isotonic saline infused at 20 ml/min for 1 hr prior to the infusion of labeled peptide. Arterial and renal venous blood were obtained before starting the infusion and then sampled at 15-sec intervals for 45–120 sec after starting the infusion. The amount of ^{14}C and AII in the infused solution, and in arterial and renal venous plasma was determined. Results were expressed as the ratio of AII in picograms per milliliter to ^{14}C in counts per minute per milliliter (AII/^{14}C). A constancy of this ratio between the infused plasma and renal venous plasma indicates that the AII has passed through the kidney unchanged. On the other hand, as shown in Table VII, in all but one of eight experiments the ratio in the renal vein was less than that in the infused plasma. This latter finding indicates that some of the ^{14}C leaving the kidney is no longer immunologically reactive and, therefore, has been altered in transit through the kidney.

DISCUSSION

The finding of an increase in the plasma AII concentration after transit through the lung is in agreement with the results of Ng and Vane (5). The lung is the only organ which has been shown to convert AI in the plasma to AII. Furthermore, while liver, kidney, and limb have been shown to inactivate AII, lung does not appear to remove AII from the plasma (11, 12). The possibility exists that AI is converted to AII in several organs and is destroyed before entering the venous effluent.

The finding by Gocke et al. of elevated AII levels in renal venous plasma in patients with renal hypertension suggests that AII is being formed within the kidney under these circumstances (7). Skinner, McCubbin, and Page reported pressor material in both renal vein and thoracic duct lymph after acutely reducing the renal perfusion pressure in dogs (13). These authors contended that the pressor material was AII. Lever and Peart previously reported increased concentrations of renin and pressor material in renal lymph but not in renal venous plasma during similar conditions (14).

In the present acute experiments elevated levels of AII in renal vein were not demonstrated during either hemorrhage or reduced renal perfusion pressure (Table III). However, the concentration of AII in renal lymph was clearly elevated with these maneuvers and was always greater than that in plasma (Tables IV and V).

Several factors might explain both the high lymph concentration of AII and the increase during hemorrhage and reduced perfusion pressure. The rise in AII concentration in lymph cannot be ascribed to a fall in lymph flow rate (Table VII) and appears to be due to the ad-

TABLE VII

Ratio of AII to ^{14}C in Infused Plasma and in Renal Venous Plasma in Eight Experiments

Date	Infused AII/^{14}C*	Renal vein AII/^{14}C*
2–9	2.4	<0.2
2–11	2.0	1.32
2–16	1.92	1.32
2–19	1.78	0.56
2–21	1.89	1.08
2–23	2.24	0.66
2–24	1.99	1.23
2–25	1.61	1.63

* AII determined by radioimmunoassay; ^{14}C determined by liquid scintillation counting.

dition of AII to the lymph. The AII appearing in the lymph might be derived from peptide removed from the arterial plasma. This is unlikely since the levels of AII in lymph increased in the face of undetectable arterial and renal venous concentrations of AII (Tables IV and V). The concentrations in lymph do not appear to be due to differential binding of AII in renal lymph since in vitro experiments have failed to demonstrate any significant binding in either plasma or lymph. Most importantly, however, when ^{14}C-labeled AII was continuously infused into the renal artery during aortic constriction, the specific activity of AII in renal lymph was much less than that in the renal arterial plasma (Table VI). This clearly demonstrates that AII in renal lymph is a consequence of *de novo* synthesis of the peptide within the kidney rather than extraction of AII from the plasma.

The most likely explanation of the experimental findings is that AII is generated within the kidney through the interaction of angiotensinogen, renin, and converting enzyme and that the concentration of AII in lymph is a reflection of the concentration of free AII in the renal interstitium. The finding by Hosie et al. that the concentration of angiotensinogen in renal lymph is low while renin concentration may be higher than arterial or renal venous plasma further suggests that the AII found in lymph is generated within the kidney (15). The apparent independance of the AII levels in lymph and plasma suggest that the hormone in renal tissue is not in equilibrium with that in plasma.

High AII concentration in renal tissue might come about in several ways. First, renin may be released from the juxtaglomerular cells into both the circulation and the renal extracellular space at the same rate. The difference in volume of distribution between the plasma and renal interstitial space might then result in a difference in renin concentration and, therefore, the amount of AII formed. Second, AII may be generated and stored within cells in the kidney and released selectively into the extracellular space. The present data do not distinguish between these two alternatives.

Despite the fact that AII is present in lymph in concentrations greater than that in either arterial or renal venous plasma, the kidney extracts 40–70% of AII in plasma during a single transit. Part of the mechanism by which AII is extracted from the arterial plasma by kidney may be explained by the present studies. Since the fractional extraction of AII by kidney (40–70%) is greater than the expected filtration fraction, the mechanism must involve more than filtration and urinary excretion alone; insignificant amounts of ^{14}C appear in the urine after infusion of ^{14}C-labeled AII into the renal artery. Removal in lymph does not appear to play an important part since virtually none of the injected AII-^{14}C appeared in renal lymph. The finding of a decrease in the AII/^{14}C ratio in the renal venous plasma suggests that the AII is being hydrolyzed during transit through the kidney and, therefore, becomes immunologically unreactive. The data of Cain, Catt, and Coghlan indicate that some of the AII breakdown products in venous plasma may react with antibody directed against AII (16), suggesting that the assay may overestimate the venous levels; renal extraction may in fact be greater than the data indicate. The fact that 40–70% of arterial AII can be removed from plasma during transit through the kidney (Table III) in association with the finding that AII appears to be hydrolyzed rather than excreted by the kidney suggests that the removal process is mediated by intravascular angiotensinase, probably located in the renal vascular endothelium.

Several important physiological implications can be drawn from the present studies. First, exceedingly high tissue concentrations of AII suggest that this hormone plays an important intrarenal regulatory role during hemorrhage and reduced renal perfusion pressure. Second, the intrarenal concentrations of AII are in no way reflected in the arterial and renal venous levels. The peptide in the renal interstitium is, therefore, in a compartment which is not in equilibrium with plasma. Third, a large fraction of either naturally generated AII or exogenous AII in arterial plasma is removed in a single passage through the kidney, presumably by hydrolysis; consequently little of the hormone in plasma appears to reach the important sites of intrarenal action other than vascular smooth muscle.

The exact physiological function subserved by the generation of high intrarenal concentrations of AII is not clear. One possibility is that AII generated in the kidney reaches the systemic circulation via the renal lymph and thoracic duct and thus contributes to the control of systemic AII levels. This possibility cannot be completely evaluated by the present experiments since the concentration of AII above and below the entrance of the thoracic duct into the vena cava was not determined. However, the large increase in AII concentration during transit through the lung indicates that pulmonary conversion of AI to AII is the primary mechanism determining the circulating systemic level of AII (11).

Other investigators have suggested that the intrarenal release of AII plays a role in the control of tubular sodium reabsorption or mediates autoregulation of renal blood flow and glomerular filtration rate (1–3, 17). The present studies do not permit evaluation of these two possibilities.

ACKNOWLEDGMENTS

This work was supported in part by Grant 1 PO HE 11662 and Training Grant 5TO1 AM 05028 (Dr. Bailie), both from the U. S. Public Health Service.

REFERENCES

1. Britton, K. E. 1968. Renin and renal autoregulation. *Lancet.* **2:** 329.
2. Thurau, K., J. Schnermann, W. Nagel, M. Horster, and M. Wahl. 1967. Composition of the tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. *Hypertension.* **15:** 79.
3. Leyssac, P. P. 1967. Intrarenal function of angiotensin. *Fed. Proc.* **26:** 55.
4. Lowitz, H.-D., K. O. Stumpe, and B. Ochwadt. 1969. Micropuncture study of the action of angiotensin-II on tubular sodium and water reabsorption in the rat. *Nephron.* **6:** 173.
5. Ng, K. K. F., and J. R. Vane. 1967. Conversion of angiotensin I to angiotensin II. *Nature (London).* **216:** 762.
6. Bakhle, Y. S., A. M. Reynard, and J. R. Vane. 1969. Metabolism of the angiotensins in isolated perfused tissues. *Nature (London).* **222:** 956.
7. Gocke, D. M., J. Gerten, L. M. Sherwood, and J. L. Laragh. 1969. Physiological and pathological variations of plasma angiotensin II in man. Correlation with renin activity and sodium balance. *Hypertension.* **17:** 131.
8. Goodfriend, T. L., L. Levine, and G. D. Fasman. 1964. Antibodies to bradykinin and angiotensin: a use of carbodiimides in immunology. *Science (Washington).* **144:** 1344.
9. Herbert, V., K.-S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25:** 1375.
10. Keyl, M. J., J. B. Scott, J. M. Dabney, F. J. Haddy, R. B. Harvey, R. D. Bell, and H. E. Ginn. 1965. Composition of canine renal hilar lymph. *Amer. J. Physiol.* **209:** 1031.
11. Hodge, R. L., K. K. F. Ng, and J. R. Vane. 1967. Disappearance of angiotensin from the circulation of the dog. *Nature (London).* **215:** 138.
12. Leary, W. P., and J. G. Ledingham. 1969. Removal of angiotensin by isolated perfused organs of the rat. *Nature (London).* **222:** 959.
13. Skinner, S. L., J. W. McCubbin, and I. H. Page. 1963. Angiotensin in blood and lymph following reduction in renal arterial perfusion pressure in dogs. *Circ. Res.* **13:** 336.
14. Lever, A. F., and W. S. Peart. 1962. Renin and angiotensin-like activity in renal lymph. *J. Physiol.* **160:** 548.
15. Hosie, K. F., J. J. Brown, A. M. Harper, A. F. Lever, R. F. Macadam, J. MacGregor, and J. I. S. Robertson. 1970. The release of renin into the renal circulation of the anaesthetized dog. *Clin. Sci.* **38:** 157.
16. Cain, M. D., K. J. Catt, and J. P. Coghlan. 1969. Effect of circulating fragments of angiotensin II on radioimmunoassay in arterial and venous blood. *J. Clin. Endocrinol. Metab.* **29:** 1639.
17. Thurau, K. 1966. Influence of sodium concentration at macula densa cells on tubular sodium load. *Ann. N.Y. Acad. Sci.* **139:** 388.