IN VIVO PRODUCTION OF ANGIOTENSINS I AND II

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IN VIVO PRODUCTION

OF ANGIOTENSINS I AND II

(Productie van Angiotensine I en II in vivo)

PROEFSCHRIFT

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Peter Jan Jacobus Admiraal

geboren te Bergen (NH)

Promotor:	Prof. Dr. M.A.D.H. Schalekamp
Promotiecommissie:	Prof. Dr. W.H. Birkenhäger Prof. Dr. P.R. Saxena Prof. Dr. P.D. Verdouw

Titles are shadows, crowns are empty things.
The good of subjects is the end of kings.

Daniel Defoe, 1701

"The road not taken"

Two roads diverged in a wood, and I -
I took the one less traveled by
and that has made all the difference.

Robert Frost

Voor mijn ouders
aan Maddie
voor Alex

This thesis is based on the following articles:

Chapter 2. Admiraal PJJ, Derkx FHM, Danser AHJ, Pieterman H, Schalekamp MADH. Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. *Hypertension* 1990;15:44-55

Chapter 3. Admiraal PJJ, Derkx FHM, Danser AHJ, Pieterman H, Schalekamp MADH. Intrarenal de novo production of angiotensin I in subjects with renal artery stenosis. *Hypertension* 1990;16:555-563

Chapter 4. Admiraal PJJ, Danser AHJ, Jong MS, Pieterman H, Derkx FHM, Schalekamp MADH. Regional angiotensin II production in essential hypertension and renal artery stenosis. *Hypertension* 1993;21:173-184

Chapter 5. Admiraal PJJ, Danser AHJ, Pieterman H, Derkx FHM, Schalekamp MADH. Effect of stimulation of renal renin secretion by diuretic treatment on regional production of angiotensins I and II. Submitted

Chapter 6. Danser AHJ, Koning MMG, Admiraal PJJ, Derkx FHM, Verdouw PD, Schalekamp MADH. Metabolism of angiotensin I by different tissues in the intact animal. *Am J Physiol* 1992,263:H418-H428

Chapter 7. Danser AHJ, Koning MMG, Admiraal PJJ, Sassen LMA, Derkx FHM, Verdouw PD, Schalekamp MADH. Production of angiotensins I and II at tissue sites in the intact pig. *Am J Physiol* 1992,263:H429-H437

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Chapter 1

General Introduction

The renin-angiotensin system plays a central role in the maintenance of blood pressure and in fluid and electrolyte homeostasis, as well as in the etiology of some forms of hypertension. In this chapter, following an introduction into the history of the renin-angiotensin system and a brief outline of the classic concept of the renin-angiotensin system, as a circulating endocrine system, an overview will be given of recent evidence, that has changed our views on the system, and now includes generation of angiotensins at tissue sites as well as in the circulating blood. At the end of this chapter the objective of our studies is formulated.

History

The first component of the renin-angiotensin system, which was discovered in 1898 by Tigerstedt and Bergman[1], was a substance in saline extracts of rabbit kidneys that raised blood pressure when injected into the bloodstream. The pressor substance was named 'renin'. The importance of this observation was recognized only after Goldblatt and co-workers[2] showed that clamping of the renal arteries produced a chronic sustained hypertension. They proposed that a pressor substance, renin, was released from the kidney with the constricted artery. Later it was shown that renin was not a pressor substance by itself but that it was an enzyme. By renin's action on a plasma protein the pressor substance was formed, which was originally named 'hypertensin'[3] or 'angiotonin',[4] and which was later renamed 'angiotensin'. The other components of what is nowadays known as the renin-angiotensin system; angiotensinogen (renin substrate), angiotensin I (Ang I) and angiotensin II (Ang II), and angiotensin I-converting enzyme (ACE), were characterized in subsequent years.[5-9] After the discovery of ACE, it took more than a decade before it came clear that this enzyme plays a critical role in the renin-angiotensin system and that the pulmonary circulation is an important site for the conversion of Ang I into Ang II.[10] Later it was shown that ACE is located on the luminal side of the endothelial lining of the pulmonary vascular bed,[11] a location ideal for the interaction with circulating peptides.

Classic View of the Renin-Angiotensin System

In the classic concept of the renin-angiotensin system, kidney-derived renin circulating in the blood acts on circulating liver-derived angiotensinogen to release the inactive decapeptide Ang I, which is then converted into the active octapeptide Ang II mainly by ACE on the pulmonary endothelium during the passage of blood through the lungs (Figure 1). The Ang II thus formed is conveyed by arterial blood to the peripheral tissues, where it exerts its effects by interaction with Ang II receptors,[12] and where it is hydrolyzed by angiotensinases into inactive peptide fragments.

According to the classic view, the primary function of the renin-angiotensin system is the delivery, via the bloodstream, of the effector peptide Ang II to target sites in the tissues. The renin-angiotensin system is thus perceived as a circulating hormonal system.

The renin-catalyzed formation of Ang I is the first rate-determining reaction of the cascade that results in the formation of Ang II, and the body exerts control of circulating Ang II levels by regulating the rate of renin secretion from the kidney. Renin is released into the bloodstream from storage vesicles in the cells of the juxtaglomerular apparatus of the kidney. Renin secretion is stimulated by hypotension, by diminished delivery of sodium to the macula densa cells, which also belong to the juxtaglomerular apparatus, and by ß-adrenoceptor stimulation. Renin secretion is suppressed by ß-adrenoceptor blockade, by a high sodium-load to the macula densa, and by Ang II.[13,14] Thus the renin-angiotensin system acts as a closed-loop mechanism which tends to stabilize itself by feedback inhibition of renin release via Ang II.

The importance of renin-angiotensin system for blood pressure control, is clearly demonstrated by the blood pressure lowering properties of agents that specifically decrease the plasma level of Ang II, that is inhibitors of ACE, or by agents that compete with Ang II for binding to the Ang II receptor, for instance [Sar^1, Val^5, Ala^8]-Ang II, saralasin.

Actions of Angiotensin II

Ang II is a potent vasoconstrictor but it also has other effects, such as stimulation of release of the sodium retaining steroid hormone aldosterone from the adrenal cortex, stimulation of prostaglandin release from various cells, modulation of transmission in sympathetic nerve terminals, stimulation of vasopressin release from the hypothalamus, and direct stimulation of sodium and bicarbonate reabsorption in the proximal tubule of the kidney. Ang II also has a

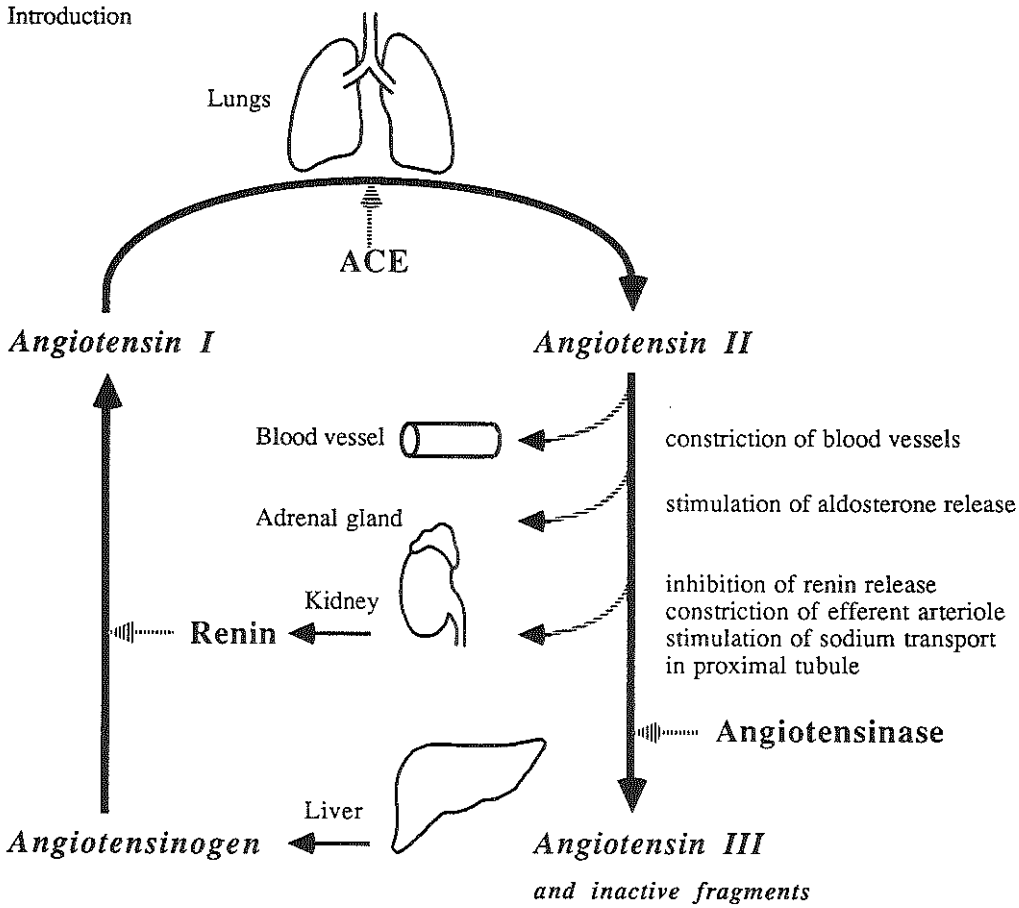

FIGURE 1
The classic concept of the renin-angiotensin system.
Angiotensinogen: *Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Asn-Glu-...R*
R=437 amino acids
Angiotensin I: *Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu*
Angiotensin II: *Asp-Arg-Val-Tyr-Ile-His-Pro-Phe*

positive inotropic effect on heart muscle, evokes thirst when injected into the ventricle of the brain, stimulates the expression of proto-oncogenes, may cause hypertrophy and/or proliferation of vascular smooth muscle cells, and may stimulate angiogenesis.[15-19]

Ang II acts through binding with specific receptors located on the plasma membrane of various cells. In addition Ang II binding sites have been found in hepatic cytosol, in nuclear preparations, and possibly on nuclear chromatin.[20-25]

The development of Ang II receptor antagonists has revealed the existence of

at least two distinct Ang II receptors, AT_1 and AT_2.[26,27] The human and bovine AT_1 receptors have been cloned and characterized. Activation of this receptor, which shows high affinity for the antagonist losartan (DuP 753), appears to be responsible for the well known physiological responses to Ang II.[28-30] Binding of Ang II to the AT_1 receptor leads, via interaction with a G-protein, to activation of phospho-inositide specific phospholipase C with subsequent formation of inositol-1,4,5-triphosphate and diacylglycerol, causing mobilization of intracellular calcium stores, and activation of protein kinase C. Recent evidence suggests that in rat kidney, adrenal, and brain, AT_1 receptors consist of two slightly different subtypes, AT_{1A} and AT_{1B}, which exhibit 95% similarity in their amino acid sequence.[31,32] Activation of the former may induce vascular smooth muscle contraction, whereas the latter may be involved in adrenal aldosterone secretion, in the regulation of drinking, and in pituitary ACTH and prolactin secretion.[32]

The function of the AT_2 receptor, which shows a high affinity for the antagonists CGP 42,112A and PD 123,177, and the second messengers of this receptor are not precisely known. On the basis of guanine nucleotide and pertussis toxin sensitivity it has been suggested recently that AT_2 receptor in the rat brain consists of two subtypes.[33] Activation of the AT_2 receptor reduces intracellular cGMP levels in neuronal cell cultures from neonatal rat brain,[34] and may alter membrane conductance by modulation of calcium channels.[35] Stimulation of AT_2 receptors in rat adrenal zona glomerulosa cells and PC12W cells inhibits particulate guanylate cyclase, and a phosphotyrosine phosphatase appears to be implicated in this process.[36] In neonatal and young rats the AT_2 receptor may be involved during differentiation and development, because AT_2 receptors, which are abundant in neonatal brain are much less abundant or absent in many areas in the adult rat brain.[37] In the chick chorio-allantoic membrane activation of the AT_2 receptor may promote neovascularization.[38] AT_2 receptor blockade appears to inhibit neointima formation in balloon-injured rat carotid arteries.[39]

Local Renin-Angiotensin Systems

From the classic point of view of the renin-angiotensin system, there is the somewhat surprising notion that the great diversity of physiological activities of Ang II is regulated by the same level of Ang II; namely, the arterial Ang II concentration that bathes all tissues. Thus mechanisms were searched for by which local tissues would be able to modulate the action of arterially delivered Ang II. In the past two decades results from several lines of investigation have been collected which challenge the concept of the renin-angiotensin system as an endocrine hormonal system. The evidence in favor of local renin-angiotensin

systems, as opposed to the circulating system, is arranged, quite arbitrarily, into four categories; *1*) clinical studies, *2*) pharmacokinetic and pharmacological experiments, *3*) biochemical evidence, and *4*) molecular biological evidence.

1) Evidence from clinical studies: The evidence from this category stems from observations of a discrepancy between the duration of the hypotensive effect of ACE and renin inhibitors, and the time during which these compounds suppress the plasma concentration of Ang II. The duration of the blood pressure lowering effect of both ACE and renin inhibitors is longer than their effect on plasma Ang II.[40-42] This has led to the assumption that the level of Ang II within the tissues may be affected differently by these agents than Ang II in circulating plasma, and it has been proposed that the tissue level of Ang II is the predominant determinant of local vascular tone.

2) Pharmacokinetic and pharmacological experiments: Results from a study in humans, in which the classic clearance concept was used to estimate the production rate of Ang II, showed that the metabolism of infused Ang II was rapid, and indicated that the generation of Ang II was probably not confined to the plasma compartment.[43] Extensive metabolism of Ang I and II was found also in the systemic vascular beds of sheep.[44,45] Despite the rapid metabolism in these beds, the plasma levels of immunoreactive Ang I and II in the veins were approximately equal to the levels in the artery, indicating an equally rapid generation of angiotensin peptides. The primitive extraction, separation, and detection techniques used at that time prevented a further analysis but nonetheless the results from these studies indicated that a major fraction of Ang I and II in the circulating plasma might be derived from local generation.[46]

In addition, results from pharmacological studies showed that isolated perfused rat hindlimbs and blood vessels are able to generate Ang II. The generation of Ang II in these preparations could be modulated by ACE- and renin inhibitors, and by ß-adrenoceptor agonists.[47-52]

3) Biochemical evidence: In numerous studies it was found that the enzymatic activities of renin and ACE were not confined to the kidney and the lungs, but were also present in other tissues. Also by immunohistochemical techniques, renin, ACE, angiotensinogen, and sometimes angiotensins could be localized, although the (subcellular)-localization might be quite distinct for each component.[53-63] In general, these studies do not answer the question from where the different components are derived from; local synthesis or uptake from the blood. Additional evidence for local synthesis was obtained from studies that showed the production of renin and angiotensins in cultured endothelial cells and adrenal glomerulosa cells, and the secretion of angiotensins from these cells into the culture medium.[64,65]

Generation of Ang I and II in vivo may not be limited to the enzymatic action

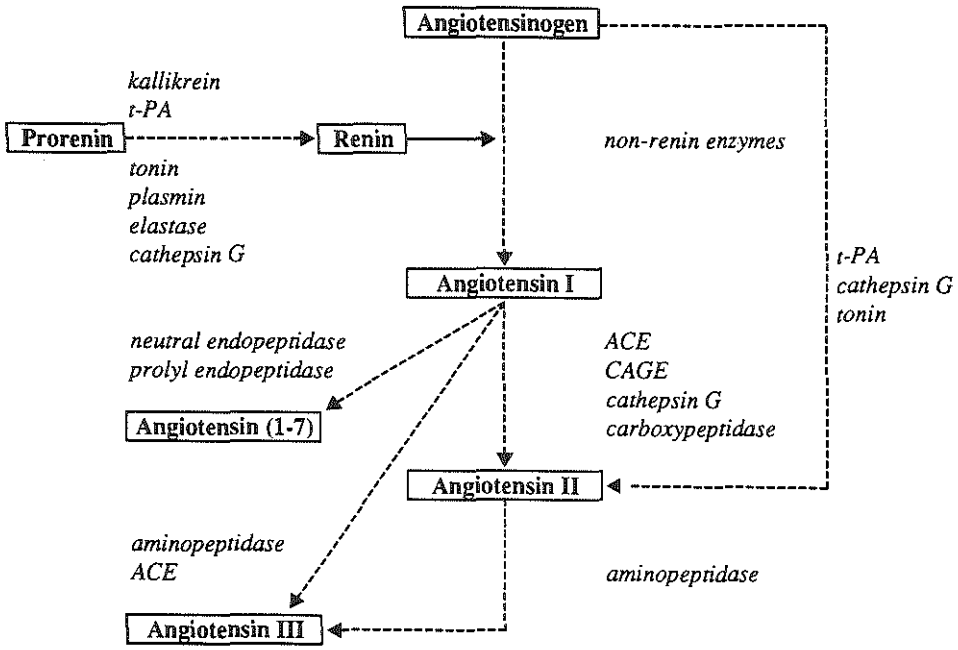

FIGURE 2
Schematic outline of pathways of prorenin activation and angiotensin peptide metabolism. ACE, angiotensin converting enzyme; CAGE, chymostatin-sensitive angiotensin II-generating enzyme; t-PA, tissue plasminogen activator.

of renin and ACE. Potentially several other enzymes, for instance chymostatin-sensitive angiotensin II-generating enzyme (CAGE), tonin, carboxypeptidase, cathepsin G, human mast cell-chymase I, and tissue-plasminogen activator (t-PA), are able to generate Ang I or Ang II, either directly from angiotensinogen or from Ang I, at least in vitro (Figure 2). These enzymes, although they do not appear to be of major importance for the formation of circulating angiotensin peptides, may contribute to the formation of angiotensins within tissues.[66-69]

Recently it has been shown that prorenin, the inactive precursor of renin, becomes reversibly catalytically active under certain experimental conditions.[70-72] This transient activation of prorenin may be due to a conformational change of the prorenin molecule. The prosegment is thought to detach itself from the enzymatic cleft of the protein and swivel away, thereby exposing the catalytic apparatus and rendering the prorenin molecule in an enzymatically active form. Although there has been some speculation about the physiological consequences of this finding, it is at present far from clear if a reversibly active form of prorenin

exists in vivo, and even so, if this form has any physiologically relevant activity.[73] In binephrectomized subjects the levels of active renin and Ang I and II that are measured in the plasma are less than 5% of normal. The circulating level of prorenin in these subjects is almost normal whereas the circulating level of angiotensinogen is greatly elevated.[74] Thus, even under these conditions the contribution of prorenin to the circulating levels of Ang I and II appears to be very low. Again this leaves intact the possibility that within tissues or within individual cells conditions may exist, for instance high acidity in lysosomes, that favor the activation of prorenin. Under such conditions activated prorenin may play an important role in the local formation of angiotensins.

4) Evidence from molecular biology: Most studies in this category have been aimed at detecting the messenger-RNA's for renin, ACE, and angiotensinogen. Indeed, these mRNA's have been detected in various tissues using Northern blot, in situ hybridization, or polymerase chain reaction technique.[75] Most tissues however do not express all the components of the renin-angiotensin system in the same cell type. For instance, in the vasculature renin mRNA is primarily localized in the smooth muscle cell layer, angiotensinogen mRNA can be found in the smooth muscle cells and in the adventitia (probably in perivascular fat).[76,77] In the brain, angiotensinogen gene and protein expression occurs predominantly in glial cells, whereas renin and ACE appear to be localized mainly in neuronal cells. In the vasculature ACE is located mainly on the endothelial membrane. ACE is found also in the deeper layers of the large arteries, where it is associated with the endothelial lining of the vasa vasorum.[78] The abundance of renin mRNA in the kidney may be modulated by sodium status, by treatment with ACE inhibitors and some renin inhibitors, and by the presence of renal artery stenosis.[79-82] The presence of the mRNA's of the components of the renin-angiotensin system is strong evidence for their local expression, and for the local production of the encoded proteins.

Another approach using molecular biological techniques is the study of the expression of imported genes of components of the renin-angiotensin system in transgenic animals. Studies in rats, which were transfected with the mouse Ren-2 gene, showed that the mouse renin encoded by this gene has a high level of expression in the adrenal gland, and renin gene transcripts were also detectable in the testes, the thymus and the small intestine. Furthermore these rats developed fulminant hypertension within 6 to 8 weeks after birth.[83] The circulating levels of renin and Ang II are low in these rats but circulating prorenin is high. These findings support importance of local angiotensin formation possibly via locally formed prorenin. The pathophysiological basis for the early development of hypertension in the transgenic rats is still unclear. Beginning at four months the rats develop hypertension related alterations and pathological lesions in various

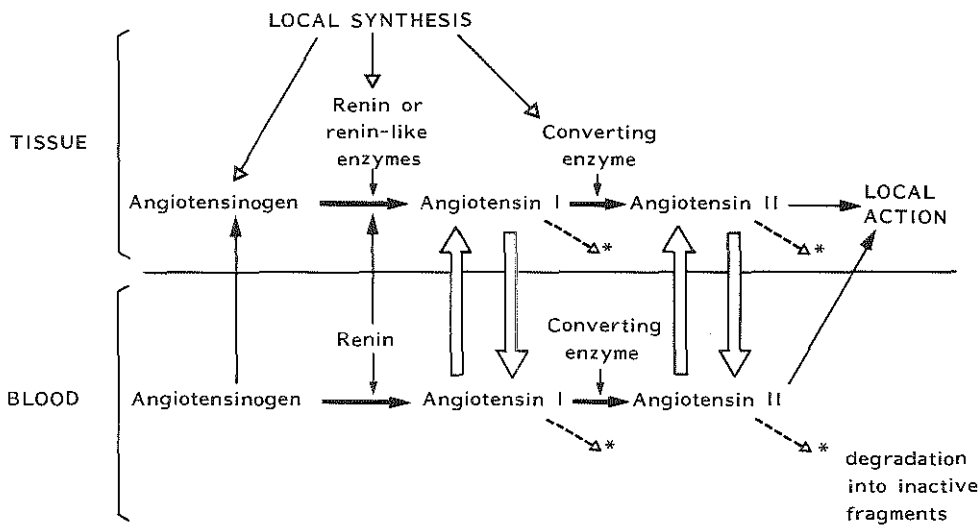

FIGURE 3
Possible integration of circulating and local renin-angiotensin systems.

tissues.[84] Transgenic mice carrying the rat angiotensinogen gene also developed hypertension. The transgene in these mice is expressed in the liver and the brain, and both plasma angiotensinogen and Ang II were elevated.[85]

Thus a large body of information has accumulated in the last decade in favor of regional angiotensin-generating mechanisms or local renin-angiotensin systems. Today the primary function of the circulating renin-angiotensin system is thought to be the delivery of renin and angiotensinogen to the tissues, rather than the delivery of the effector peptide Ang II to target tissues (Figure 3). Locally formed Ang II (or Ang I), after entry into the circulation, may contribute to the level of circulating Ang II (endocrine mode of action), or may also act at the site of its formation. These local actions may include effects on the cells adjacent to the site of Ang II formation (paracrine action), on the cells of synthesis itself (autocrine action), and even within the cells of synthesis (intracrine action, Figure 4).[86,87]

Convincing as the evidence in support of local renin-angiotensin systems appears, it is not known to what degree local and circulating renin-angiotensin systems contribute to the plasma levels of Ang I and II. Little is known about the physiological importance of local angiotensin production relative to angiotensin production in the circulation, and knowledge of the regulation of angiotensin production and metabolism in tissues is lacking. The main reason for this may

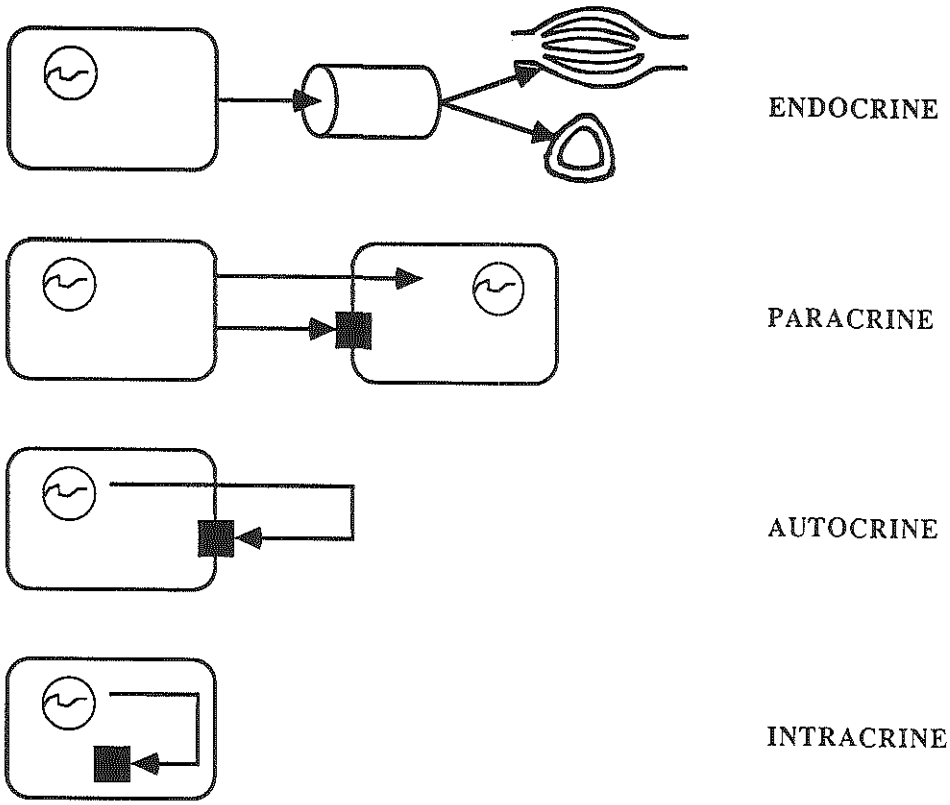

FIGURE 4
Possible mechanisms of action of locally formed angiotensin II.
Endocrine: *The peptide enters the circulation and produces its effects at target organs located at a distance.*
Paracrine: *After secretion, the peptide acts on receptors located on adjacent cells.*
Autocrine: *After release, the peptide acts on receptor located on the same cell.*
Intracrine: *The peptide acts within the cell of its own synthesis.*
(Modified after M. Paul et al., Trends Cardiovasc Med 1992;2:94-99)

originate in the technical difficulties encountered by the determination of Ang I and II in biological matrices.

The development of high-performance liquid chromatography (HPLC), and the development and miniaturization of reversed-phase HPLC columns in the 1970's and 1980's have made it possible to separate Ang I and Ang II from their metabolites and from substances that interfered with the radioimmunoassay (RIA) of angiotensins. Nussberger et al.[88,89] described a combined technique of solid-

phase extraction, HPLC separation, and RIA detection for the determination of Ang II from human plasma. The high recovery of this method, and the use of antibodies with high affinity, enabled the detection of as little as 0.4 femtomole Ang II octapeptide per ml plasma.

This methodology made it feasible to study the generation and metabolism of angiotensin peptides in intact subjects. This thesis describes the results from some studies we have undertaken in this field.

Aim of the Thesis

The aim of this thesis was to assess the metabolism of angiotensins in various tissues in subjects with hypertension, and to determine the degree of angiotensin production in these tissues and in circulating plasma. The in vivo metabolism of endogenous Ang I and II was studied using ^{125}I-Ang I and II as radiolabeled tracers. For this purpose we modified the separation and detection techniques described above, to enable us to determine simultaneously radiolabeled Ang I and II, and endogenous Ang I and II in plasma. In chapter 2 this methodology is described, together with our first study on Ang I production and metabolism in subjects with essential hypertension who were treated with the ACE-inhibitor captopril. Chapter 3 deals with similar experiments undertaken in captopril-treated subjects with unilateral renal artery stenosis. Chapter 4 describes a study of Ang II generation and Ang I-II conversion in subjects with essential hypertension and subjects with hypertension associated with unilateral renal artery stenosis. Chapter 5 deals with a similar study in subjects who were treated with the diuretic furosemide.

In humans the radiolabeled tracers were given by intravenous infusion. A major disadvantage of this approach is the large 'first-pass' effect due to metabolism in the pulmonary vascular bed. To overcome this limitation we did comparable studies of angiotensin production and metabolism in pigs. This animal model was chosen because blood samples are easily obtained from various sites, and because great expertise with this model is present at the Department of Experimental Cardiology of the Erasmus University. In these animals the radiolabeled tracer was infused directly into the left cardiac ventricle. This set-up allowed the determination of angiotensin clearance and production rates. In chapters 6 and 7 these studies are described. A summary of all these studies is given in chapter 8.

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Chapter 2

Metabolism and Production of Angiotensin I in Different Vascular Beds in Subjects With Hypertension

Summary

To study the metabolism and production of angiotensin I, highly purified monoiodinated ^{125}I-angiotensin I was given by constant systemic intravenous infusion, either alone (n=7) or combined with unlabeled angiotensin I (n=5), to subjects with essential hypertension who were treated with the angiotensin converting enzyme inhibitor captopril (50 mg, twice daily). Blood samples were taken from the aorta and the renal, antecubital, femoral and hepatic veins. ^{125}I-Angiotensin I and angiotensin I were extracted from plasma, separated by high-performance liquid chromatography, and quantitated by gamma counting and radioimmunoassay. Plasma renin activity was measured at pH 7.4. The plasma decay curves after discontinuation of the infusions of ^{125}I-angiotensin I and unlabeled angiotensin I were similar for the two peptides. The regional extraction ratio of ^{125}I-angiotensin I was 47±4% (mean±SEM) across the forearm, 59±3% across the leg, 81±1% across the kidneys, and 96±1% across the hepatomesenteric vascular bed. These results were not different from those obtained for infused unlabeled angiotensin I. Despite the rapid removal of arterially delivered angiotensin I, no difference was found between the venous and arterial levels of endogenous angiotensin I across the various vascular beds, with the exception of the liver where angiotensin I in the vein was 50% lower than in the aorta. Thus 50-90% of endogenous angiotensin I in the veins appeared to be derived from regional de novo production. The blood transit time is 0.1-0.2 minute in the limbs and in the kidneys and 0.3-0.5 minute in the hepatomesenteric vascular bed. This is too short for plasma renin activity to account for the measured de novo angiotensin I production. It was calculated that less than 20-30% in the limbs and in the kidneys and in the hepatomesenteric region approximately 60% of de novo-produced angiotensin I could be accounted for by circulating renin. These results indicate that a high percentage of plasma angiotensin I may be produced locally (i.e. not in circulating plasma).

Introduction

Renin (EC 3.4.23.15), an aspartyl protease synthesized mainly by the kidneys, acts on renin substrate (angiotensinogen) to form the decapeptide angiotensin I (Ang I), which is then converted to the octapeptide angiotensin II (Ang II) by angiotensin converting enzyme (ACE). All these components of the renin-angiotensin system are present in plasma. Plasma renin activity (PRA), which is the rate of Ang I formation in vitro, is considered a measure of the production of plasma Ang I in vivo. However, there is evidence from animal studies to suggest that the rate of angiotensin metabolism is too high for PRA to maintain the actual plasma levels of Ang I and II.[1-3] Thus, part of plasma Ang I may be formed locally rather than in circulating plasma.

The source of renin involved in local Ang I production is not known. It is possible that the circulating renin-angiotensin system not only serves to deliver Ang I to the blood vessel wall for conversion to Ang II but that it also serves to deliver renin for local Ang I production. Through binding or uptake of plasma renin by vascular tissue or through local activation of plasma prorenin, the local concentration of renin may reach levels higher than in circulating plasma. Another possibility is that local production of Ang I depends on locally synthesized rather than systemically delivered renin.

Renin is synthesized by various tissues apart from the kidney, and there is evidence for local angiotensin generation in some of these tissues.[2,4] In culture, bovine and canine vascular smooth muscle cells and bovine endothelial cells synthesize Ang II and secrete this peptide into the medium.[5,6] Isolated rat mesenteric arteries, when perfused with solutions free of renin and renin substrate, release Ang II into the perfusate,[7] and in similar experiments, isolated rat hindlegs were found to release both Ang I and Ang II.[8] Thus, part of Ang I and Ang II circulating in plasma may have been generated locally in the interstitium or in the cells of vascular tissue, and it has been postulated that a vascular renin-angiotensin system is important for the regulation of vascular tone and the maintenance of hypertension.[9-12]

The production rate of angiotensins in different vascular beds cannot be simply derived from the regional blood flow and the arteriovenous concentration gradient because a high rate of production may be matched by a high rate of metabolism. Studies using tracer amounts of radiolabeled angiotensins for measuring Ang I-II conversion and Ang I and II degradation are scarce because of the difficulty to measure the angiotensins separately from their metabolites and also because of the instability of some earlier radiolabeled iodine angiotensin preparations. More recent studies in dogs with highly purified monoiodinated ^{125}I-Ang I have demonstrated that this preparation can be used for measurement of

intrarenal Ang I-II conversion and Ang I degradation.[13,14] Monoiodinated ^{125}I-Ang II is a potent, full agonist analogue of Ang II.[15,16]

The present study was undertaken to explore the possibility of measuring the rate of metabolism of Ang I in humans by intravenous infusion of monoiodinated ^{125}I-Ang I. These infusions were given to hypertensive subjects at the time renal vein renin sampling was performed as part of the diagnostic workup for renovascular hypertension while the subjects were treated with the ACE inhibitor captopril. ^{125}I-Ang I and ^{125}I-Ang II and endogenous Ang I and Ang II were isolated from aortic plasma and from renal, antecubital, femoral and hepatic venous plasma, and separated by high-performance liquid chromatography (HPLC). The isolated radiolabeled angiotensins were quantitated in the gamma counter, and the concentrations of endogenous angiotensins were measured by radioimmunoassay. Regional extraction of Ang I was calculated from measurements of the arterial and venous levels of ^{125}I-Ang I. From this and from measurements of the concentrations of endogenous Ang I, information could be obtained on Ang I production in different vascular beds. Parallel measurements of ^{125}I-Ang II provided information on Ang I-II conversion.

Methods

Chemicals

[Ile^5]-Ang-(1-10) decapeptide, [Ile^5]-Ang-(1-8) octapeptide, [Ile^5]-Ang-(2-8) heptapeptide and [Ile^5]-Ang-(1-7) heptapeptide, which are denoted here as Ang I, Ang II, Ang III and Ang-(1-7) respectively, were obtained from Bachem, Bubendorf, Switzerland. [Ile^5]-Ang-(2-10) nonapeptide was from Senn Chemicals, Dielsdorf, Switzerland. [Ile^5]-Ang-(3-8) hexapeptide, [Ile^5]-Ang-(4-8) pentapeptide and Ang-(1-4) tetrapeptide were from Peninsula Labs., Belmont, CA, USA. The World Health Organization (WHO) standards of Ang I and Ang II (86/536 and 86/538) were from the National Institute for Biological Standards and Control, London, UK. Methanol and ortho-phosphoric acid (both analytical grade) were from Merck, Darmstadt, FRG. Bovine serum albumin was from Sigma Chemical Co., St. Louis, MO, USA. Human serum albumin was from Merieux, Lyon, France. Water for HPLC was prepared with a Milli-Q system from Waters Chromatography Div., Milford, Mass, USA. The statine containing renin inhibitor CGP 29,287 was a kind gift of Dr. K. Hofbauer (Ciba-Geigy, Basel, Switzerland).

Preparation of Angiotensin Antisera

Ang I and Ang II antisera were produced in New Zealand White rabbits by intracutaneous injections of 330 μg of either Ang I or Ang II coupled to bovine serum albumin with 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide HCl and suspended in Freund's complete adjuvant. Booster injections of the conjugates suspended in Freund's incomplete adjuvant were given at 4-week intervals. The Ang I antiserum was used in the radioimmunoassay in a final dilution of 1:250,000. This antiserum gave a B/Bo ratio of 0.9 at 2 fmol Ang I and a B/Bo ratio of 0.5 at 20 fmol Ang I. The antiserum also reacted (100%) with Ang-(2-10) nonapeptide but did not react (less than 0.1%) with Ang II, Ang III, Ang-(3-8) hexapeptide, or Ang-(4-8) pentapeptide.

The Ang II antiserum was used in a final dilution of 1:200,000. It gave a B/Bo ratio of 0.9 at 1.5 fmol Ang II and a B/Bo ratio of 0.5 at 16 fmol Ang II. This antiserum also reacted with Ang III (55%), Ang-(3-8) hexapeptide (73%), and Ang-(4-8) pentapeptide (100%) but virtually not with Ang I (0.3%) and Ang-(2-10) nonapeptide (0.2%). The renin inhibitor CGP 29,287, in concentrations up to 100 μM, did not react with either Ang I or Ang II antisera.

Blood Sampling

Blood for angiotensin measurements was rapidly drawn (7-13 seconds) with a plastic syringe containing the following inhibitors (0.5 ml inhibitor solution in 10 ml blood), 6.25 mM disodium EDTA, 1.25 mM 1,10-phenantroline, and 100 nM of the renin inhibitor CGP 29,287 (final concentrations in blood) and transferred into prechilled polystyrene tubes. These inhibitors were used in order to prevent Ang I generation, Ang I-II conversion, and Ang I and II degradation during blood collection and handling of the samples. The blood samples were centrifuged at 3,000*g* for 10 minutes at 4° C. Plasma was stored at -70° C, extracted within 2 days, and assayed within 2 weeks. The renin inhibitor CGP 29,287 is a renin substrate analogue. At a concentration of approximately 1 nM it inhibits PRA by 50%.[17] We chose to use the inhibitor in a final concentration of 100 nM on the basis of experiments in which we added the inhibitor in concentrations ranging from 0.1 to 1,000 nM to a plasma sample with normal PRA (12 $pmol.l^{-1}.min^{-1}$) and to plasma samples with elevated PRA (77 and 260 $pmol.l^{-1}.min^{-1}$). Ang I formation during incubation was inhibited by 98% at 37° C and by 100% at 20° C, both at an inhibitor concentration of 100 nM.

Blood for PRA measurements was collected into polystyrene tubes containing EDTA (0.2 ml disodium EDTA in 10 ml blood, final concentration 6.25 mM). The samples were centrifuged at room temperature at 3,000*g* for 10 minutes, and plasma was stored at -20° C.

Extraction of Angiotensins

Solid phase extraction cartridges (Sep-Pak C18, Waters Chromatography Div.) were conditioned with 4 ml methanol and then equilibrated two times with 4 ml cold water. Thawed plasma samples (2 ml) were applied to the cartridges. After the bound peptides were washed two times with 4 ml cold water, they were eluted with 2 ml methanol and collected into conical polypropylene tubes. The methanol extracts were evaporated at 4° C with a Savant Speed Vac concentrator (Savant Instrs., Farmingdale, NY, USA). Recovery of labeled and unlabeled Ang I and II from the Sep-Pak cartridges was 95-98%.

Separation of Angiotensins by High-Performance Liquid Chromatography

Separation of the peptides in the methanol extracts was performed with HPLC by the method of Nussberger et al[18] with some modifications. We used a reversed-phase Nucleosil C18 steel column of 250×4.6 mm and 10 μm particle size equipped with a direct-connect guard column (Alltech, Eke, Belgium). The HPLC equipment consisted of an LKB 2150 pump, a 2152 controller, a 2122 fraction collector and a 2155 column oven (LKB, Bromma, Sweden). Samples were injected with a Rheodyne 7125 injection valve equipped with a 250 μl loop (Rheodyne Inc., Cotati, CA, USA). Mobile phase A was 0.085% ortho-phosphoric acid containing 0.02% sodium azide (pH 2.33). Mobile phase B was methanol. The flow was 1 ml/min, and the working temperature was 45° C. The column was calibrated with pure Iodine-125-labeled standards of Ang I and Ang II and with unlabeled Ang I, Ang II, Ang III, and some of their metabolites. The vacuum-dried plasma extracts were dissolved in 100 μl HPLC solvent (65% A/35% B, vol/vol), centrifuged, and injected with a 100 μl Hamilton syringe (Hamilton, Bonaduz, Switzerland). Elution was performed as follows: 65% A/35% B from 0 to 9 minutes followed by a linear gradient to 45% A/55% B until 18 minutes. The eluate was collected in 0.5-minute fractions into polystyrene tubes coated with bovine serum albumin.

The concentrations of ^{125}I-Ang I and ^{125}I-Ang II in the HPLC fractions were measured in the gamma counter for 20 minutes. The fractions containing Ang I and Ang II were pooled separately. They were neutralized with 0.5 M sodium hydroxide and evaporated in the Savant concentrator. The concentrations of Ang I and Ang II were measured by radioimmunoassay. Recoveries for the HPLC separation alone were 90-95% for both labeled and unlabeled Ang I and Ang II. The overall recoveries of Ang I and Ang II added to plasma samples were 85±7% and 84±8% (mean±SD, n=6). Similar values were obtained for ^{125}I-Ang I and ^{125}I-Ang II. Results were not corrected for incomplete recovery.

Radioimmunoassay of Angiotensins

The dried HPLC fractions were dissolved in 0.25 M Tris buffer, pH 7.4, containing 0.35% bovine serum albumin and 0.02% sodium azide. They were then incubated with ^{125}I-Ang I or ^{125}I-Ang II and the appropriate antiserum at 4° C for 3 days. Separation of bound and free angiotensin was achieved by charcoal adsorption, and both the supernatant and the pellet were counted to a counting error of less than 1%. The concentrations of Ang I and Ang II (from Bachem) in the standard solutions of the radioimmunoassay were checked by comparison with the WHO Ang I and Ang II reference standards. The concentrations of angiotensins are expressed as picomoles per liter plasma. The lower limit of detection (2×SD difference from Bo) was 1.2 fmol/tube for Ang I and 0.7 fmol/tube for Ang II. The normal level of Ang I in antecubital venous plasma from healthy individuals in supine position was 20.9 pmol/l (geometric mean), range 12.3-42.4 pmol/l (n=18), and the normal level of Ang II in these individuals was 4.8 pmol/l, range 2.5-8.2 pmol/l (n=18).

Measurement of Plasma Renin Activity

PRA was measured by incubating plasma at 37° C for 0, 15, 30, and 60 minutes. During the 60-minute incubation period, pH varied from 7.1 at t=0 minutes to 7.5 at t=60 minutes. PRA measurements under these conditions gave results not different from measurements in the presence of 100 mM imidazol buffer, pH 7.4. With this buffer, pH remained 7.4 during incubation. To prevent Ang I-II conversion, Ang I degradation, prorenin-renin conversion, and bacterial growth, the following mixture of inhibitors was added to plasma before incubation (35 µl inhibitor solution/ ml plasma): 5 mM disodium EDTA, 3.4 mM 8-hydroxyquinoline sulfate, 2.4 mM phenylmethyl-sulfonyl fluoride, 2.2 nM aprotinin and 1 mg/ml neomycin sulfate (final concentrations in plasma).

The inhibitors do not interfere with the reaction of renin with substrate. The kinetic constants, V_{max} and K_m, of this reaction are not modified when these inhibitors are added to mixtures of highly purified human renin and human substrate. The measured levels of PRA are not different from those calculated from these kinetic constants and from the concentrations of renin and substrate measured in plasma.[19] Ang I that was generated during incubation was quantitated by radioimmunoassay. Ang I generation in the PRA assay was linear in the first 30 minutes of incubation, but in some samples, the rate of Ang I generation was somewhat lower in the following 30 minutes. Only the first linear part of the Ang I generation curve was used for calculation of PRA. The recovery of renin or Ang I added to plasma before to assay was 98-100%.

PRA is expressed as picomoles Ang I per liter plasma per minute of incubation (pmol.l^{-1}.min^{-1}). The normal level of PRA in antecubital venous plasma was 12.7 pmol.l^{-1}.min^{-1} (geometric mean), range 2.3-66.7 pmol.l^{-1}.min^{-1} (n=74). This is in close agreement with the results obtained by the "antibody-trapping" technique.[20,21]

Measurement of Renal Plasma Flow

Renal plasma flow was determined by measuring the clearance of ^{131}I-hippurate (Amersham Intl., Amersham, UK), which was given by constant intravenous infusion at the time the procedure of renal vein sampling was performed. Renal plasma flow was calculated by dividing

the clearance of ^{131}I-hippurate by the renal extraction ratio.[22]

Preparation of Radiolabeled Angiotensin I for Intravenous Infusion

Ang I was radiolabeled by the chloramine T method.[23] All solutions were made in sterile pyrogene free water. The whole procedure was carried out under aseptic conditions. In short, 4 nmol Ang I in 20 µl of 0.25 M sodium phosphate buffer, pH 7.5, was mixed with 1 mCi (37 MBq) ^{125}I-sodium iodide (Amersham Intl.). The reaction, which occurred at room temperature, was started by the addition of 20 µl of 7.0 mM chloramine T. After 25-30 seconds, the reaction was stopped with 20 µl of 11.0 mM sodium metabisulfite. Free ^{125}I-iodide and labeled peptide were separated on a 5×50 mm Dowex-AG-I-X8 column (50-100 mesh, Bio-Rad Labs., Richmond, CA, USA). Elution was carried out with 0.1 M acetic acid containing 0.1% human serum albumin. Labeled Ang I does not bind to the column under these conditions. Fractions of 0.5 ml were collected and the fractions containing labeled peptide were pooled.

To obtain monoiodinated ^{125}I-Ang I, the labeled peptide was applied to a 1.6×100 cm Biogel P-4 column (200-400 mesh, Bio-Rad Labs.) and eluted with 0.05 M acetic acid containing 0.1 M NaCl and 0.1% human serum albumin. Fractions of 2.5 ml were collected and counted in a gamma counter. The ^{125}I-Ang I was eluted from the Biogel P-4 column in two separate peaks. The first peak represented monoiodinated ^{125}I-Ang I, and the second peak consisted of di-iodinated ^{125}I-Ang I. Fractions from the first peak were pooled, sterilized by filtration through a 0.22 µm Millipore membrane filter (Waters), and stored at -20° C until use. The specific radioactivity of the ^{125}I-Ang I preparation was approximately 3.6×10^6 cpm/pmol (74 kBq/pmol). To obtain information about the purity of the monoiodinated ^{125}I-Ang I, a sample of the pooled fractions of the first peak was injected into the HPLC column. The HPLC elution profile of the radioactive material indicated that 98% consisted of monoiodinated ^{125}I-Ang I.

Subjects

Ten subjects with hypertension (four men, six women, mean age 48, range 26-67 years) were studied at the time they were undergoing renal vein sampling followed by renal angiography for diagnostic purposes. The renal vein renin ratio was normal (<1.5) as was the renal angiogram. Blood pressure in the outpatient clinic was repeatedly above 160/100 mm Hg despite antihypertensive medication. Routine urine analysis, serum creatinine, and serum electrolytes were normal, and the subjects were considered to have essential hypertension. Seven subjects received an infusion of ^{125}I-Ang I, and three subjects received an infusion of both ^{125}I-Ang I and Ang I. Two additional subjects with essential hypertension (one woman 47 years old and one man 58 years old) were studied at the end of a 24-hour period of intra-arterial blood pressure recording. Renal vein renin sampling and renal angiography had been performed some months before and were normal. These two subjects received an infusion of both ^{125}I-Ang I and Ang I.

All subjects received 5 ml of a 1% potassium iodide solution/day from 2 days before to 4 days after the angiotensin infusion. The subjects were treated with the ACE inhibitor captopril (50 mg p.o., twice daily). The infusion studies were performed 4-6 hours after administration of the morning dose of captopril.

Infusion Protocol

^{125}I-Ang I was infused at a rate of approximately 3.0×10^6 cpm/min, and Ang I at a rate of approximately 1.2 nmol/min for 20 minutes. An indwelling needle for blood sampling was placed in the antecubital vein of the left arm. After insertion of the catheters into the abdominal aorta and the inferior caval vein via the femoral artery and vein by the Seldinger technique, angiotensin was infused via the antecubital vein of the right arm. A blood sample was taken from the abdominal aorta before infusion. Ten minutes after the infusion had been started, samples were taken from the

aorta, the hepatic vein, and the antecubital vein of the left arm. In the following 10 minutes, a second and third series of samples were taken simultaneously from the aorta, the antecubital vein, and a renal vein, first from one kidney and then from the other. A sample from the femoral vein was taken shortly before discontinuation of the infusion. The arterial plasma levels of ^{125}I-Ang I, ^{125}I-Ang II, Ang I and Ang II remained constant between 8 and 20 minutes after the start of the infusion (coefficient of variation <4%). For measurement of the elimination half-time of ^{125}I-Ang I and infused Ang I, samples were taken from the antecubital vein or from the aorta at approximately 0.5, 1.0, 2.0, 3.0, and 4.0 minutes after the infusion had been stopped. After the last blood sample had been collected, the radiocontrast injection for renal angiography was given.

The two subjects studied some months after renal vein catheterization received an intravenous infusion of ^{125}I-Ang I and Ang I in one arm, and blood samples were taken from the brachial artery and the antecubital vein of the other arm before the infusion and at 10, 15 and 20 minutes after the start of the infusion. For measurement of the elimination half-time of the infused peptides, samples were taken from the brachial artery at the previously mentioned times after the infusion had been stopped.

Urinary excretion of radioactivity was followed over a period of 96 hours. Ninety percent of the administered radioactivity was excreted within 24 hours, and 98% was recovered in the 96-hour period. The calculated exposure to radioactivity was 0.24 μGy (or 0.6 mrad) from the ^{125}I-Ang I infusion and 12 μGy (or 30 mrad) from the ^{131}I-hippurate infusion.[24] The protocol was approved by the Hospital Ethics Review Committee.

Calculations

The regional extraction ratio of ^{125}I-Ang I was calculated as follows

$$ER = 1-(^{125}\text{I-Ang I}_{ven}/^{125}\text{I-Ang I}_{art})$$

The same formula was used for calculation of the extraction ratio of infused Ang I. In this case, the plasma level of Ang I before infusion was subtracted from the level during infusion.

The venous plasma level of Ang I derived from de novo regional production was calculated as

$$\text{Ang I}_{ven} \text{ from de novo production} = \text{Ang I}_{ven}-(1-ER)\times\text{Ang I}_{art}$$

For the renal vascular bed, it was possible to calculate the net de novo production rate of Ang I because we determined not only the renal extraction ratio of ^{125}I-Ang I and the renal arteriovenous difference in endogenous Ang I but also the renal extraction ratio and clearance of ^{131}I-hippurate. The renal extraction ratio of ^{131}I-hippurate was calculated as follows

$$ER_{hip} = 1-(^{131}\text{I-hippurate}_{ven}/^{131}\text{I-hippurate}_{art})$$

ER_{hip} was 0.72±0.02 for the left kidney and 0.75±0.03 for the right kidney ($p>0.05$). If it is assumed that the renal plasma flow is equal on both sides, the single kidney plasma flow can be calculated as follows

$$\text{Single kidney plasma flow} = \text{clearance of } ^{131}\text{I-hippurate}/(ER_{hip\ left}+ER_{hip\ right})$$

The single kidney net de novo production of Ang I is then obtained by multiplying single kidney plasma flow by the renal venous plasma level of intrarenally de novo-produced Ang I.

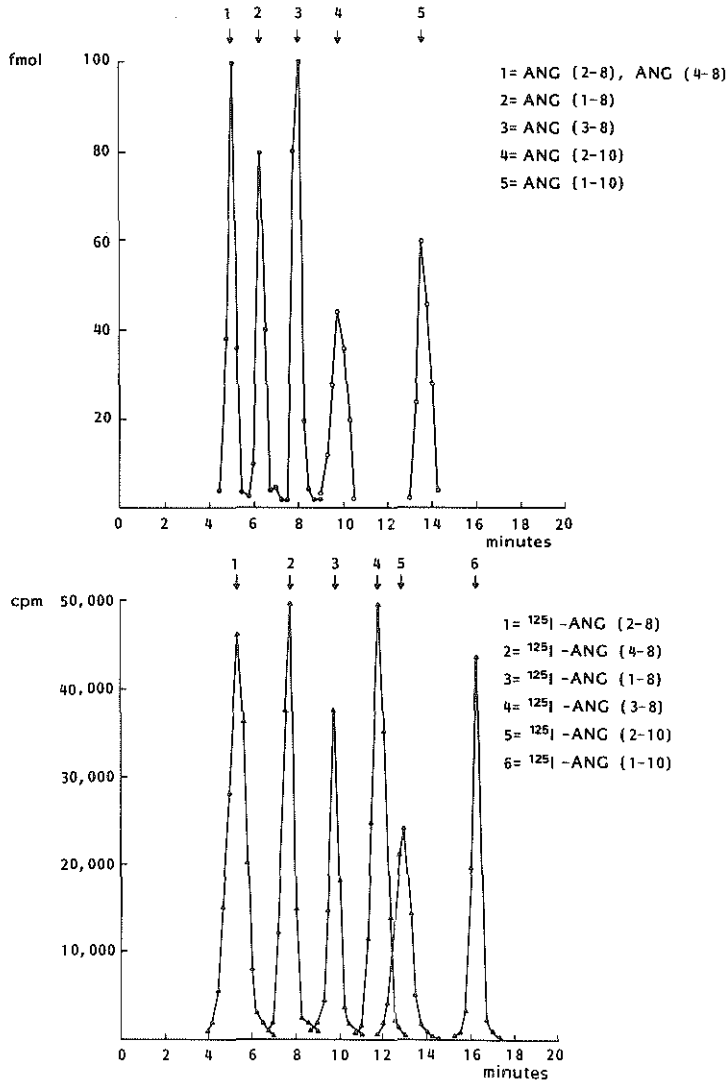

FIGURE 1

Line graph showing separation of standard angiotensin peptides (upper panel) *and Iodine-125-labeled angiotensin peptides* (lower panel) *by reversed-phase high-performance liquid chromatography. Stationary phase: Nucleosil C18, 10 µm, 250X4.6 mm. Mobile phase: methanol/orthophosphoric acid 0.085% (containing sodium azide 0.02%), 35%/65% (vol/vol) from 0 to 9 minutes, and a linear gradient from 35%/65% to 55%/45% from 9 to 18 minutes. Detection by radioimmunoassay with angiotensin I antiserum (peaks 4 and 5) or with angiotensin II antiserum (peaks 1-3) (upper panel) or by gamma counting (lower panel).*

Results

High-Performance Liquid Chromatography Separation of Angiotensin Peptides

Under the conditions specified in "Methods", satisfactory separations were obtained between Ang I, Ang II, and their metabolites as well as their radiolabeled counterparts (Figure 1). Apart from the metabolites shown in Figure 1, we also tested ^{125}I-Ang-(1-4) and ^{125}I-Ang-(1-7). These metabolites had a retention time of approximately 4 minutes. Assessment of injection-to-injection and day-to-day variability of the retention times of ^{125}I-Ang I and ^{125}I-Ang II demonstrated excellent stability of the chromatographic conditions. Separation patterns for radiolabeled angiotensins of plasma extracts from subjects who had received an intravenous infusion of ^{125}I-Ang I showed four peaks with retention times of 4.2, 9.9, 13.0, and 16.2 minutes (Figure 2). Until now, the material in the first peak has not been identified. The other peaks were ^{125}I-Ang II, ^{125}I-Ang-(2-10), and ^{125}I-Ang I. The retention times of ^{125}I-Ang I and ^{125}I-Ang II differed from the retention times of any of the metabolites we tested, and the difference was sufficient to obtain adequate separations.

Regional Extraction of Angiotensin I

An important question is whether the rate of metabolism of ^{125}I-Ang I in our subjects differed from the metabolism of unlabeled Ang I. To answer this question, unlabeled Ang I was infused together with ^{125}I-Ang I. Results obtained with these combined infusions are summarized in Table 1. Because of ACE inhibitor treatment, the infusions of Ang I did not result in a significant reduction of PRA. The preinfusion level of Ang I was therefore taken as a measure of the contribution of endogenous Ang I to the level of Ang I during infusion of this peptide. Thus, for calculation of the regional extraction of infused Ang I, the preinfusion level of Ang I was subtracted from the level during infusion. From measurements of the arterial and venous plasma levels of ^{125}I-Ang I and unlabeled Ang I, it appeared that during infusion of the two peptides the regional extraction ratios for labeled and unlabeled Ang I were not different (Figure 3). In most patients, the plasma concentrations of ^{125}I-Ang I and unlabeled Ang I fell monoexponentially after discontinuation of the infusion. Some curves, however, showed a delay. This delay can be explained, at least in part, by the passage time of blood from the infusion site to the sampling site, but the delay could also represent the exit of ^{125}I-Ang I and Ang I from tissue sites. Estimates of the elimination half-time of ^{125}I-Ang I and Ang I were therefore made from the steepest straight portion of the decay curves, and the results were not different for the two peptides (Figure 4). The estimated elimination half-time of ^{125}I-Ang I, which was determined in all subjects, was 0.73±0.04 minute

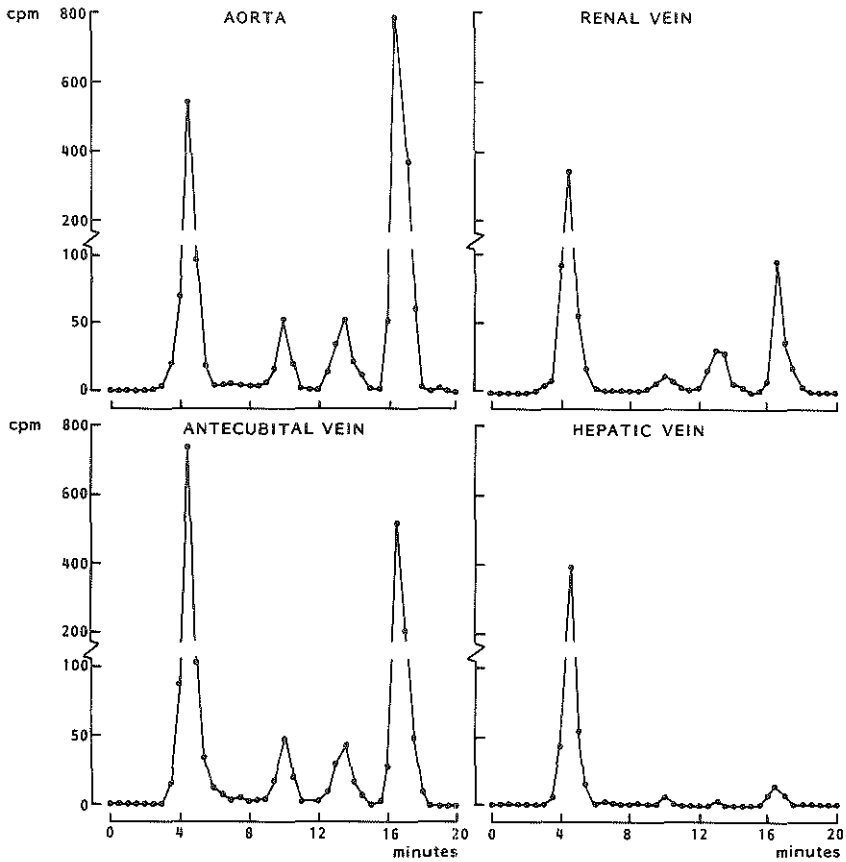

FIGURE 2

Line graph showing high-performance liquid chromatography separation of Iodine-125-labeled angiotensin (Ang) in plasma of subject who received a constant intravenous infusion of ^{125}I-Ang I. Stationary phase: Nucleosil C18, 10 μm, 250X4.6 mm. Mobile phase: methanol/ortho-phosphoric acid 0.085% (containing (0.02% sodium azide), 35%/65% (vol/vol) from 0 to 9 minutes, and a linear gradient from 35%/65% to 55%/45% from 9 to 18 minutes. Detection by gamma counting. Peaks with retention times of 9.9, 13.0, and 16.2 minutes correspond with ^{125}I-Ang-(1-8)=^{125}I-Ang II, ^{125}I-Ang-(2-10), and ^{125}I-Ang-(1-10)=^{125}I-Ang I, respectively. Material in first peak with a retention time of 4.2 minutes was not identified.

(mean±SEM).

PRA and angiotensin levels vary widely among subjects with essential hypertension. This was also the case in our study. PRA in the aorta ranged from 3.8-122 pmol.l^{-1}.min^{-1}. The plasma levels of ^{125}I-Ang I and II and endogenous Ang I and II in the aorta and in the veins of the kidneys, forearm, leg, and liver during infusion of ^{125}I-Ang I are presented in Table 2.

Table 1
Plasma Levels of ^{125}I-Angiotensin I, Angiotensin I and Plasma Renin Activity During Constant Intravenous Infusion of Both ^{125}I-Angiotensin I and Angiotensin I

	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5	
Infusion rates	^{125}I-Ang I cpm/min×10^6	Ang I nmol/min	^{125}I-Ang I cpm/min×10^6	Ang I nmol/min	^{125}I-Ang I cpm/min×10^6	Ang I nmol/min	^{125}I-Ang I cpm/min×10^6	Ang I nmol/min	^{125}I-Ang I cpm/min×10^6	Ang I nmol/min
	3.14	1.38	2.70	1.59	3.02	0.68	3.05	1.54	3.50	1.50

	Subject 1			Subject 2			Subject 3			Subject 4			Subject 5		
Plasma levels	PRA pmol/l/min	^{125}I-Ang I cpm/l ×10^3	Ang I pmol/l	PRA pmol/l/min	^{125}I-Ang I cpm/l ×10^3	Ang I pmol/l	PRA pmol/l/min	^{125}I-Ang I cpm/l ×10^3	Ang I pmol/l	PRA pmol/l/min	^{125}I-Ang I cpm/l ×10^3	Ang I pmol/l	PRA pmol/l/min	^{125}I-Ang I cpm/l ×10^3	Ang I pmol/l
Before infusion															
Artery	7.6	...	5.1	5.2	...	9.5	3.8	...	6.8	63	...	50	62	...	76
During infusion															
Artery	6.8	1,536	723	4.3	1,021	665	4.0	1,254	307	57	696	613	47	810	382
Hepatic vein	...	96	52	...	32	32	...	53	12	...	...	...	...	...	...
Left renal vein	...	260	105	...	215	124	...	235	58	...	...	...	...	...	...
Right renal vein	...	258	106	...	242	130	...	325	79	...	...	...	...	...	...
Antecubital vein	...	n.d.	n.d.	...	503	313	...	528	130	...	401	439	...	500	236
Femoral vein	...	901	404	...	518	319	...	495	143	...	...	...	...	...	...

In subjects 1-3, 'artery' is the abdominal aorta. In subjects 4-5, 'artery' is the brachial artery. Ang I, angiotensin I; Ang II, angiotensin II; PRA, plasma renin activity; n.d., not determined.

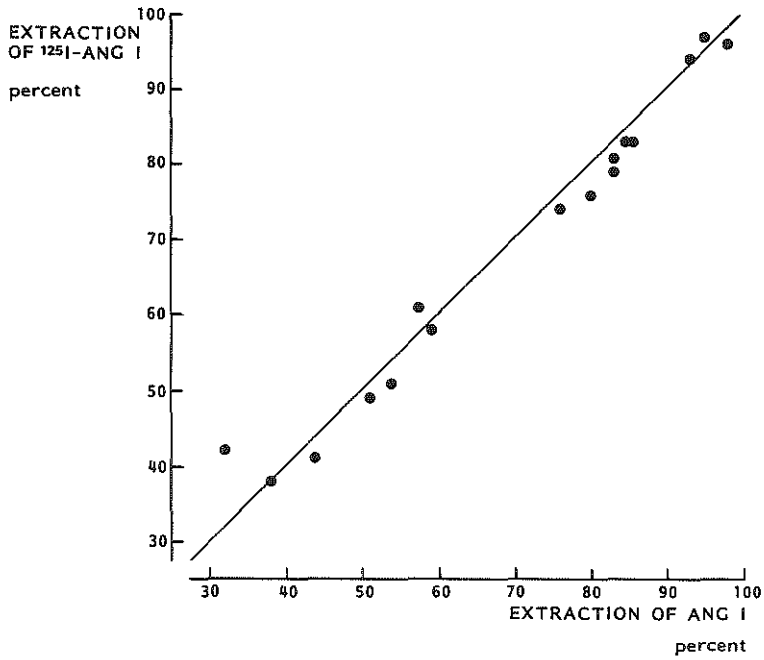

FIGURE 3
Scatter plot showing comparison of regional extraction of ^{125}I-angiotensin I (Ang I) with that of Ang I during constant intravenous infusion of the two peptides in five subjects. Data are expressed as a percentage of arterial plasma concentration.

limbs is 0.1-0.2 minute or less.[25,26] From this and from the measurements of PRA, the extraction of arterially delivered Ang I, and the arteriovenous difference in the plasma levels of Ang I, it was calculated that less than 20-30% of Ang I produced in the kidneys, the forearm, and the leg could be accounted not measure the ^{125}I-Ang I levels in mixed venous plasma, but an approximation was made by assuming an overall extraction of systemically delivered ^{125}I-Ang I of 60%. The mixed venous level downstream from the site where the infused ^{125}I-Ang I enters the central venous compartment can then be obtained by dividing the infusion rate by the cardiac output, which was taken to be 3 l plasma/min/1.73 m^2 body surface area, and by adding to this quotient 40% of the arterial level of ^{125}I-Ang I. In this way, it was calculated that approximately 30-40% of the ^{125}I-Ang I delivered to the cardiopulmonary circulation was extracted by this vascular bed. From this and from a systemic extraction of 60%, a whole body extraction of

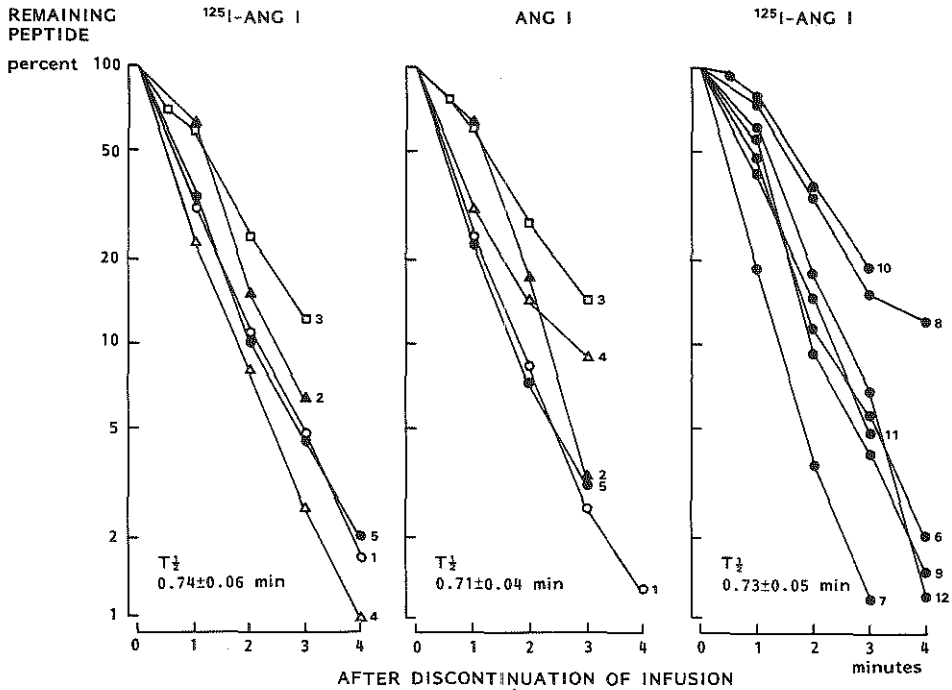

FIGURE 4

Line graphs showing in vivo elimination of ^{125}I-angiotensin I (Ang I) and Ang I after discontinuation of a constant intravenous infusion of both ^{125}I-Ang I and Ang I (left and middle panel) or constant intravenous infusion of ^{125}I-Ang I only (right panel). Plasma Ang I levels in the middle panel were corrected by preinfusion Ang I levels. Each patient in left and middle panel is denoted by different symbol and number. Numbers in these panels refer to the subjects in Table 1. Blood was sampled from the aorta in subjects 1, 3, 6, and 7, from the brachial artery in subjects 4 and 5, or from the antecubital vein in subjects 2 and 8-12.

about 75% can be calculated. Because of this high extraction rate, the "true" half-time of Ang I in the body must be shorter than the whole body transit time of blood (about 0.5 minute). The true half-time of Ang I must therefore be shorter than the half time (0.73 minute) we estimated from the plasma ^{125}I-Ang I and Ang I decay curves after the infusion of these peptides had been stopped. This difference again may represent the exit of ^{125}I-Ang I and Ang I from tissue sites.

Regional Angiotensin I-II Conversion

We did not measure the ^{125}I-Ang II levels in mixed venous plasma, but they are likely to be lower than in aortic plasma, because the ^{125}I-Ang II levels we measured in the renal, antecubital, femoral, and hepatic veins were lower than in

Table 2
Arterial and Venous Plasma Levels of Angiotensins in Seven Hypertensive Subjects During Constant Intravenous Infusion of ^{125}I-Angiotensin I

Plasma levels	^{125}I-Ang I cpm/l×10^3	Ang I pmol/l	^{125}I-Ang II cpm/l×10^3	Ang II pmol/l
Aorta	661 (439-1,335)	34 (8.9-142)	133 (86-234)	2.1 (0.8-5.0)
Antecubital vein	341* (234-872)	34 (7.8-143)	114* (72-199)	2.3 (1.1-5.8)
Left renal vein	123* (62-225)	33 (9.0-124)	23* (14-33)	0.8* (0.4-1.5)
Right renal vein	129* (61-191)	32 (9.1-120)	21* (11-38)	0.8* (0.4-1.9)
Femoral vein	265* (158-617)	34 (7.8-134)	109* (62-199)	1.8 (0.8-4.3)
Hepatic vein (n=5)	31* (22-44)	17* (4.9-59)	5* (2-7)	0.4* (0.2-0.6)

^{125}I-Angiotensin I infusion rate was 3.28 (2.46-4.31)×10^6 cpm/min. Values are geometric mean (range). Ang I, angiotensin I; Ang II, angiotensin II.
*Significantly different from corresponding aortic levels (p<0.02, Wilcoxon signed rank test).

the aorta (Table 2) and because observations in animals demonstrated extraction of Ang II in the circulation of the head.[1] It may therefore be assumed that ^{125}I-Ang I-II conversion had occurred in the cardiopulmonary region, presumably the lungs, despite the fact that our subjects were on ACE inhibitor treatment. Because the extraction of ^{125}I-Ang II by the various vascular beds was not measured, it was not possible to calculate the degree of ^{125}I-Ang I-II conversion in these regions.

Regional Production of Angiotensin I

In spite of the rapid extraction of arterially delivered Ang I, the arterial and venous plasma levels of endogenous Ang I across the kidneys and the limbs showed little difference (Figure 5). The vein-to-artery Ang I concentration ratio was 0.98±0.05 across the kidneys, 1.01±0.03 across the forearm, and 1.00±0.03 across the leg. Ang I concentration in the hepatic vein was lower than in the aorta. The vein-to-artery Ang I concentration ratio across the hepatomesenteric vascular bed was 0.52±0.03. From these data and from the regional extraction ratio of Ang I, the venous concentration of Ang I derived from regional de novo production could be calculated. Figure 6 clearly illustrates the point that a high proportion of venous Ang I originated from regional de novo production. For the renal vascular bed, it was possible to calculate the net de novo production rate of Ang I because renal plasma flow was measured. Single kidney plasma flow was

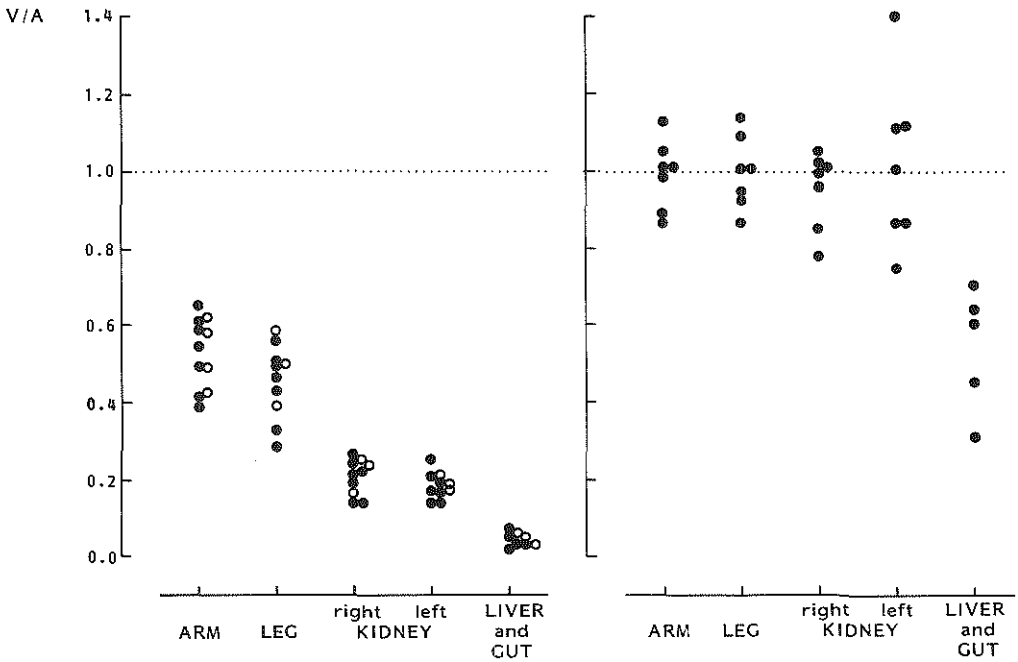

FIGURE 5
Scatter plots showing vein-to-artery ratios of ^{125}I-angiotensin I (Ang I) (left panel) and endogenous Ang I (right panel) across various vascular beds during constant intravenous infusion of ^{125}I-Ang I. ○ represent data obtained during infusion of both ^{125}I-Ang I and Ang I.

0.283±0.020 l/min, and single kidney net de novo production of Ang I was calculated to be 7.1 pmol/min (geometric mean), range 2.4-29.5 pmol/min.

Contribution of Plasma Renin Activity to Regional Production of Angiotensin I

The mean transit time of blood is 0.1-0.2 minute in the kidneys and the limbs and 0.3-0.5 minute in the hepatomesenteric vascular bed.[25,26] From this and the measured levels of PRA, we can calculate the contribution of circulating PRA to the total regional production of Ang I. In the classic concept of the renin-angiotensin system, circulating renin should fully account for this production. The calculations, however, showed that circulating PRA only accounted for less than 20-30% of Ang I production in the kidneys, the forearm, and the leg. In the hepatomesenteric vascular bed, circulating PRA accounted for about 60% of the Ang I production in this region (Table 3).

Discussion

Circulating Ang I and Ang II are rapidly metabolized by the action of peptidases. Ang I is converted to what is considered to be the most important biologically active product of the renin-angiotensin system, Ang II. Both Ang I and Ang II are degraded into smaller inactive peptides. The term "metabolism" is used here to denote both conversion and degradation. There are very few reports on measurements of Ang I metabolism in intact animals,[13,14,27] and we are not aware of any such data in humans.

The present study addressed the question whether in humans the rate at which Ang I is generated by the reaction of circulating renin with circulating renin substrate is sufficiently high to maintain the actual plasma level of Ang I in the face of the rapid metabolism of this peptide. We studied Ang I metabolism in different vascular beds by measurement of the extraction of arterially delivered ^{125}I-Ang I during constant intravenous infusion of this peptide. Again, the term "extraction" includes the processes of conversion and degradation. In our hypertensive subjects on captopril treatment, the regional extraction of ^{125}I-Ang I by the kidneys, the limbs, and the hepatomesenteric vascular bed was not different from the extraction of simultaneously infused unlabeled Ang I. Also, the plasma decay curves after discontinuation of the ^{125}I-Ang I and Ang I infusions were very similar. This supports the validity of using our measurements of ^{125}I-Ang I metabolism for estimation of the metabolism and production of endogenous Ang I, at least in subjects on ACE inhibitor treatment. With such treatment, Ang I metabolism is mainly due to degradation into smaller inactive fragments. Apparently, there is little or no difference in the rate of degradation between labeled and unlabeled Ang I. Whether the conversion rates of the two peptides are also similar has to be established by measurements in subjects not on ACE inhibitor treatment.

Extraction of arterially delivered Ang I by the vascular beds we studied ranged from 47 to 96%. The plasma levels of ACE and angiotensinase activity are by far too low to account for the rapid removal of Ang I. Thus, it appears that most of Ang I metabolism takes place at the surface of blood vessels or in the vascular tissue. In studies in dogs, ^{125}I-Ang I was infused into the renal artery, and it was found that about 80% was removed during a single passage of blood through the kidney.[13] This was for the most part due to degradation rather than conversion. Our results in humans are in agreement with these data.

From the regional extraction ratios of Ang I and from the arteriovenous differences in Ang I, it was calculated that approximately 50-90% of Ang I in the regional veins was not derived from arterially delivered Ang I, but from regional de novo production. In the dog, it was found that 70-80% of Ang I in the renal

Table 3
Contribution of Plasma Renin Activity to Venous Levels of Angiotensin I in Seven Hypertensive Subjects

	Kidney	Forearm	Leg	Liver and Gut*
Mean blood transit time (min)	0.17	0.17	0.17	0.42
Arterial PRA ($pmol.l^{-1}.min^{-1}$)	24 (7.3-117)	24 (6.7-122)	25 (8.9-119)	see 'Forearm'
Venous PRA ($pmol.l^{-1}.min^{-1}$)	31 (7.3-211)	25 (7.2-119)	26 (8.1-135)	23 (6.2-82)
Venous Ang I originating from de novo production (pmol/l)	26 (7.4-102)	16 (4.3-63)	20 (4.9-71)	16 (4.5-53)
Venous Ang I produced by circulating PRA (pmol/l)	5.3 (1.3-33)	4.2 (1.2-20)	4.1 (1.1-18)	8.9 (2.5-42)
Percent of de novo-produced Ang I	21±3	27±3	22±3	62±9

Values are geometric mean (range) or mean±SEM. Mean blood transit time values are from the literature.[25,26] Three arterial samples were collected from the abdominal aorta during the steady-state phase: sample 1 simultaneously with the antecubital vein and hepatic vein samples, sample 2 simultaneously with a left or right renal vein sample, and sample 3 simultaneously with a renal vein sample on the opposite side. The femoral vein sample was taken immediately after aortic sample 3. The left and right renal vein levels of PRA and Ang I were averaged as were the two corresponding aortic levels of PRA. Ang I, angiotensin I; PRA , plasma renin activity. *n=5.

veins was derived from de novo production in the kidney,[13] which is similar to what we found for the human kidney.

With the exception of the hepatomesenteric vascular bed, where the plasma level of Ang I in the hepatic vein was found to be much lower than in the aorta, there was little or no arteriovenous difference in Ang I. Thus, the rapid removal of plasma Ang I was matched by a high rate of production. By taking into consideration the blood transit time in the various vascular beds, it can easily be seen that circulating levels of PRA were not sufficient to account for this high rate of Ang I production. The mean transit time of blood in the kidneys and the limbs is 0.1-0.2 minute or less.[25,26] From this and from the measurements of PRA, the extraction of arterially delivered Ang I, and the arteriovenous difference in the plasma levels of Ang I, it was calculated that less than 20-30% of Ang I produced in the kidneys, the forearm, and the leg could be accounted

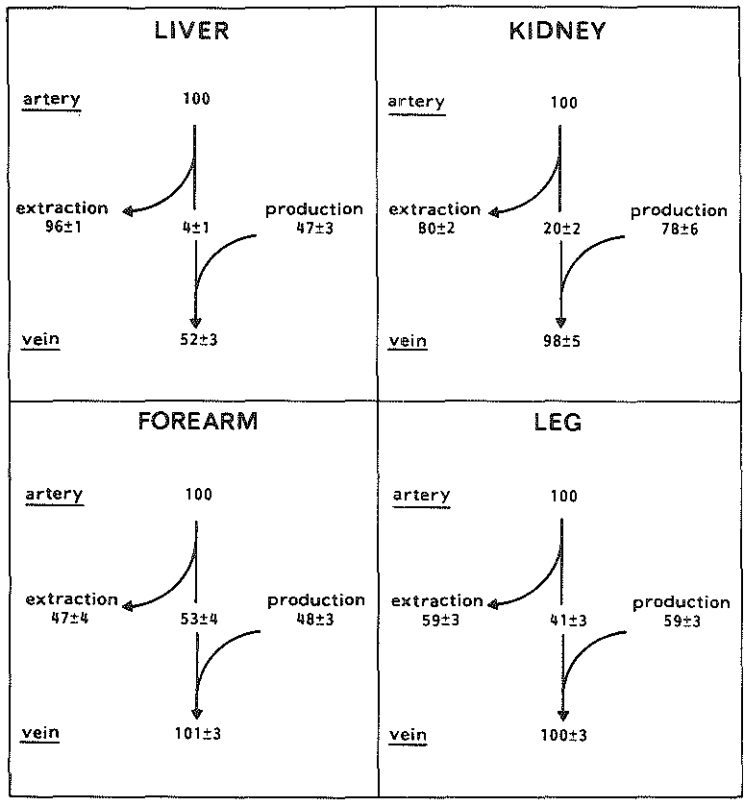

FIGURE 6
Schematic drawing showing regional venous plasma concentration of angiotensin I (Ang I) originating from de novo production of Ang I in various vascular beds. Data (mean±SEM) are expressed as a percentage of arterial plasma concentration of Ang I.

for by the generation of Ang I by circulating PRA (Table 3). For the renal vascular bed, it was possible to calculate the net de novo production rate of Ang I because renal plasma flow was known. The single kidney intravascular plasma volume is approximately 40 ml.[25] From this and from the level of PRA in the renal vein, it was calculated that less than 10% of Ang I produced in the kidney could be accounted for by circulating PRA. The calculated contribution of PRA is a maximum value because it is quite likely that part of regionally produced Ang I is metabolized before it can reach the veins. The blood transit time in the hepatomesenteric vascular bed is 0.3-0.5 minute,[26] and in this region, the Ang I production could be largely accounted for by circulating PRA.

The conclusion of this study, that a high proportion of Ang I in plasma appears not to be generated by the action of circulating renin on circulating renin

substrate, depends on the validity of using PRA as a precise measure of the generation of Ang I by plasma in vivo. We therefore performed the PRA assay at near physiological pH under conditions that did not cause denaturation of renin, activation of prorenin, and conversion and degradation of Ang I, and did not interfere with the reaction of renin with its substrate.

From the data presented in Table 2, it is evident that a discrepancy exists between the ^{125}I-Ang I/^{125}I-Ang II and Ang I/Ang II ratios. One possibility might be that the ^{125}I-Ang II peak is contaminated by some metabolite. We are confident, however, that the ^{125}I-Ang II peak is not contaminated by the possible aminopeptidase breakdown products, ^{125}I-Ang-(2-8), ^{125}I-Ang-(3-8), ^{125}I-Ang-(4-8), and ^{125}I-Ang-(2-10), nor by the prolyl- or neutral-endopeptidase breakdown products, ^{125}I-Ang-(1-4) and ^{125}I-Ang-(1-7). However, we did not measure the retention time of ^{125}I-Ang-(1-9), a possible product of carboxypeptidase attack on ^{125}I-Ang I. We did not observe any heterogeneity in the ^{125}I-Ang II peak when plasma samples were analyzed under different chromatographic conditions, also indicating that ^{125}I-Ang II was the only radiolabeled peptide in this peak. Because we did not compare the rates of metabolism of ^{125}I-Ang II and Ang II, our study only provides semiquantitative information of Ang I-II conversion in the various vascular beds. At any rate, our measurements of the arterial and venous plasma concentrations of ^{125}I-Ang I and ^{125}I-Ang II indicate that, despite ACE inhibitor treatment, Ang I-II conversion had occurred in the cardiopulmonary vascular bed, presumably the lungs.

The results of the present study add new evidence to previously published data supporting the hypothesis that a major fraction of Ang I in circulating plasma is produced locally rather than in circulating plasma itself. The local production of Ang I may depend on synthesis of renin in situ. Binding or uptake of plasma renin or prorenin and subsequent activation of prorenin and local production of Ang I are other possibilities. Whatever the exact mechanism, vascular production of Ang I may contribute to the control of vascular tone, and this contribution may be independent, at least in part, of the circulating levels of renin and renin substrate.

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Chapter 3

Intrarenal De Novo Production of Angiotensin I in Subjects With Renal Artery Stenosis

Summary

To estimate the renal extraction and de novo production of angiotensin I and to assess the contribution of blood-borne renin to renal angiotensin I production, the aortic and renal venous plasma levels of renin and intact ^{125}I-angiotensin I and endogenous angiotensin I during continuous systemic intravenous infusion of monoiodinated ^{125}I-angiotensin I were measured in subjects with unilateral renal artery stenosis (n=8) who were treated with captopril (50 mg, twice daily). Results demonstrated that 80% of angiotensin I delivered by the renal artery was extracted both by the affected and the unaffected kidney and that on both sides a major part of angiotensin I in the renal vein was derived from intrarenal de novo production. Production of plasma angiotensin I was in excess over extraction (p<0.01) on the affected side, whereas extraction was in excess over production (p<0.01) on the contralateral side. The plasma level of de novo intrarenally produced angiotensin I in the renal vein was seven times higher on the affected side than the contralateral side. This difference was by far too big to be explained by a difference in the transit time of blood between the two kidneys, or by an augmented production of angiotensin I in the circulating blood passing through the affected kidney due to the higher level of venous plasma renin activity in that kidney, or by the combination of both. We conclude that intrarenal production of angiotensin I in a compartment outside the circulating blood is a major source of this peptide in the renal vein, and that in kidneys with artery stenosis, in situ synthesized renin is an important component of this source. To the extent that local angiotensin I production by intrarenally synthesized renin is a sine qua non for a local physiological role of renin, our results support the hypothesis that renin may have such a role.

Introduction

Angiotensin II (Ang II) in the kidney subserves important functions in the control of renal blood flow, glomerular filtration rate, and tubular sodium handling. It does so by acting on specific receptors located on blood vessels and mesangial and tubular cells.[1-3] These receptors are stimulated by Ang II delivered from the systemic circulation by the renal artery and most likely also by Ang II formed within the kidney. Intrarenally formed Ang II in turn originates from conversion of arterially delivered angiotensin I (Ang I) and possibly also from locally produced Ang I. Intrarenally produced Ang I may also arise from two sources, namely arterially delivered renin or in situ synthesized renin. The interactions between systemically delivered and locally produced components of the renin-angiotensin system in the kidney are not well understood. Detailed information on how much and where Ang I and Ang II are produced in the kidney would help to answer this question.

In a previous study in subjects with essential hypertension, we demonstrated that the levels of radiolabeled Ang I in arterial and venous plasma, during systemic intravenous infusion of purified monoiodinated ^{125}I-Ang I can be used for calculating the Ang I extraction rate in different vascular beds, including the kidney, at least under conditions of angiotensin converting enzyme (ACE) inhibition.[4] Circulating Ang I is rapidly metabolized by the tissues, and this process includes degradation into inactive peptides as well as conversion to Ang II. By combining data on regional ^{125}I-Ang I extraction with results of measurements of endogenous Ang I, information can be obtained on regional Ang I production.

The present study was aimed at collecting quantitative data on intrarenal Ang I production in subjects with renal artery stenosis. For this purpose ^{125}I-Ang I was administered systemically, which enabled us to collect information on Ang I metabolism and production in the kidney affected by artery stenosis as well as in the contralateral unaffected kidney.

Methods

Chemicals

[Ile^5]Ang-(1-10) decapeptide (Ang I), [Ile^5]Ang-(1-8) octapeptide (Ang II), [Ile^5]Ang-(2-8) heptapeptide (Ang III), and [Ile^5]Ang-(1-7) were obtained from Bachem, Bubendorf, Switzerland. [Ile^5]Ang-(2-10) nonapeptide was from Senn Chemicals, Dielsdorf, Switzerland. [Ile^5]Ang-(3-8) hexapeptide, [Ile^5]Ang-(4-8) pentapeptide, and Ang-(1-4) tetrapeptide were from Peninsula Laboratories, Belmont, CA, USA. The World Health Organization standards of Ang I and Ang II (86/536 and 86/538) were from the National Institute for Biological Standards and Control,

London, UK. Methanol and ortho-phosphoric acid (both analytical grade) were from Merck, Darmstadt, FRG. Bovine serum albumin was from Sigma Chemical Co., St. Louis, MO, USA. Water for high-performance liquid chromatography (HPLC) was prepared with a Milli-Q system from Waters Chromatography Div., Milford, MA, USA. The statine containing renin inhibitor CGP 29,287 was a kind gift of Dr. K. Hofbauer (Ciba-Geigy, Basel, Switzerland).

Blood Sampling

Blood for angiotensin measurements was rapidly drawn (7-13 seconds) with a plastic syringe containing the following inhibitors (0.5 ml inhibitor solution in 10 ml blood), 6.25 mM disodium EDTA, 1.25 mM 1,10-phenantroline and 100 nM of the renin inhibitor CGP 29,287 (final concentrations in blood) and transferred into prechilled polystyrene tubes. The blood samples were centrifuged at 3,000*g* for 10 minutes at 4° C. Plasma was stored at -70° C, extracted within 2 days and assayed within 2 weeks.

Blood for plasma renin activity (PRA) measurements was collected in polystyrene tubes containing EDTA (0.2 ml disodium EDTA in 10 ml blood, final concentration 6.25 mM). The samples were centrifuged at 3,000*g* for 10 minutes at room temperature and plasma was stored at -20° C.

Separation of Angiotensins by High-Performance Liquid Chromatography

Angiotensins and their metabolites were extracted from plasma by reversible adsorption to octadecylsilyl-silica (Sep-Pak C18, Waters) and separated by reversed-phase HPLC, according to the method of Nussberger et al[5] with some modifications as described by Admiraal et al[4] Separations were performed on a reversed-phase Nucleosil C18 steel column of 250X4.6 mm and 10 µm particle size (Alltech, Eke, Belgium). Mobile phase A was 0.085% ortho-phophoric acid containing 0.02% sodium azide. Mobile phase B was methanol. The flow was 1 ml/min and the working temperature 45° C. Extracts of 2 ml plasma were dissolved in 100 µl HPLC solvents and injected. Elution was performed as follows: 65% A/35% B (vol/vol) from 0 to 9 minutes followed by a linear gradient to 45% A/55% B until 18 minutes. The eluate was collected in 0.5 minute fractions into polystyrene tubes coated with bovine serum albumin. The concentrations of ^{125}I-Ang I and ^{125}I-Ang II and their metabolites in the HPLC fractions were measured in the gamma counter. The fractions containing Ang I and Ang II were neutralized with 0.5 M sodium hydroxide and vacuum dried at 4° C.

Assays of Angiotensins, Renin and Renin Substrate

The concentrations of Ang I and Ang II in the HPLC fractions were measured by radioimmunoassay.[4] The Ang I antiserum cross-reacted with Ang-(2-10) nonapeptide (100%) but not (<0.1%) with Ang II, Ang III, Ang-(3-8) hexapeptide, Ang-(4-8) pentapeptide, Ang-(1-7) heptapeptide, or Ang-(1-4) tetrapeptide. The Ang II antiserum cross-reacted with Ang III (55%), Ang-(3-8) hexapeptide (73%), and Ang-(4-8) pentapeptide (100%) but virtually not (<0.2 %) with Ang I, Ang-(2-10) nonapeptide, Ang-(1-7) heptapeptide, or Ang-(1-4) tetrapeptide.

The plasma levels of enzymatically active renin and PRA were determined by the enzyme-kinetic method, in which generated Ang I was measured by radioimmunoassay.[6] In the assay of plasma renin concentration Ang I was generated by incubation with sheep renin substrate, and in the assay of PRA by incubation with endogenous substrate. Both incubations were carried out at pH 7.4 and 37° C. Renin substrate was determined as the maximal quantity of Ang I generated during incubation of plasma with an excess of purified human kidney renin.[6]

Estimations of Renal Plasma Flow and Split Renal Function

The clearance of ^{131}I-iodohippurate was determined as a measure of total effective renal

plasma flow (right plus left kidney). The single kidney extraction ratio of ^{131}I-iodohippurate was also measured.

The single kidney uptake of ^{99m}Tc-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) was determined by scintillation camera renography.[7] The increase in radioactivity over the kidney region during the second minute after intravenous injection of ^{99m}Tc-DTPA was expressed as activity ratio, that is, right/(right+left) ratio. This ratio is a measure of the single kidney contribution to the total glomerular filtration rate.

Subjects

Subjects with hypertension received an intravenous infusion of ^{125}I-Ang I at the time they were undergoing renal vein sampling followed by renal angiography for diagnostic purposes. Here we report the results obtained in eight subjects (six men, two women, 22-70 years old), who proved to have unilateral renal artery stenosis (60-90% stenosis) on their angiographic examination. The results in these subjects were compared with those obtained in a group of seven subjects with essential hypertension and a normal angiogram. The latter group was described in a previous paper.[4] The subjects were studied while they were treated with the ACE inhibitor captopril (50 mg orally, twice daily). The infusions were given 4-6 hours after administration of the morning dose of captopril. The subjects had given their informed consent and the study was approved by the Hospital Ethics Review Committee.

Infusion Protocol

Highly purified monoiodinated ^{125}I-Ang I was prepared as described previously.[4] After insertion of the catheters into the abdominal aorta and the inferior caval vein via the femoral artery and vein by the Seldinger technique, ^{125}I-Ang I was infused via an antecubital vein, at a constant rate of approximately 3×10^6 cpm/min (approximately 1 pmol/min) for 20 minutes. Blood samples were taken from the right and left renal vein between 10 and 20 minutes after the infusion was started. An aortic sample was taken simultaneously with each renal vein sample. The arterial and venous plasma levels of ^{125}I-Ang I and II remained constant during this period (coefficient of variation <4%).

Calculations and Statistics

The renal extraction ratio (ER) of Ang I was calculated as follows:

$$\text{ER of Ang I} = 1-(^{125}\text{I-Ang I}_{ven}/^{125}\text{I-Ang I}_{art})$$

where ven is renal venous and art is aortic.

The renal venous plasma level of Ang I (Ang I_{ven}) derived from renal de novo production was calculated as follows:

$$\text{Ang I}_{ven} \text{ from de novo production} = \text{Ang I}_{ven}-(1-\text{ER})\times\text{Ang I}_{art}$$

in which ER is the extraction ratio of Ang I.

Results of measurements of angiotensins and renin were not normally distributed and are presented as medians and ranges. The Wilcoxon rank sum test was used to compare angiotensin and renin levels between groups. The Wilcoxon signed rank test was used for paired data.

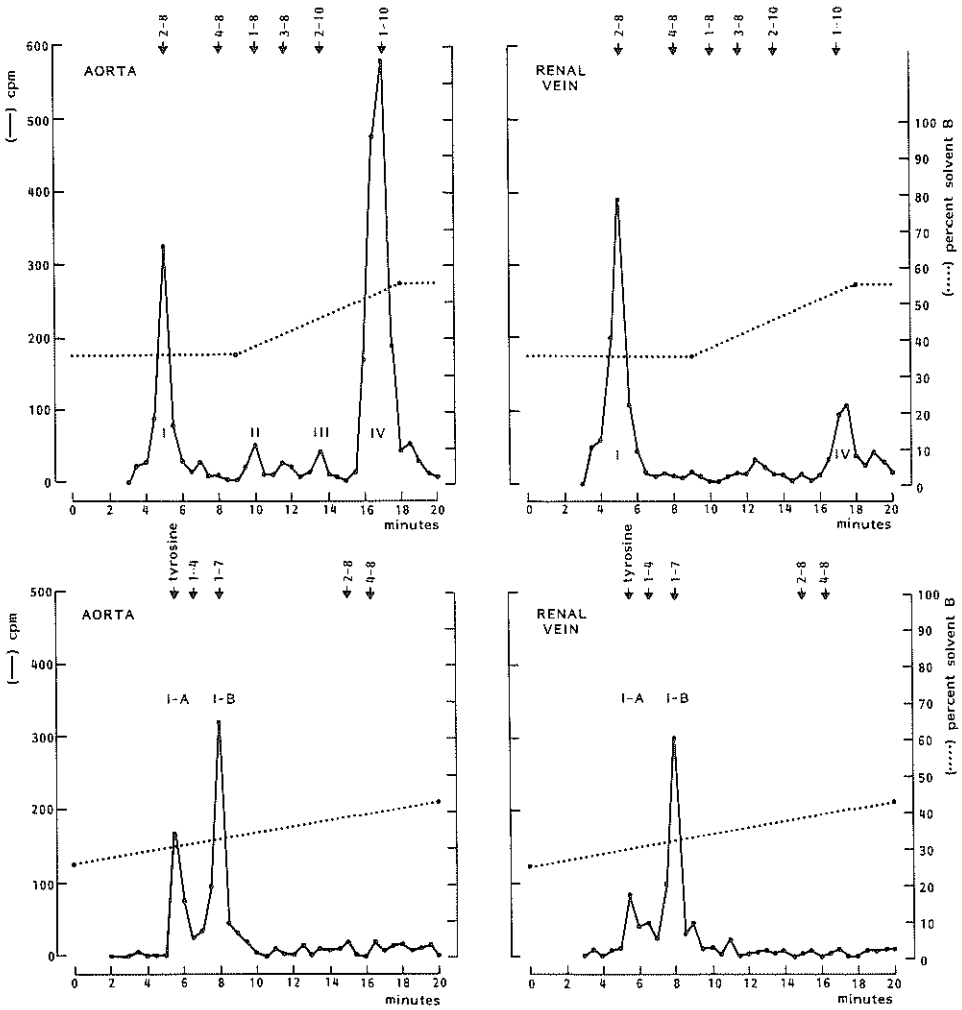

FIGURE 1

Line graphs show high-performance liquid chromatography of iodine-125-labeled angiotensins (Ang) in aortic and renal venous plasma on affected side of subject with renal artery stenosis who received a constant intravenous infusion of ^{125}I-Ang I. Peak I (upper panels) was rechromatographed using a different elution program (lower panels). Stationary phase, Nucleosil C18, 10 µm, 250×4.6 mm. Mobile phase A, ortho-phosphoric acid 0.085% (containing 0.02% sodium azide). Mobile phase B, methanol. Detection by gamma counting. Peaks II, III, and IV (upper panels) with retention times of 10.0, 13.5, and 16.5 minutes correspond with ^{125}I-Ang-(1-8)= ^{125}I-Ang II, ^{125}I-Ang-(2-10), and ^{125}I-Ang-(1-10)=^{125}I-Ang I, respectively. Retention times of peaks I-A and I-B (lower panels) correspond with those of ^{125}I-tyrosine and ^{125}I-Ang-(1-7), respectively. Arrows indicate retention times of standard radiolabeled angiotensin peptides, for instance, 1-8=^{125}I-Ang-(1-8) and tyrosine=^{125}I-tyrosine.

Results

Identification of Angiotensins and Their Metabolites in Plasma by High-Performance Liquid Chromatography

With the standard elution program, the radiolabeled peptides in the Sep-Pak extracts of aortic and renal venous plasma from subjects to whom ^{125}I-Ang I was given with constant intravenous infusion, were separated by HPLC into four major peaks (peaks I to IV, Figure 1). Material in these peaks was quantitatively bound to Sep-Pak and was completely recovered by elution with methanol. Peaks II, III, and IV with a retention time of 10.0, 13.5, and 16.5 minutes were identified as ^{125}I-Ang-(1-8)=^{125}I-Ang II, ^{125}I-Ang-(2-10), and ^{125}I-Ang-(1-10)= ^{125}I-Ang I, respectively. The identification was not only based on their retention time but also on their reactivity with the Ang I and Ang II antisera. The Ang I antiserum, which reacted with Ang I and Ang-(2-10) but virtually not with Ang II, bound 90-100% of the radioactivity in peaks III and IV and bound less than 3% of the radioactivity in peak II. In contrast, the Ang II antiserum bound more than 90% of the radioactivity of peak II and less than 5% of the radioactivity of peaks III and I.

The retention times of peaks II and IV were sufficiently different from each other and from the retention times of ^{125}I-Ang-(2-8)=^{125}I-Ang III, ^{125}I-Ang-(3-8), ^{125}I-Ang-(4-8), ^{125}I-Ang-(1-7), ^{125}I-Ang-(1-4), and ^{125}I-tyrosine to obtain satisfactory separations. By removing cross-reacting angiotensin peptides, the HPLC separations allowed us to perform specific measurements of unlabeled Ang I and Ang II by radioimmunoassay.

By using lower starting concentrations of methanol in the mobile phase, it was possible to separate peak I into two to three peaks (Figure 1, lower panels). One of these, peak I-B, accounting for 30-50% of the total radioactivity of peak I, had a retention time identical to that of ^{125}I-Ang-(1-7). On the basis of its chromatographic behavior, this peak could be differentiated from all the other angiotensin peptides we tested, but the conclusion that this peak is indeed ^{125}I-Ang-(1-7) needs further confirmation. A second subfraction, peak I-A, could be separated from peak I. This subfraction had the retention time of ^{125}I-tyrosine and could also be differentiated from the other angiotensin metabolites we tested.

Split Renal Function and Renal Venous Plasma Levels of Renin and Angiotensins

The total (right plus left kidney) glomerular filtration rate, as indicated by the serum level of creatinine, was reduced in the subjects with renal artery stenosis as compared to essential hypertension (Table 1). The total effective renal plasma flow was also reduced. The presence of unilateral renal artery stenosis was reflected by differences between the two kidneys in ^{99m}Tc-DTPA uptake and

Table 1
Blood Pressure and Renal Function in Subjects With Renal Artery Stenosis as Compared With Essential Hypertension

Variables	Essential hypertension (n=7)	Renal artery stenosis (n=8)	p value
Age (yr)	42±12	54±16	NS
Sex (male/female) (n)	2/5	6/2	
Body surface area (m^2)	1.88±0.22	1.94±0.15	NS
BP systolic (mm Hg)	160±24	155±18	NS
BP diastolic (mm Hg)	99±9	99±14	NS
Serum creatinine (μmol/l)	83±25	119±36	$p<0.05$
DTPA uptake			
right/left kidney (%)	51±6/49±6		
affected/unaffected kidney (%)		18±11/82±11	$p<0.01/p<0.01$
Extraction ratio ^{131}I-hippurate			
right/left kidney (%)	75±3/72±2		
affected/unaffected kidney (%)		37±18/80±5	$p<0.01$/NS
^{131}I-hippurate clearance (l/min)	0.388±0.071	0.284±0.095	$p=0.063$

Data are mean±SD. Significance levels for differences with essential hypertension were analyzed with the Student's t test. The subjects with essential hypertension were described in a previous paper and data on the renal extraction of ^{131}I-hippurate are from that paper.[4] BP, blood pressure; DTPA, ^{99m}Tc-diethylenetriamine pentaacetic acid.

^{131}I-iodohippurate elimination. As judged from ^{99m}Tc-DTPA uptake, the contribution of the kidney affected by artery stenosis to the total glomerular filtration rate was approximately 20%.

As expected, the aortic plasma levels of renin were higher in the subjects with renal artery stenosis than in those with essential hypertension (Table 2). Renin substrate concentration was lower in the renal artery stenosis group than in essential hypertension group. For calculating PRA, the levels of renin and renin substrate and the values of K_m=1.2 μmol/l and V_{max}=1.6 pmol Ang I/min per milliunit renin were entered into the Michaelis-Menten equation.[6] Results of this calculation were close to the actually measured values of PRA (Table 2), which indicates that the incubate of the PRA assay was free of substances interfering with the enzymatic activity of renin. Data on the renal vein PRA levels in the individual subjects with renal artery stenosis are given in Table 3.

In the renal artery stenosis group, the renal vein-to-aorta ratio of PRA was

Table 2
Aortic Plasma Levels of Renin, Renin Substrate, Plasma Renin Activity and Angiotensins in Subjects With Renal Artery Stenosis as Compared With Essential Hypertension

Variables	Essential hypertension (n=7)		Renal artery stenosis (n=8)	
Renin (milliunits/l)	22	(6.2-117)	272	(28-463)
Renin substrate (μmol/l)	1.11	(0.84-1.33)	0.71	(0.41-1.17)*
PRA calculated (pmol.l^{-1}.min^{-1}) †	21	(6.1-101)	126	(32-272)**
PRA measured (pmol.l^{-1}.min^{-1})	25	(7.3-117)	128	(35-430)**
Angiotensin I (pmol/l)	46	(8.9-142)	187	(55-308)**
Angiotensin II (pmol/l)	2.2	(0.8-5.0)	10.6	(2.7-73)**

Data are medians and ranges. The subjects with essential hypertension were described in a previous paper and data on plasma renin activity (PRA) and angiotensin I (Ang I) and II are from that paper.[4]

*p<0.05 and **p<0.01 for difference from essential hypertension.

† K_m=1.2 μmol/l and V_{max}=1.6 pmol Ang I/min per milliunit of renin were entered into the Michaelis-Menten equation to calculate PRA.

1.92 (median) on the affected side, which was significantly (p<0.01) higher than 1.00 (Figure 2). Contralaterally this ratio was 1.05, which was not significantly different from 1.00. Similar results were obtained for renin. Thus, there was net secretion of renin into the circulation on the affected side but not on the contralateral side. In essential hypertension, the renal vein-to-aorta ratio of PRA is known to be higher than 1.00 and is approximately 1.25.[8] The low renin ratio of the contralateral kidney in subjects with renal artery stenosis is likely to reflect the suppressed production of renin by that kidney.

The aortic plasma levels of Ang I and Ang II were higher in the subjects with renal artery stenosis than in those with essential hypertension (Table 2). As expected in subjects treated with ACE inhibitor, the plasma levels of Ang II were low, particularly in relation to the high levels of Ang I. Data on the renal venous plasma levels of Ang I and Ang II in the individual subjects with renal artery stenosis are given in Table 3.

In the renal artery stenosis group, the renal vein-to-aorta concentration ratio of Ang I was 1.97 on the affected side and 0.67 contralaterally (Figure 2). On the affected side, the ratio was higher than 1.00 (p<0.01). Contralaterally it was lower than 1.00 (p<0.01), indicating net extraction of Ang I from the systemic

Table 3
Levels of Plasma Renin Activity and Angiotensins in Subjects With Renal Artery Stenosis

Subjects	Angiotensin I (pmol/l)			Angiotensin II (pmol/l)			PRA (pmol.l^{-1}.min^{-1})		
	Artery	Vein affected kidney	Vein unaffected kidney	Artery	Vein affected kidney	Vein unaffected kidney	Artery	Vein affected kidney	Vein unaffected kidney
1	246	495	49	39.8	16.1	2.8	170	292	135
2	142	274	87	16.6	6.4	3.5	127	240	139
3	105	111	76	4.6	1.7	1.7	103	149	13
4	308	467	241	19.5	12.0	10.5	430	833	400
5	55	67	39	3.5	2.1	1.2	35	48	34
6	83	242	53	3.3	2.3	2.4	62	364	130
7	289	2,115	268	72.5	15.5	7.2	280	1,032	346
8	232	546	69	2.9	2.9	2.9	128	309	128
Median	187	370	73	10.6	4.6	2.8	128	301	134

PRA, plasma renin activity.

circulation. This contrasts with essential hypertension, where the renal vein-to-aorta ratio of Ang I on both sides was not significantly different from 1.00.[4] The renal vein-to-aorta ratio of Ang II in the subjects with renal artery stenosis was less than 1.00 ($p<0.01$) both on the affected side and contralaterally. Thus, there was net extraction of Ang II from the circulation by both the affected and unaffected kidney.

The presence of Ang-(2-8)=Ang III in plasma has been reported.[9-11] When ^{125}I-Ang III was added to plasma before extraction, it was completely recovered in the Sep-Pak extract. However, without the addition of ^{125}I-Ang III, very little of this radioactive metabolite was found in the plasma of our subjects.

Renal Extraction and Production of Angiotensin I

Data on the renal venous plasma levels of ^{125}I-Ang I and ^{125}I-Ang II in the individual subjects with renal artery stenosis are given in Table 4. The renal extraction ratio of ^{125}I-Ang I in this group was 0.82 both on the affected side and the contralateral side (Figure 2). This is similar to the ratio we found in essential hypertension.[4] The renal vein level of Ang I derived from intrarenal de novo

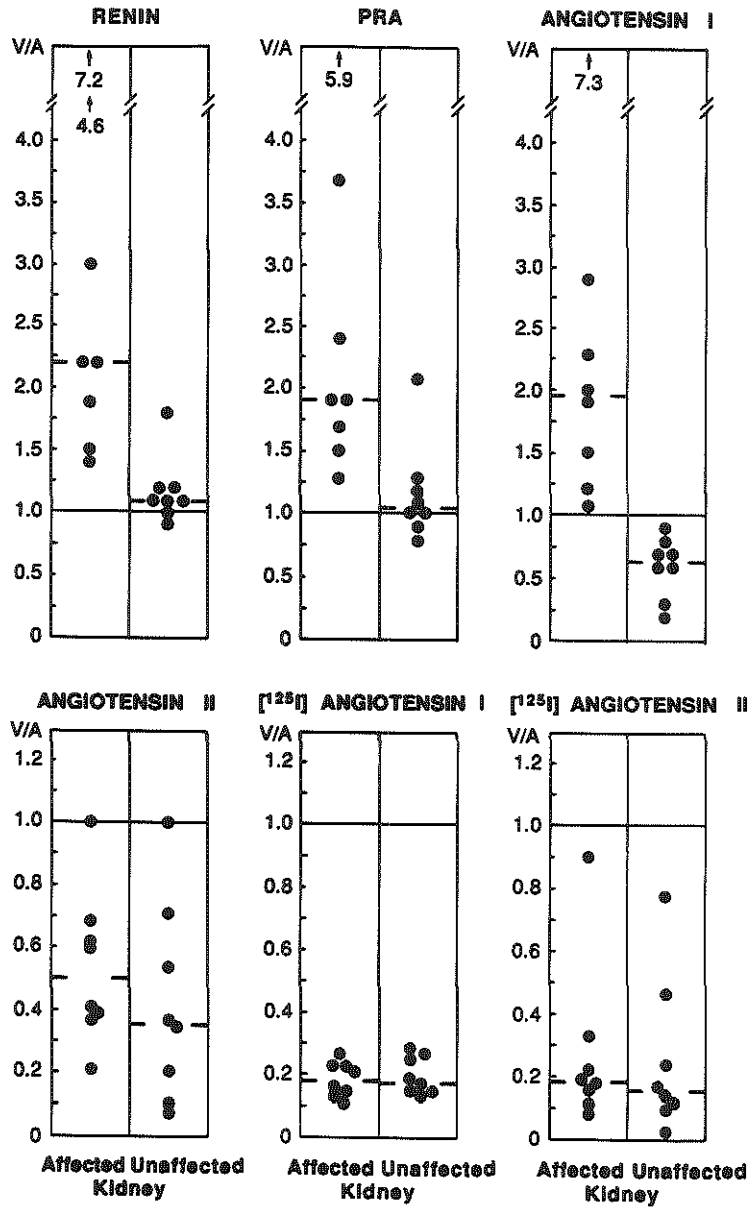

FIGURE 2
Scatter plots showing renal vein-to-aorta ratios (V/A) of plasma renin concentration, plasma renin activity (PRA), and plasma concentrations of radiolabeled and endogenous angiotensins in subjects with renal artery stenosis.

Table 4
Levels of Radiolabeled Angiotensins in Subjects With Renal Artery Stenosis

Subject	^{125}I-Angiotensin I (cpm/l×10^3)			^{125}I-Angiotensin II (cpm/l×10^3)			^{125}I-Angiotensin I infusion rate (cpm/min×10^6)
	Artery	Vein affected kidney	Vein unaffected kidney	Artery	Vein affected kidney	Vein unaffected kidney	
1	1,119	173	187	716	81	21	4.00
2	396	58	61	196	33	34	3.00
3	690	92	174	67	5	7	3.30
4	306	68	81	86	19	21	3.36
5	700	157	131	137	24	19	2.68
6	689	182	101	83	75	65	1.90
7	554	60	76	712	134	86	3.59
8	767	161	221	36	12	17	2.35
Median	690	125	116	112	29	21	3.15

production was calculated from the extraction of ^{125}I-Ang I and from the venous and arterial levels of Ang I (see "Methods"). The renal vein level of intrarenally produced Ang I in the subjects with renal artery stenosis appeared much higher on the affected side than the contralateral side (Figure 3). It was calculated that 91% (82-98%) (median and range) of Ang I in the renal vein on the affected side was derived from intrarenal production, as compared to 70% (2-85%) on the contralateral side ($p<0.01$).

Contribution of Circulating Renin to the Renal Production of Angiotensin I

The mean transit time of plasma has been reported to be 4-10 seconds (0.07-0.17 minutes) for kidneys of hypertensive patients without renal artery stenosis as well as for kidneys with a degree of artery stenosis similar to that in our subjects.[12-14] In our subjects with renal artery stenosis, the minimum time required for circulating PRA to maintain the renal vein level of Ang I was much longer; it was 1.10 (0.48-2.01) minutes (median and range) on the affected side and 0.39 (0.01-0.84) minutes on the contralateral side. To estimate the renal vein level of Ang I derived from intrarenal production by circulating PRA, we used a

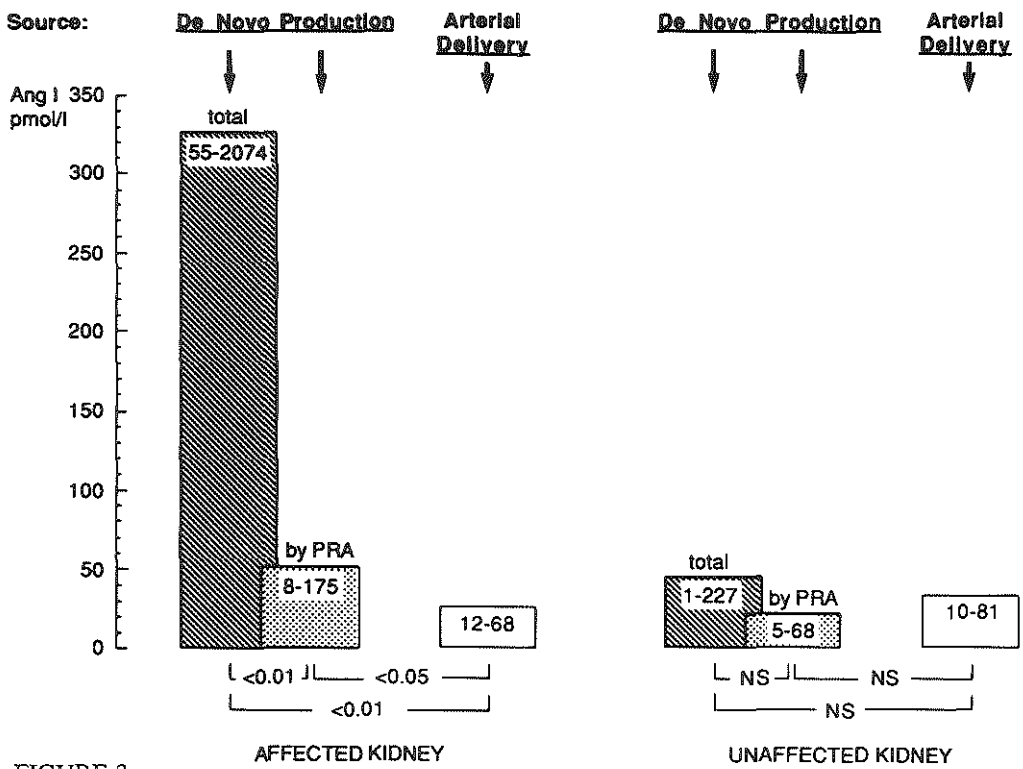

FIGURE 3

Bar graphs showing renal venous plasma levels (median values) of angiotensin I (Ang I) in subjects with renal artery stenosis. Various sources of Ang I in the renal vein (i.e., arterial delivery, total intrarenal de novo production, and intrarenal production due to circulating plasma renin activity [PRA]) are indicated by separate bars. Ranges of Ang I levels for each bar and p values for differences between bars are given. Renal venous plasma level of Ang I derived from circulating PRA was calculated assuming a mean transit time of blood of 0.17 minute (see text). NS, not significant.

transit time of 0.17 minutes and multiplied this value with the measured renal venous level of PRA. The results are presented in Figure 3. It appeared that on the affected side most of the intrarenally produced Ang I in renal venous plasma could not be accounted for by circulating PRA. On the unaffected side about half of Ang I in the renal vein could be accounted for by circulating PRA. In two subjects, virtually all Ang I in the renal vein on the unaffected side could be accounted for by circulating PRA. This might indicate that the transit time we used to calculate the intrarenal Ang I production by circulating PRA was too high. An alternative explanation might be that part of Ang I produced by circulating PRA is extracted before it can reach the renal vein.

Renal Angiotensin I-II Conversion

Despite ACE inhibitor treatment, some ^{125}I-Ang I-II conversion occurred in our subjects (Table 4), who were studied 4-6 hours after oral dosing of 50 mg captopril. At that time part of plasma ACE activity is already restored. Assuming the degradation rates for ^{125}I-Ang I and ^{125}I-Ang II to be equal, the results of this study, showing similar renal vein-to-aorta ratios of ^{125}I-Ang I and ^{125}I-Ang II (Figure 2) would suggest that little ^{125}I-Ang I-II conversion had occurred in the kidneys. However, no firm conclusion on Ang I-II conversion can be drawn from our results because we did not study Ang II metabolism. Moreover, while the in vivo degradation rates of ^{125}I-Ang I and unlabeled Ang I show little or no difference,[4] it is not known whether the conversion rates of the two peptides are also not different.

Discussion

Measurements of immunoreactive Ang I-like material in renal venous and arterial plasma reported by others provide evidence for net release of Ang I into the systemic circulation by kidneys affected by renal artery stenosis but not by kidneys with normal arteries.[15] Measurements in dogs of the arterial and renal venous plasma levels of true Ang I (separated from cross-reacting immunoreactive material) demonstrated net release of this peptide into the systemic circulation when renal perfusion pressure was reduced by clamping the renal artery.[16] Our study extends these data by measurement of the renal extraction of arterially delivered radiolabeled Ang I, which enabled us to collect information on the de novo production of Ang I in the kidney. In a previous study, we validated the use of systemic intravenous infusion of monoiodinated ^{125}I-Ang I for measuring the extraction of arterially delivered Ang I by different vascular beds, including the kidney, in subjects given captopril.[4] In the present study, which concerned subjects with unilateral renal artery stenosis, the renal extraction ratio of Ang I appeared independent of renal plasma flow; it was about 80% both on the affected side and contralaterally, and this is not different from what we found in essential hypertension.[4]

From the renal extraction ratio of Ang I and from the measured levels of intact endogenous Ang I in the aorta and renal vein, it could be calculated that in the subjects with renal artery stenosis 70-90% of Ang I in the renal vein was derived from intrarenal production and not from delivery via the renal artery, both on the affected side side and on the contralateral side. Again, this is similar to what we found in essential hypertension.[4] The level of intrarenally produced Ang I in the renal vein in the subjects with renal artery stenosis was seven times

higher on the affected side than on the contralateral side and 10 times higher than in essential hypertension.[4] It has been reported that in hypertensive subjects the plasma flow per unit weight of kidneys with a degree of artery stenosis similar to that in our subjects is no less than half the flow of kidneys with normal arteries.[12] Thus, the observed differences in the levels of intrarenally produced Ang I in the renal vein were likely to be due, at least in part, to differences in the production rate of this peptide.

One assumption is that these differences were caused by the higher levels of renin in the renal circulation in subjects with renal artery stenosis as compared with those with essential hypertension. By taking into account the measured levels of PRA and the reported values of the mean transit time of blood for kidneys with and without artery stenosis, it was possible to estimate the contribution of circulating PRA to the intrarenal production of Ang I. We took the highest value of the renal transit time of blood (0.17 minute) reported in the literature[13,14] to avoid underestimation of the contribution of circulating PRA. It appeared that in our subjects with renal artery stenosis less than 10-20% of intrarenally produced Ang I on the affected side could be accounted for by circulating PRA. Also, in essential hypertension only a minor part, less than 20-30%, of intrarenally produced Ang I can be accounted for by circulating PRA.[4] These figures are indeed maximum values, not only because the transit time values we chose were on the high side but also because not all intrarenally produced Ang I may have reached the renal vein.

Locally synthesized renin contributes to the level of PRA in the renal circulation and thereby to the production of Ang I in plasma during its passage through the kidneys. From the data above, however, we have to conclude that most Ang I in the renal vein is not derived from the action of circulating renin on circulating renin substrate but is produced in a compartment outside the circulating blood. Ang I in this compartment is likely to be produced by in situ synthesized renin because the renal venous level of Ang I derived from this compartment, in our subjects with renal artery stenosis, was higher on the affected side than on the contralateral side, a difference that was much too big to be explained solely by a difference in the renal transit time of blood.

The anatomical localization of this compartment is unknown, but our data suggest two possibilities. One possibility is the vascular wall. Multiple lines of evidence support the existence of a vascular renin-angiotensin system. Vascular renin appears to originate from binding or uptake of plasma renin or prorenin, but there is also evidence for synthesis of renin in situ.[17-19] Binding of plasma renin to vascular tissue may result in a high local concentration of this enzyme and a high local production rate of angiotensins. Vascular renin in the kidney may originate from renin that is delivered to the renal circulation via the renal

artery as well as from renin that is locally secreted into the renal circulation.

Alternatively, Ang I may be formed by renin that is secreted by the juxtaglomerular cells into the renal interstitium.[20,21] Renal lymph contains renin in high concentrations[22] and interstitial renin is thought to enter the blood compartment at the level of the peritubular capillaries.[1] These sites may therefore represent the compartment from which most of Ang I in the renal vein is derived.

^{125}I-Ang II, ^{125}I-Ang-(2-10), and possibly ^{125}I-Ang-(1-7) were identified in addition to ^{125}I-Ang I in both aortic and renal venous plasma. Very little if any ^{125}I-Ang III could be detected. ^{125}I-Ang-(2-10) is the aminopeptidase product of ^{125}I-Ang I. In the kidney, neutral endopeptidase 24.11 (enkephalinase) is thought to inactivate bradykinin by cleaving the Pro-Phe bond of this peptide, and the enzyme is also capable of cleaving this bond in Ang I, thereby forming Ang-(1-7).[23]

The results of this study seem to indicate that in our subjects who were receiving ACE inhibitor treatment little Ang I-II conversion occurred in the kidneys. However, as explained under "Results", our data on Ang I-II conversion are incomplete. In untreated dogs receiving ^{125}I-Ang I via infusion into the renal artery, about 20% of radioactivity in the renal venous plasma of the infused kidney was recovered in the form of ^{125}I-Ang II.[16] Intrarenally infused unlabeled Ang I in these animals was also partly converted to Ang II, and the formed Ang II had significant effects on renal hemodynamics.[24] Ang II is not only present in the blood compartment of the kidney; this peptide has also been detected in renal lymph.[25] The levels that were measured in renal lymph were higher as compared to plasma, but there is some doubt on these measurements because of the possibility that part of Ang II was formed during the procedure of lymph collection.

To the extent that local Ang I production by intrarenally synthesized renin is a sine qua non for a local physiological role of renin, the results of our study support the hypothesis that renin may have such a role.

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Chapter 4

Regional Angiotensin II Production in Essential Hypertension and Renal Artery Stenosis

Summary

To study regional metabolism and production of angiotensin II, we measured steady-state plasma levels of ^{125}I-angiotensin I and II and endogenous angiotensin I and II in the aorta and the antecubital, femoral, renal, and hepatic veins during systemic infusion of ^{125}I-angiotensin I or II. Extraction of arterially delivered angiotensin II ranged from 30-50% in the limbs to 80-100% in the renal and hepatomesenteric vascular beds both in essential hypertension (n=13) and in unilateral renal artery stenosis (n=7). Across the limbs, 20-30% of arterially delivered angiotensin I was converted to angiotensin II in both groups, and there was no arteriovenous gradient in endogenous angiotensin II. No conversion of arterially delivered angiotensin I was detected across the renal and hepatomesenteric beds, and there was net extraction of angiotensin II from the systemic circulation by these beds. Although regional production of angiotensin I at tissue sites made a significant contribution to its level in the veins, little of this locally produced angiotensin I reached the regional veins in the form of angiotensin II, even in the kidney with artery stenosis, where the venous levels of locally produced angiotensin I were particularly high. These results provide no evidence for a source of circulating angiotensin II other than blood-borne angiotensin I and illustrate the high degree of compartmentalization of angiotensin I and II production.

Introduction

According to the classic view of the renin-angiotensin system, circulating renin acts on circulating angiotensinogen to form the decapeptide angiotensin I (Ang I). During the passage of blood through the lungs, Ang I is converted to the octapeptide angiotensin II (Ang II) by angiotensin converting enzyme (ACE) that

is bound to the luminal surface of the pulmonary vascular endothelium. Ang II is then transported by the bloodstream to peripheral target sites to exert its physiological actions.

This view of the renin-angiotensin system, however, is an oversimplification. Observations in animals and humans have clearly demonstrated that considerable conversion of plasma Ang I occurs in organs other than the lungs,[1-6] but particularly in humans, it is not known to what extent extrapulmonary conversion of Ang I to II contributes to the circulating levels of Ang II.

Moreover, the concept of the renin-angiotensin system as a circulating endocrine system is now being challenged.[7-10] Animal studies, in which pharmacological doses of Ang II were systemically infused, have indicated that this peptide is rapidly eliminated from the circulation,[11-13] and it has been postulated that the plasma levels of Ang II cannot be accounted for by the conversion of plasma Ang I that is formed by the catalytic action of circulating renin on circulating angiotensinogen.[9] In these animal studies, Ang II was measured with a radioimmunoassay that was not entirely specific for intact Ang II octapeptide. In recent studies in humans, we infused tracer doses of ^{125}I-Ang I, and we used specific assays of intact ^{125}I-Ang I and endogenous Ang I. The results showed that, indeed, a major fraction of Ang I present in plasma is produced outside the bloodstream. These observations were made in hypertensive subjects who were receiving ACE inhibitor treatment.[14,15]

In the present study, we infused tracer doses of ^{125}I-Ang I or ^{125}I-Ang II into subjects with essential hypertension (EHT) and subjects with hypertension associated with unilateral renal artery stenosis (URAS) who were not receiving ACE inhibitor treatment. By measuring the arterial and venous levels of intact ^{125}I-Ang I and ^{125}I-Ang II as well as the levels of endogenous Ang I and Ang II across a number of vascular beds, we were able to quantify the degree of Ang I-II conversion in these beds and to examine whether the conversion of blood-borne Ang I is the only source of circulating Ang II.

Methods

Chemicals

[Ile^5]Ang-(1-10) decapeptide (Ang I) and [Ile^5]Ang-(1-8) octapeptide (Ang II) were obtained from Bachem, Bubendorf, Switzerland. The World Health Organization Ang I and Ang II standards (86/536 and 86/538) were from the National Institute for Biological Standards and Control, London, UK. Methanol and ortho-phosphoric acid (both analytical grade) were from E. Merck, Darmstadt, FRG. Water for high-performance liquid chromatography was prepared with a Milli-Q system from Waters Chromatography Division, Milford, Mass, USA. Bovine serum albumin and ACE from rabbit lung were from Sigma Chemical Co., St. Louis, Mo, USA. Human

kidney ACE was a gift of Dr. F. Alhenc-Gelas and Dr. P. Corvol (Institute National de la Santé et de la Recherche Médicale, Paris, France). The statine-containing renin inhibitor CGP 29,287 was a gift of Dr. K. Hofbauer (Ciba-Geigy, Basel, Switzerland). The ACE inhibitor lisinopril was a gift of Dr. J. Bouman (Merck Sharp & Dohme, Haarlem, The Netherlands).

Blood Sampling

Blood for angiotensin measurements was rapidly drawn (within approximately 10 seconds from the aorta and the antecubital and femoral veins, and within approximately 15 seconds from the renal veins) with a plastic 10-ml syringe containing 0.5 ml inhibitor solution (6.25 mM disodium EDTA, 1.25 mM 1,10-phenantroline, and 100 nM CGP 29,287, final concentrations in blood) and was transferred into prechilled plastic tubes. The blood samples were centrifuged at 3,000*g* for 10 minutes at 4° C. Plasma was stored at -70° C, and was extracted within 2 days.

Blood for plasma renin activity (PRA) measurements was collected into 10-ml plastic tubes containing 0.2 ml disodium EDTA (final concentration, 6.25 mM). The samples were centrifuged at 3,000*g* for 10 minutes at room temperature, and plasma was stored at -20° C.

Blood for in vitro Ang I-II conversion studies was collected from the antecubital vein of healthy volunteers into tubes containing 0.04 ml sodium heparin (final concentration, 200 USP units in 10 ml blood). Samples were centrifuged at 3,000*g* and 4° C, and the plasma was immediately used.

Separation and Assay of Angiotensins

Angiotensins were extracted from 2 ml plasma by reversible adsorption to Sep-Pak C18 cartridges (Waters) and were separated by reversed-phase high-performance liquid chromatography according to the method of Nussberger et al[16] with some modifications as described previously.[14] Concentrations of ^{125}I-Ang I, ^{125}I-Ang II, and their metabolites in collected chromatographic fractions were measured directly in a 12-channel gamma counter (Multigamma 1260, LKB-Wallac, Turku, Finland). Fractions containing Ang I and Ang II were neutralized with sodium hydroxide and dried at reduced pressure. The Ang I and Ang II concentrations in these fractions were measured by radioimmunoassay. Characteristics of Ang I and Ang II antisera that were used in these radioimmunoassays and the way these assays were performed have been described previously.[14] The lower limit of detection (2×SD difference from blank) was 1.0 fmol per tube for Ang I and 0.4 fmol per tube for Ang II. Recovery for Ang I and Ang II that were added to plasma was 85±7% and 84±8% (mean±SD, n=6), respectively. Similar values were obtained for ^{125}I-Ang I and ^{125}I-Ang II. Results of the angiotensin measurements were not corrected for incomplete recovery.

Comparison Between Conversion of Angiotensin I to II and ^{125}I-Angiotensin I to II In Vitro

Solutions of ^{125}I-Ang I (20×10^9 cpm/l), unlabeled Ang I (150 nM), the renin inhibitor CGP 29,287 (100 μM), and the ACE inhibitor captopril (25 mM) were prepared in water supplemented with bovine serum albumin (1 g/l). ^{125}I-Ang I (50 μl) or unlabeled Ang I (50 μl) and CGP 29,287 (50 μl) were added to 7 ml fresh normal plasma. The mixture was incubated in the presence or absence of captopril (100 μl) at 37° C. Samples were taken from the incubate at 0, 1, 2, 5, 10, and 15 minutes to determine the half-life of added labeled and unlabeled Ang I. The half-life of added labeled and unlabeled Ang II was also measured.

In vitro conversion rates for ^{125}I-Ang I and unlabeled Ang I were also compared by incubating ^{125}I-Ang I (20×10^6 cpm) or unlabeled Ang I (10 pmol) with purified ACE (5 milliunits). Two ACE preparations were used, namely, ACE from rabbit lung (Sigma) or ACE from human kidney.[17] Incubations were carried out at 37° C and pH 7.5 in the presence of 300 mM KCl, 1 μM $ZnCl_2$, 200 mM Tris, and 2 g/l bovine serum albumin. Total volume of incubate was

200 µl. Samples (25 µl) were taken after 0, 1, 2, 5, 10, 20, and 30 minutes of incubation. The samples were diluted in 1 ml inhibitor solution containing 13 mM disodium EDTA and 75 µM of the ACE inhibitor lisinopril. Radiolabeled and unlabeled angiotensins were measured after extraction and separation.

Subjects

Regional metabolism of Ang I and Ang II was studied in 27 hypertensive subjects, 13 men and 14 women, aged 49.6±12.2 years (mean±SD). Combined infusions of ^{125}I-Ang I and unlabeled Ang I were given to six subjects with EHT. Infusions of ^{125}I-Ang I only were given to seven subjects with URAS and seven subjects with EHT. Infusions of ^{125}I-Ang II were given to seven subjects with EHT. Subjects were not receiving antihypertensive treatment, except four subjects who received an infusion of ^{125}I-Ang II while they were treated with the ACE inhibitor captopril, 25 mg twice daily.

All subjects were studied at the time they were undergoing renal vein renin sampling followed by renal angiography for diagnostic purposes. URAS was found in seven subjects. They proved to have a 60-90% stenosis of the renal artery. The remaining subjects showed no abnormalities on their angiogram and were diagnosed as having EHT.

Infusion Protocol

Angiotensin infusions were started after insertion of catheters into the abdominal aorta and the inferior caval vein via the femoral artery and vein with the Seldinger technique. Seven subjects with URAS and seven with EHT received an intravenous infusion of ^{125}I-Ang I at a constant rate of 4.2±1.5×10^6 cpm/min (mean±SD; specific activity, approximately 74 TBq/mmol) for 20 minutes in the antecubital vein of the right arm. In the period between 8 and 20 minutes after the start of the infusion, when arterial and venous levels of ^{125}I-Ang I and ^{125}I-Ang II had reached a plateau,[14] blood samples were drawn from the veins of the left forearm, the leg, the liver, and both kidneys. Simultaneously with each venous sample, a sample from the aorta was taken. At 0.25, 0.5, 0.75, 1.1, and 1.5 minutes after discontinuation of the infusion, samples were taken from the aorta for determination of the biological half-life of infused angiotensins.

Six subjects with EHT received an intravenous infusion of a mixture of ^{125}I-Ang I (4.3±0.9 ×10^6 cpm/min) and unlabeled Ang I (0.8±0.1 nmol/min), and seven subjects with EHT received an intravenous infusion of ^{125}I-Ang II (3.4±0.4×10^6 cpm/min; specific activity, approximately 74 TBq/mmol). Blood samples were taken from the aorta and the various regional veins as described above.

The calculated exposure to radioactivity due to radiolabeled angiotensin infusion was approx. 0.4 µGy (0.8 mrad).[18] All subjects gave their informed consent, and the study was approved by the Hospital Ethics Review Committee.

Calculations

Regional extraction of angiotensins I and II. The regional extraction ratio of ^{125}I-Ang I, $^*E_I$, was defined as follows

$$^*E_I = 1-(^{125}\text{I-Ang } I_{out}/^{125}\text{I-Ang } I_{in}) \quad (1)$$

in which ^{125}I-Ang I_{in} and ^{125}I-Ang I_{out} are the concentrations in inflowing (arterial) and outflowing (venous) plasma, respectively, at steady state during the ^{125}I-Ang I infusion.

The regional extraction ratio of ^{125}I-Ang II, $^*E_{II}$, was defined as follows

$$^*E_{II} = 1-(^{125}\text{I-Ang } II_{out}/^{125}\text{I-Ang } II_{in}) \quad (2)$$

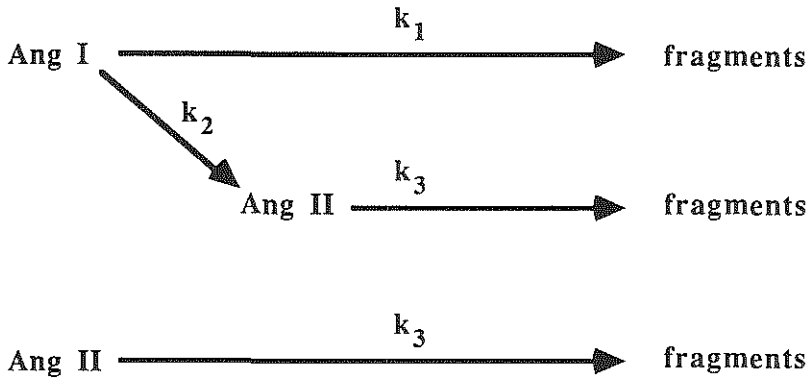

FIGURE 1
Schematic drawing shows metabolism of angiotensins I and II (Ang I, Ang II). k_1, first order rate constant of Ang I degradation; k_2, first order rate constant of Ang I-II conversion; k_3, first order rate constant of Ang II degradation.

in which ^{125}I-Ang II_{in} and ^{125}I-Ang II_{out} are the concentrations in inflowing (arterial) and outflowing (venous) plasma, respectively, at steady state during the ^{125}I-Ang II infusion.

Regional degradation and conversion of angiotensin I. Regional extraction of arterially delivered ^{125}I-Ang I at steady state during the ^{125}I-Ang I infusion is the result of rapid metabolism, which occurs via two pathways, i.e., conversion into ^{125}I-Ang II and degradation of ^{125}I-Ang I into other peptides (Figure 1). Regional extraction of arterially delivered ^{125}I-Ang II is the result of degradation only. Both conversion and degradation are enzymatic processes, and it may be assumed that the overall metabolism follows first-order reaction kinetics, according to the formula $C_t = C_o \times e^{-kt}$, in which C_t is the concentration at time t of the substance that is metabolized, C_o is the concentration at time zero, and k is the first-order reaction rate constant.

Regional metabolism of arterially delivered ^{125}I-Ang I can be described by this formula as follows

$$^{125}\text{I-Ang } I_{out} = {}^{125}\text{I-Ang } I_{in} \times e^{-*kt} \quad (3)$$

in which $*k$ is the first-order rate constant of ^{125}I-Ang I metabolism, and t is the time the arterially delivered ^{125}I-Ang I is exposed to the converting and degrading enzymes during the passage of blood. Thus, $*kt$ is given by the equation

$$*kt = -\ln(1-*E_I) \quad (4)$$

^{125}I-Ang I metabolism is the result of both degradation and conversion, and it may be assumed that

$$*kt = *k_1 t + *k_2 t \quad (5)$$

in which $*k_1$ is the first-order rate constant for ^{125}I-Ang I degradation, and $*k_2$ is the first-order rate constant for ^{125}I-Ang I-II conversion. Equation 4 can therefore be rewritten as

$$*k_1t+*k_2t = -\ln(1-*E_I) \quad (6)$$

in which $*E_I$ is given by Equation 1.

According to the reaction scheme presented in Figure 1, and assuming the first-order rate constant for ^{125}I-Ang II degradation to be equal to the first-order rate constant for ^{125}I-Ang I degradation, $*k_1t$ and $*k_2t$ can be expressed as a function of the ^{125}I-Ang I and ^{125}I-Ang II concentrations in inflowing and outflowing plasma

$$*k_1t = -\ln\{(^{125}\text{I-Ang I}_{out}+^{125}\text{I-Ang II}_{out})/(^{125}\text{I-Ang I}_{in}+^{125}\text{I-Ang II}_{in})\} \quad (7)$$

and, subsequently $*k_2t$ can be calculated by subtracting $*k_1t$, as given by Equation 7, from $*k_1t+*k_2t$ as given by Equation 6:

$$*k_2t = \ln\{(^{125}\text{I-Ang I}_{out}+^{125}\text{I-Ang II}_{out})/(^{125}\text{I-Ang I}_{in}+^{125}\text{I-Ang II}_{in})\} - \ln(^{125}\text{I-Ang I}_{out}/^{125}\text{I-Ang I}_{in}) \quad (8)$$

Similar to $*k_1t+*k_2t$ for ^{125}I-Ang I metabolism (degradation plus conversion), $*k_3t$ for ^{125}I-Ang II metabolism (degradation only) can be expressed as

$$*k_3t = -\ln(^{125}\text{I-Ang II}_{out}/^{125}\text{I-Ang II}_{in}) \quad (9)$$

The $*k_1t$ values calculated with Equation 7 from results obtained during ^{125}I-Ang I infusion were not different from the $*k_3t$ values calculated with Equation 9 from results obtained during ^{125}I-Ang II infusion. These findings support the validity of calculating $*k_1t$ and $*k_2t$ by using Equations 7 and 8, in which it is assumed that $*k_3t=*k_1t$.

We compared the metabolism of unlabeled Ang I with that of ^{125}I-Ang I by combined infusion of the two peptides. As with $*k_1t$ and $*k_2t$, k_1t and k_2t for infused Ang I can be expressed as

$$k_1t = -\ln\{(\text{Ang I}_{out}+\text{Ang II}_{out})/(\text{Ang I}_{in}+\text{Ang II}_{in})\} \quad (10)$$

and

$$k_2t = \ln\{(\text{Ang I}_{out}+\text{Ang II}_{out})/(\text{Ang I}_{in}+\text{Ang II}_{in})\} - \ln(\text{Ang I}_{out}/\text{Ang I}_{in}) \quad (11)$$

in which Ang I_{in} and Ang I_{out} are the concentrations of infused Ang I in inflowing (arterial) and outflowing (venous) plasma, and in which Ang II_{in} and Ang II_{out} are the concentrations of Ang II derived from conversion of infused Ang I.

The concentration of infused Ang I and the concentration of Ang II derived from infused Ang I can be calculated by subtracting the levels of endogenous Ang I and II measured before infusion from the levels measured during infusion. Ang I infusion causes extra Ang II formation, which reduces renin secretion from the kidney and consequently reduces the level of endogenous Ang I. However, during the short infusion period the levels of endogenous Ang I, measured at 2 minutes after the discontinuation of the combined infusion, were only marginally suppressed (see "Results"). We therefore did not correct for this small reduction in endogenous Ang I.

Analogous to $*k_3t$, k_3t for infused Ang II can be expressed as

$$k_3t = -\ln(\text{Ang II}_{out}/\text{Ang II}_{in}) \quad (12)$$

From the results obtained during the combined ^{125}I-Ang I and Ang I infusion, it appeared that

the values of k_1t for infused Ang I, which were calculated with Equation 10, were not significantly different from the values of $*k_1t$ for ^{125}I-Ang I, which were calculated with Equation 7 (see "Results"). However, k_2t for infused Ang I was 0.65 times $*k_2t$ for ^{125}I-Ang I. Thus

$$k_1t = *k_1t \tag{13}$$

and

$$k_2t = 0.65 \times *k_2t \tag{14}$$

The fractional regional degradation of arterially delivered endogenous Ang I is defined as

$$\text{Fractional Ang I degradation} = E_I \times k_1t/(k_1t+k_2t) \tag{15}$$

The fractional regional conversion of arterially delivered endogenous Ang I is defined as

$$\text{Fractional Ang I conversion} = E_I \times k_2t/(k_1t+k_2t) \tag{16}$$

Fractional Ang I degradation and conversion could be calculated from the results obtained during infusion of ^{125}I-Ang I alone as follows: First, $*k_1t$ and $*k_2t$ were calculated according to Equations 7 and 8; then, Equations 13 and 14 were used to calculate k_1t and k_2t; and finally, these calculated values of k_1t and k_2t were used to obtain E_I with the equation

$$k_1t+k_2t = -\ln(1-E_I) \tag{17}$$

which is analogues to Equation 6.

Sources of endogenous angiotensins I and II in regional venous plasma. Endogenous Ang I in regional venous plasma originates from arterial delivery and from de novo production. De novo production of Ang I occurs at tissue sites where part of this locally produced Ang I reaches the circulation.[14,15] In addition, some de novo production of Ang I occurs in circulating plasma by PRA, during the passage of blood from the arterial to the venous side of the vascular bed.

Regional venous Ang I derived from arterial delivery could be calculated from the results obtained during infusion of ^{125}I-Ang I alone as follows

$$\text{Ang } I_{out} \text{ from Ang } I_{in} = \text{Ang } I_{in} \times (1-E_I) \tag{18}$$

in which Ang I_{in} is the concentration of endogenous Ang I in inflowing (arterial) plasma, and E_I is given by Equation 17.

Regional venous Ang I derived from regional production by circulating PRA was calculated as

$$\text{Ang } I_{out} \text{ from PRA} = \text{PRA} \times \text{blood transit time} \tag{19}$$

This equation does not take into account the regional metabolism of Ang I and therefore leads to an overestimation of the level of regional venous Ang I that is regionally produced by circulating PRA.

Endogenous Ang II in regional venous plasma also originates from arterial delivery and de novo production. De novo production depends, at least in part, on the conversion of arterially delivered Ang I. Regional venous Ang II derived from arterial delivery was calculated as

$$\text{Ang II}_{out} \text{ from Ang II}_{in} = \text{Ang II}_{in} \times (1 - E_{II}) \quad (20)$$

in which Ang II_{in} is the concentration of endogenous Ang II in inflowing (arterial) plasma, and E_{II} is given by the equation

$$k_3 t = -\ln(1 - E_{II}) \quad (21)$$

which is analogous to Equation 6. With Equation 21, E_{II} could be calculated from the results obtained during infusion of ^{125}I-Ang I alone by assuming that $k_3 t = {}^*k_3 t = {}^*k_1 t$ (see "Results").

According to the reaction scheme shown in Figure 1 and assuming $k_3 t = k_1 t$ (see "Results"), regional venous Ang II derived from conversion of arterially delivered Ang I was calculated as

$$\text{Ang II}_{out} \text{ from conversion of Ang I}_{in} = \text{Ang I}_{in} \times (E_I - E_{II}) \quad (22)$$

Statistical Evaluation

Statistical differences were evaluated with the paired or unpaired Student's t test. Whenever data were not normally distributed, the non-parametric Wilcoxon tests was used instead. Significance was assumed at a value of $p<0.05$.

Results

In Vitro Studies

Conversion of ^{125}I-angiotensin I to II versus angiotensin I to II in plasma. Freshly obtained normal human plasma, to which the renin inhibitor CGP 29,287 had been added, was used in these experiments (see "Methods"). The concentration of the inhibitor was high enough to block Ang I generation completely. Experiments were carried out in triplicate.

In the absence of ACE inhibition, that is, without the addition of captopril (see "Methods"), the half-life of ^{125}I-Ang I in plasma ranged from 1.7 to 2.6 minutes, and the half-life of Ang I that had been added to the plasma ranged from 2.8 to 3.2 minutes. The first-order elimination rate constant for ^{125}I-Ang I, $*k$, was 0.35 min^{-1} (mean, n=3), and the first-order elimination rate constant for Ang I, k, was 0.23 min^{-1}.

In the presence of the ACE inhibitor captopril, the plasma half-life ranged from 11 to 16 minutes for ^{125}I-Ang I and from 13 to 18 minutes for added Ang I. In the presence of captopril, conversion is virtually zero, so that under these conditions the first-order elimination rate constants, $*k$ and k, are equal to the first-order degradation rate constants, $*k_1$ and k_1, respectively. The first-order degradation rate constant for ^{125}I-Ang I, $*k_1$, was 0.046 min^{-1}, and the first-order degradation rate constant for Ang I, k_1, was 0.051 min^{-1}. The k_1-to-$*k_1$ ratio was 1.1.

The first-order rate constant for ^{125}I-Ang I-II conversion, $*k_2$, which was

obtained by subtracting $*k_1$ from $*k$, was 0.31 min^{-1}, and the first-order rate constant for Ang I-II conversion, k_2, was 0.18 min^{-1}. The k_2-to-$*k_2$ ratio was 0.6.

The half-life in plasma of ^{125}I-Ang II and added Ang II ranged from 11 to 15 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of ^{125}I-Ang I and Ang I. This indicates that the degradation rate constants, k_1 for Ang I, $*k_1$ for ^{125}I-Ang I, k_3 for Ang II, and $*k_3$ for ^{125}I-Ang II all have approximately the same value.

Conversion of ^{125}I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, k_2, for Ang I-II conversion was compared with the first-order rate constant, $*k_2$, for ^{125}I-Ang I-II conversion for each of the two ACE preparations. The k_2-to-$*k_2$ ratio for both preparations was 0.7 (mean, n=3). Degradation of ^{125}I-Ang I, Ang I, ^{125}I-Ang II, or Ang II was undetectable in these experiments.

In Vivo Studies

Effects of infusions of unlabeled angiotensin I on blood pressure, heart rate, and plasma levels of angiotensins I and II. Unlabeled Ang I, which was infused in combination with ^{125}I-Ang I in six subjects, had to be given in rather high quantities to minimize the contribution of endogenous Ang I to the plasma levels of Ang I measured during the infusion. At these doses, Ang I caused an increase in blood pressure without a change in heart rate. Blood pressure reached a new plateau within 5-6 minutes after the start of the infusion; systolic pressure rose from 175±28 (mean±SD) to 206±23 mm Hg, and diastolic pressure rose from 93±9 to 106±10 mm Hg (p<0.05). After discontinuation of the Ang I infusion blood pressure fell rapidly, reaching preinfusion levels within a few minutes. As expected, there were no changes in blood pressure, heart rate, and the plasma levels of endogenous angiotensins during infusion of ^{125}I-Ang I alone or ^{125}I-Ang II alone.

The arterial plasma levels of endogenous Ang I and II before infusion and the levels of Ang I and II during combined infusion of ^{125}I-Ang I and Ang I are shown in Figure 2. After the start of the infusion, the plasma levels of Ang I and II rose rapidly and reached a new plateau within a few minutes. The plasma level of Ang I in the aorta rose from 18 (8-46) pmol/l (median, range) to 98 (51-118) pmol/l, and the level of Ang II rose from 8 (4-43) pmol/l to 155 (51-185) pmol/l. The levels of Ang I and II were constant in the period between 8 and 20 minutes after the start of the infusion. After discontinuation of the infusion, the plasma levels of Ang I and II fell very rapidly and reached preinfusion levels within 2-3

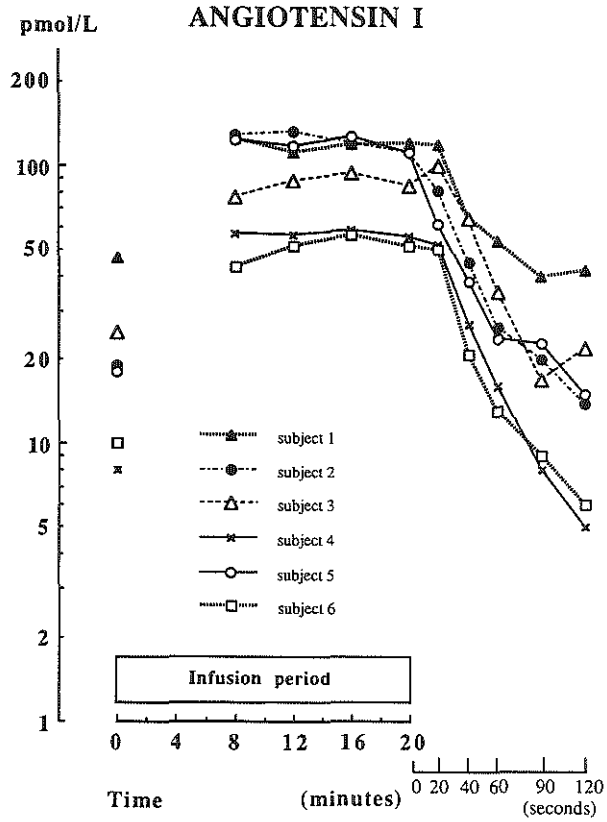

FIGURE 2

Line graphs show plasma levels of unlabeled angiotensin I (page 68) and angiotensin II (page 69) in the aorta in six subjects with essential hypertension before infusion, during combined intravenous infusion of ^{125}I-angiotensin I (4.3±0.9×10^6 cpm/min, mean±SD) and unlabeled angiotensin I (0.8±0.1 nmol/min), and after discontinuation of the infusion.

minutes. The plasma level of Ang I at 2 minutes after discontinuation of the infusion was 15 (5-43) pmol/l. The elimination half-time was 0.25±0.07 minutes (mean±SD) for ^{125}I-Ang I, and 0.25±0.08 minutes for infused unlabeled Ang I.

Regional ^{125}I-angiotensin I and II metabolism versus angiotensin I and II metabolism. The regional vein-to-artery (V/A) ratios of ^{125}I-Ang I and Ang I were determined at steady state during combined infusion of ^{125}I-Ang I and Ang I. In Table 1, results are expressed as 1–V/A. Results for ^{125}I-Ang I and Ang I were not significantly different.

The regional degradation constants for ^{125}I-Ang I and Ang I, $^*k_1$t and k_1t, were calculated with Equations 7 and 10. Results of these calculations are given in

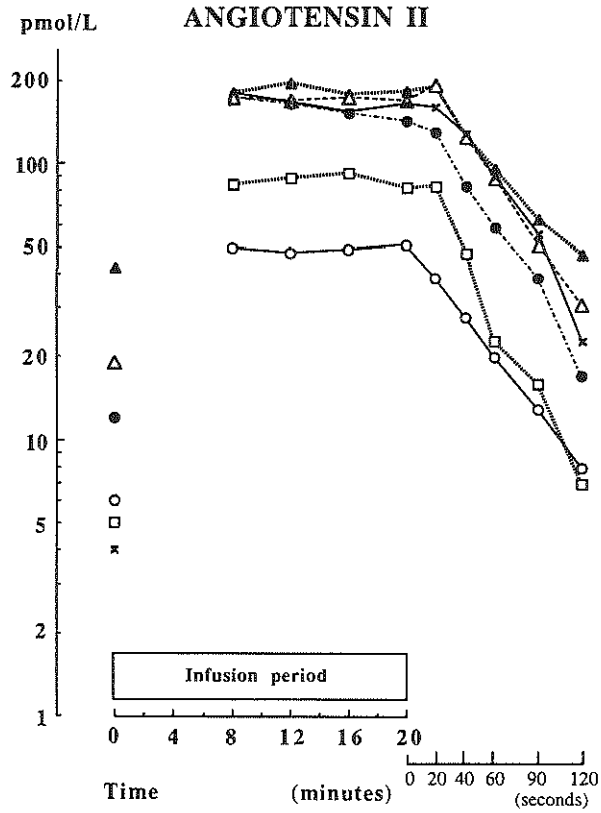

Table 2. They show no difference in degradation between ^{125}I-Ang I and Ang I. Similar calculations of $*k_1t$ were made from data obtained during the infusion of ^{125}I-Ang I alone. These $*k_1t$ values were not different from the values that were calculated from the data obtained during combined infusion of ^{125}I-Ang I and Ang I.

The regional conversion constants for ^{125}I-Ang I and Ang I, $*k_2t$ and k_2t, were calculated with Equations 8 and 11. Results for the limbs are given in Table 2. They show that $*k_2t$ was higher than k_2t. The k_2t-to-$*k_2t$ ratio was 0.65±0.36 (mean±SD, n=12). This is in good agreement with the in vitro experiments, which showed that the V_{max}/K_m ratio of ACE for Ang I was 0.6–0.7 times the

Table 1
Regional Vein-to-Artery Ratios in Subjects With Essential Hypertension

	Single infusion ^{125}I-Ang I (n=7)	Combined infusion ^{125}I-Ang I and Ang I (n=6)				Single infusion ^{125}I-Ang II (n=7)
Region	1–V/A ^{125}I-Ang I	1–V/A ^{125}I-Ang I	1–V/A Ang I	1–V/A ^{125}I-Ang II	1–V/A Ang II	1–V/A ^{125}I-Ang II
Forearm	0.64±0.18	0.67±0.19	0.65±0.20	0.39±0.14	0.35±0.19	0.33±0.11
Leg	0.84±0.06	0.86±0.06	0.79±0.13	0.47±0.19	0.43±0.21	0.58±0.09
Right kidney	0.88±0.05	0.85±0.04	0.84±0.10	0.88±0.05	0.87±0.07	0.87±0.05
Left kidney	0.85±0.05	0.87±0.03	0.82±0.16	0.87±0.02	0.85±0.05	0.86±0.05
Liver and gut	0.98±0.02	0.97±0.02	n.d.	0.98±0.01	0.96±0.01	0.99±0.01

Ang I, angiotensin I; Ang II, angiotensin II; V/A, vein-to-artery ratio; n.d., not determined.
Values are mean±SD.

V_{max}/K_m value for ^{125}I-Ang I.

Estimated values of the conversion constants in the renal and hepatomesenteric vascular beds were too low to compare between ^{125}I-Ang I and Ang I.

The regional degradation constant for ^{125}I-Ang II, $*k_3t$, was calculated with Equation 9 from data obtained during the infusion of ^{125}I-Ang II. These values of $*k_3t$ were not significantly different from the values of $*k_1t$ calculated from data obtained during infusion of ^{125}I-Ang I (Table 2).

Combined infusions of ^{125}I-Ang II and Ang II were not given in this study. However, from the values of $*k_1t$ and $*k_2t$ and of k_1t and k_2t, calculated from data obtained during the combined infusions of ^{125}I-Ang I and Ang I (Table 2), the venous concentrations of ^{125}I-Ang II and Ang II derived from arterially delivered ^{125}I-Ang II and Ang II and from conversion of arterially delivered ^{125}I-Ang I and Ang I could be calculated with Equations 20 and 22. These calculations showed that the venous concentrations of ^{125}I-Ang II and Ang II derived from arterially delivered ^{125}I-Ang II and Ang II were more than 10 times the venous concentrations of ^{125}I-Ang II and Ang II derived from arterially delivered ^{125}I-Ang I and Ang I. Thus, the contribution of this latter source could be ignored, and the degradation constants for ^{125}I-Ang II and Ang II could be calculated from data obtained during the combined ^{125}I-Ang I and Ang I infusions by using

Equations 9 and 12. Results of these calculations showed no significant difference between $*k_3t$ and k_3t (Table 2). These $*k_3t$ values were also not different from the values calculated from the data obtained during the infusion of ^{125}I-Ang II alone.

Regional Production of Angiotensins I and II

Arterial and venous plasma levels of endogenous angiotensins I and II. Tables 3 and 4 give the arterial and venous plasma levels of endogenous Ang I and II in the subjects who received an infusion of ^{125}I-Ang I (not combined with Ang I). The venous level of Ang I in the forearm was not significantly different from the arterial level, both in the subjects with URAS and in EHT. Venous levels of Ang I in the leg were significantly higher than arterial Ang I in URAS but not in EHT. There was no arteriovenous gradient of Ang II across the forearm and leg in both URAS and EHT.

In the subjects with URAS, renal venous Ang I on the affected side was approximately three times higher than arterial Ang I, whereas on the unaffected side, renal venous Ang I was similar to arterial Ang I. Thus, there was net release of Ang I from the affected kidney into the systemic circulation. On both the affected and unaffected sides in these subjects, renal venous Ang II was lower than arterial Ang II, indicating net extraction of Ang II from the systemic circulation. In EHT, renal venous Ang I on both sides was somewhat higher than arterial Ang I, whereas renal venous Ang II was lower than arterial Ang II.

In both groups of subjects, the levels of Ang I and Ang II in the hepatic vein were much lower than in the artery, illustrating the high degree of extraction of systemically delivered Ang I and II across the hepatomesenteric vascular bed.

Regional venous plasma levels of de novo produced angiotensin I. The values of $*k_1t$ and $*k_2t$ in the subjects who received an infusion of ^{125}I-Ang I (not combined with Ang I) were calculated from the data presented in Tables 3 and 4 by using Equations 7 and 8. These $*k_1t$ and $*k_2t$ values were then used for calculating the venous level of Ang I derived from arterial delivery, according to Equation 18. Subtraction of this level from the level that was actually measured gives the venous level of de novo produced Ang I. Results are shown in Figure 3. Most of de novo produced venous Ang I in forearm and leg could not be accounted for by circulating PRA, both in the group with URAS and in EHT. In the subjects with URAS, the renal venous level of intrarenally de novo produced Ang I was higher on the affected side than on the unaffected side and could also not be accounted for by circulating PRA. In contrast, in the hepatic vein, most of Ang I that is de novo produced in the hepatomesenteric vascular bed could be accounted for by circulating PRA.

Table 2
Regional Degradation and Conversion Constants in Subjects With Essential Hypertension

Region	Single infusion, ^{125}I-Ang I (n=7)		Combined infusion, ^{125}I-Ang I and Ang I (n=6)				Single infusion, ^{125}I-Ang II (n=7)		
	$*k_1$t ^{125}I-Ang I	$*k_2$t ^{125}I-Ang I	$*k_1$t ^{125}I-Ang I	k_1t Ang I	$*k_2$t ^{125}I-Ang I	k_2t Ang I	$*k_3$t ^{125}I-Ang II	k_3t Ang II	$*k_3$t ^{125}I-Ang II
Forearm	0.60±0.26	0.33±0.18	0.70±0.29	0.80±0.36	0.58±0.49	0.44±0.39*	0.51±0.25	0.47±0.33	0.43±0.19†‡
Leg	0.93±0.30	0.62±0.23	0.97±0.14	1.04±0.22	1.06±0.50	0.72±0.71*	0.69±0.33	0.62±0.36	0.89±0.23
Right kidney	2.29±0.36	<0.05	2.11±0.35	2.23±0.77	<0.05	<0.05	2.21±0.42	2.13±0.54	2.11±0.37
Left kidney	2.10±0.34	<0.05	2.03±0.16	2.04±0.50	<0.05	<0.05	2.05±0.19	1.96±0.38	2.04±0.37
Liver and gut	5.55±1.86	<0.05	3.89±0.79	n.d.	<0.05	n.d.	3.86±0.33	4.27±0.51	4.83±1.43

Ang I, angiotensin I; Ang II, angiotensin II. $*k_1$ and k_1, first-order rate constant for ^{125}I-Ang I and Ang I degradation; $*k_2$ and k_2, first-order rate constant for ^{125}I-Ang I-II and Ang I-II conversion; $*k_3$, first-order rate constant for ^{125}I-Ang II degradation; n.d., not determined. Values are mean±SD.
*p<0.05 for difference between $*k_2$t and k_2t during combined infusion.
†p<0.05 for difference from $*k_1$t during combined infusion.
‡p<0.05 for difference from k_1t during combined infusion (Student t test).

Regional venous plasma levels of de novo produced angiotensin II. The values of $*k_1t$ and $*k_2t$ in the subjects who received an infusion of ^{125}I-Ang I (not combined with Ang I) were also used for calculating the fractional regional conversion and degradation of arterially delivered endogenous Ang I (Equations 15 and 16) and for calculating the venous level of Ang II derived from arterial delivery (Equation 20). Subtraction of this level from the level that was actually measured (see Tables 3 and 4) gives the venous level of de novo produced Ang II. Results are shown in Figures 4 and 5. In the forearm, 20%, and in the leg, 30% of arterially delivered Ang I was converted to Ang II, confirming that a substantial part of the conversion of circulating Ang I occurs outside the lungs. There was no difference in fractional conversion of arterially delivered Ang I in the forearm and leg between the subjects with URAS and EHT. We could not demonstrate Ang I-II conversion in the renal and hepatomesenteric vascular beds in both groups.

Release of locally produced Ang II into the venous plasma in the forearm and leg was 25-30% of the release of locally produced Ang I, and by far the most of de novo production of Ang II could be accounted for by conversion of arterially delivered Ang I. The venous level of de novo-produced Ang II that could not be accounted for by this source was less than 1 pmol/l in the forearm and leg. Our estimate of the venous level of de novo produced-Ang II includes Ang II formed during the time taken to collect blood from the antecubital and femoral veins and also Ang II produced by conversion of Ang I that is generated by circulating PRA during passage of blood from artery to vein. However, the contribution of these two sources to our estimates of the venous level of de novo produced Ang II is small. As shown in Figure 3, the contribution of circulating PRA to de novo-produced venous Ang I in the forearm and leg accounts for less than 15-25% of total de novo-produced venous Ang I. In the forearm, 20%, and in the leg, 30% of arterially delivered Ang I was converted to Ang II. It is unlikely that Ang I generated by circulating PRA is subject to the same degree of regional conversion as arterially delivered Ang I, because the former is exposed to ACE for a shorter time than the latter. Thus, the contribution of circulating PRA to de novo-produced venous Ang II in the limbs could be calculated to be less than 6%.

In the kidney, the release of locally produced Ang II was 10-15% of the release of locally produced Ang I, and in contrast with the limbs, virtually none of de novo-produced venous Ang II in the kidney could be accounted for by conversion of arterially delivered Ang I. However, the renal venous level of de novo-produced Ang II was less than 2 pmol/l in EHT and less than 4 pmol/l on the affected side and less than 1 pmol/l on the unaffected side in URAS. Again, these figures may be overestimates because some Ang II was formed during the time it took to collect blood from the renal veins (approximately 15 seconds). At

Table 3
Plasma Levels of Plasma Renin Activity, Angiotensins I and II, and ^{125}I-Angiotensins I and II Across Various Vascular Beds During ^{125}I-Angiotensin I Infusion in Seven Subjects With Essential Hypertension

		Plasma level				
Vascular bed	*n*	PRA pmol.l^{-1}.min^{-1}	Ang I pmol/l	Ang II pmol/l	^{125}I-Ang I cpm/l×10^3	^{125}I-Ang II cpm/l×10^3
Aorta	7	31 (7.1-62)	24 (4.0-41)	13 (3.2-17)	206 (164-926)	893 (568-2,449)
Antecubital vein			25 (3.2-37)	10 (2.9-16)	71 (43-466) *	495 (403-1,630) *
Aorta	6	24 (7.2-43)	22 (3.1-25)	8.3 (2.6-18)	297 (178-823)	941 (586-2,471)
Femoral vein			22 (2.9-49)	8.6 (2.4-14)	44 (19-170) *	516 (381-717) *
Aorta	7	32 (7.2-50)	25 (3.6-40)	13 (2.9-17)	202 (149-967)	865 (549-2,584)
Right renal vein		41 (6.5-57) *	30 (5.9-48) *	3.2 (0.6-4.9) *	30 (9-129) *	100 (47-225) *
Aorta	7	30 (7.3-55)	23 (3.1-46)	13 (2.6-16)	209 (178-987)	926 (555-2,292)
Left renal vein		35 (11-69) *	36 (3.9-49) *	3.5 (0.9-9.0) *	30 (13-153) *	110 (72-206) *
Aorta	2	7.2 and 42	4.6 and 25	3.3 and 13	452 and 202	1,519 and 893
Hepatic vein		6.7 and 25	4.3 and 20	0.9 and 0.8	<5 and <5	<5 and 10

PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II. Data are median values and ranges.
*$p<0.05$ for difference between aortic and venous plasma levels (Wilcoxon signed rank test).

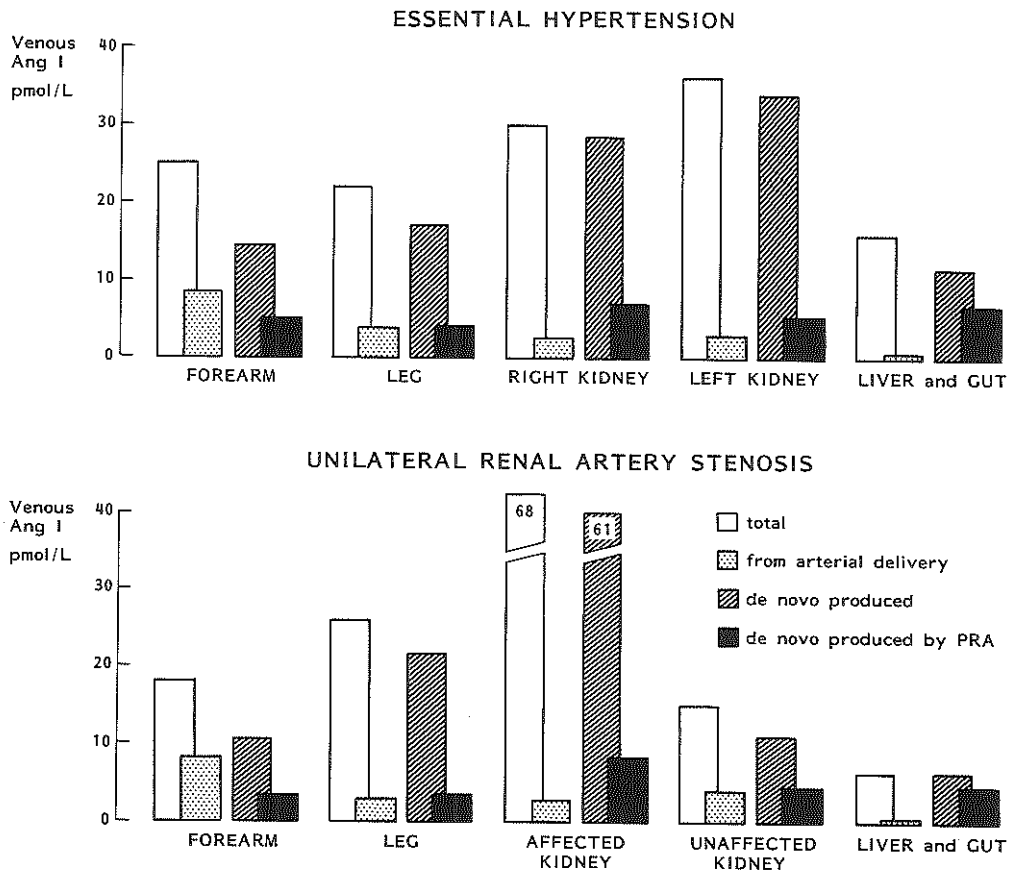

FIGURE 3

Bar graphs show venous plasma levels (median values) of angiotensin I (Ang I) in different vascular beds in subjects with essential hypertension and unilateral renal artery stenosis. Indicated are total venous Ang I (open bars) and the levels of venous Ang I originating from arterial delivery (light gray bars), from total de novo production (hatched bars), and from production by plasma renin activity (PRA) (solid bars). Venous Ang I derived from circulating PRA was calculated according to Equation 19 (see "Methods") assuming a mean transit time of blood of 0.17 minute in the limbs and in the kidney, and 0.42 minute in the hepatomesenteric vascular bed.[14] In forearm, leg, and kidneys total venous Ang I was higher than the level that could be accounted for by the sum of arterial delivery and production by PRA ($p<0.05$, Wilcoxon signed rank test).

Table 4
Plasma Levels of Plasma Renin Activity, Angiotensins I and II, and ^{125}I-Angiotensins I and II Across Various Vascular Beds During ^{125}I-Angiotensin I Infusion in Seven Subjects With Unilateral Renal Artery Stenosis

		Plasma level							
Vascular bed	*n*	PRA pmol.l^{-1}.min^{-1}		Ang I pmol/l		Ang II pmol/l		^{125}I-Ang I cpm/l×10^3		^{125}I-Ang II cpm/l×10^3	
Aorta	7	21	(7.2-64)	16	(5.6-49)	8.9	(3.0-22)	306	(120-621)	1,003	(712-1,777)
Antecubital vein				18	(4.4-59)	8.1	(2.9-22)	78	(17-236) *	699	(319-1,326) *
Aorta	7	20	(7.7-72)	16	(5.4-56)	8.7	(2.7-21)	331	(127-709)	996	(670-1,906)
Femoral vein				26	(6.3-63) *	8.9	(2.9-16)	39	(16-85) *	460	(290-661) *
Aorta	7	21	(7.7-57)	20	(5.4-45)	11	(4.1-22)	310	(134-709)	998	(735-1,906)
Renal vein *(affected kidney)*		50	(10-119) *	68	(8.5-113) *	6.1	(1.9-11) *	56	(20-106) *	172	(76-208) *
Aorta	7	26	(7.0-72)	18	(5.4-56)	9.5	(3.8-21)	363	(148-533)	1,000	(670-1,647)
Renal vein *(unaffected kidney)*		28	(7.5-71)	15	(5.4-58)	3.4	(0.9-8.5) *	77	(27-124) *	234	(106-323) *
Aorta	4	14	(7.0-39)	9.9	(5.5-23)	7.1	(4.5-8.7)	217	(196-384)	1,056	(705-1,203)
Hepatic vein		10	(5.2-35)	6.6	(3.2-18)	0.7	(0.3-1.8)	11	(<5-21)	27	(21-54)

PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II. Data are median values and ranges.
*$p<0.05$ for difference between aortic and venous plasma levels (Wilcoxon signed rank test).

the time the blood is collected, mixing with the inhibitor solution in the syringe is not complete. Given a first-order rate constant for Ang I-II conversion in plasma of 0.18 min^{-1} and a rate constant of only 0.05 min^{-1} for Ang I and Ang II degradation (see results of in vitro experiments), and given the high renal venous levels of Ang I, particularly on the affected side in URAS, the measured Ang II levels in renal vein samples may exceed the "true" Ang II levels by 1-3 pmol/l.

The levels of de novo-produced Ang I in the hepatic vein were low, probably because of the highly effective extraction of Ang I by the liver. Nearly complete extraction of Ang II may also explain the low Ang II levels in the hepatic vein. These levels were too low to make a reliable estimate of the fraction derived from de novo production in the hepatomesenteric vascular bed.

Thus, it appears that in the limbs and the kidney, little of Ang I produced at tissue sites reaches the blood in the regional veins in the form of Ang II and that most Ang II present in the circulation originates from blood-borne Ang I.

Discussion

Little is known in humans about the contribution of extrapulmonary Ang I-II conversion to the plasma level of Ang II. It has been reported that the plasma Ang II-I concentration ratio is not reduced in human subjects during extrapulmonary bypass with extracorporeal circulation, which may suggest that extrapulmonary conversion of arterially delivered Ang I is the most important source of circulating Ang II.[19] It should be noted, however, that the Ang II-I concentration ratio in plasma is not only dependent on the degree of conversion of blood-borne Ang I; it also depends on Ang I and II degradation and Ang I production, as well as on Ang II production from sources other than conversion of blood-borne Ang I.

The present study enabled us to measure directly the degree of conversion of blood-borne Ang I in extrapulmonary vascular beds. Our estimates of regional Ang I-II conversion were based on data obtained during systemic infusion of ^{125}I-Ang I. We accounted for the somewhat higher in vivo conversion rates of radiolabeled Ang I as compared with unlabeled Ang I. Our in vitro experiments indicate that this difference in conversion rate is probably due to the difference in the V_{max}/K_m ratio of ACE for the two substrates. This explains why the arterial and venous ^{125}I-Ang II/^{125}I-Ang I concentration ratios during ^{125}I-Ang I infusion were higher than the corresponding endogenous Ang II/Ang I ratios (see Tables 3 and 4). This, however, cannot be the full explanation because the discrepancy is too big. Indeed, a discrepancy was still observed when unlabeled Ang I was infused. From the data shown in Figure 2 it can be derived that, for each subject

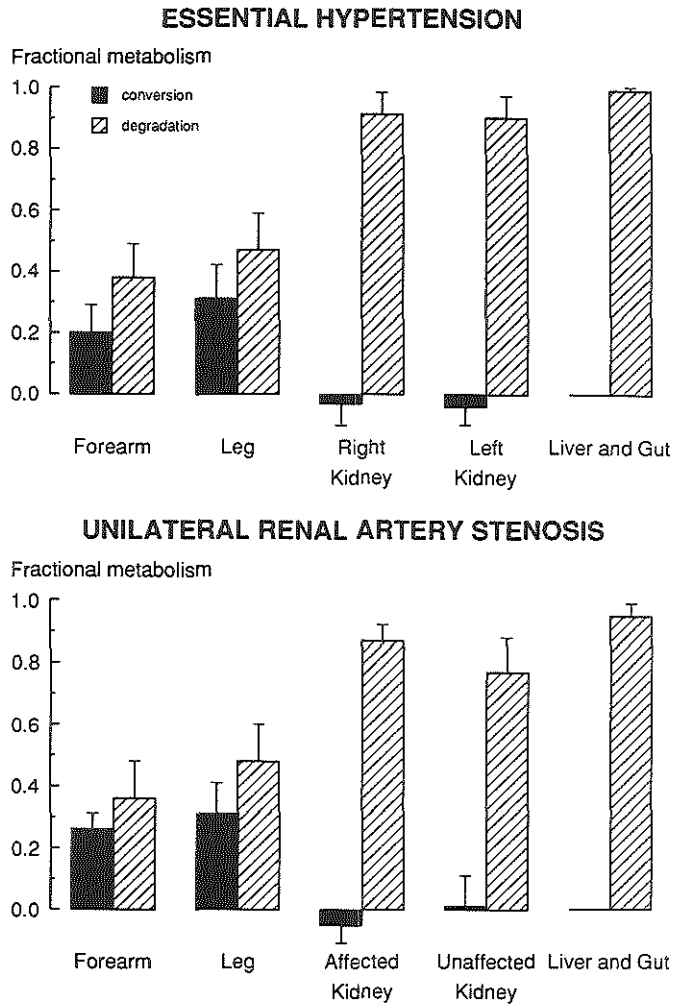

FIGURE 4
Bar graphs show fractional conversion and degradation (mean values, SD) of arterially delivered angiotensin I in different vascular beds in subjects with essential hypertension and unilateral renal artery stenosis.

receiving an infusion of unlabeled Ang I, the Ang II/Ang I ratio in the aorta was higher during the infusion than before. This difference clearly illustrates that a higher proportion of exogenous Ang I than endogenous Ang I is passing along the sites where the conversion is occurring. Circulating Ang I is probably converted mainly at the level of the arterioles, whereas Ang I is formed at all levels of the circulation. In addition, Ang I formed in the tissues may reach the circulation mainly at the level of the capillaries and venules (see also last paragraph of "Discussion").

Forearm and leg converted 20-30% of arterially delivered Ang I. We found little or no conversion of arterially delivered Ang I in the kidney and in the hepatomesenteric vascular bed. ^{125}I-Ang I infusion experiments in pigs have shown that the venous plasma levels of ^{125}I-Ang I and II in the leg during these infusions are similar to those in mixed venous plasma.[20] If the cardiac output of plasma in our subjects is assumed to be 3 l/min and if it is assumed that during the systemic infusion of ^{125}I-Ang I the venous plasma levels of ^{125}I-Ang I and II we measured in the leg are representative of the levels in mixed venous plasma, it can be calculated from our results (with Equation 16) that 40-50% of Ang I delivered to the central circulation is converted to Ang II during a single passage of blood from the right atrium to the aorta, whereas 20-30% of Ang I delivered to the systemic circulation is converted to Ang II during a single passage of blood from the aorta to the right atrium. Thus, because the plasma levels of endogenous Ang I in the aorta have been reported to be only 20% lower than in the right atrium and the peripheral veins,[21] our results indicate that 30-40% of the conversion of blood-borne Ang I occurs outside the lungs. In a similar way (with Equation 15), it can be calculated that only about 15% of Ang I delivered to the central circulation is degraded into peptides other than Ang II during a single passage of blood from the right atrium to the aorta, whereas 35-50% of Ang I delivered to the systemic circulation is degraded during a single passage of blood from the aorta to the right atrium, indicating that at least 70-80% of the degradation of blood-borne Ang I occurs outside the lungs. Our estimates of Ang I conversion and degradation in the central vascular bed are in good agreement with results in numerous studies showing in the lungs approximately 50% conversion of arterially delivered Ang I and little degradation of arterially delivered Ang I and II.[11,12, 21-29]

We were unable to detect any Ang I-II conversion in the kidneys both in URAS and in EHT. In URAS on the affected side and in EHT on both sides, our estimates of conversion yielded even negative values. A somewhat higher degradation rate for Ang II than for Ang I in the kidney could explain these negative values of conversion. In our calculations of regional Ang I-II conversion and Ang I degradation, the first-order rate constant of Ang I degradation, k_1, was

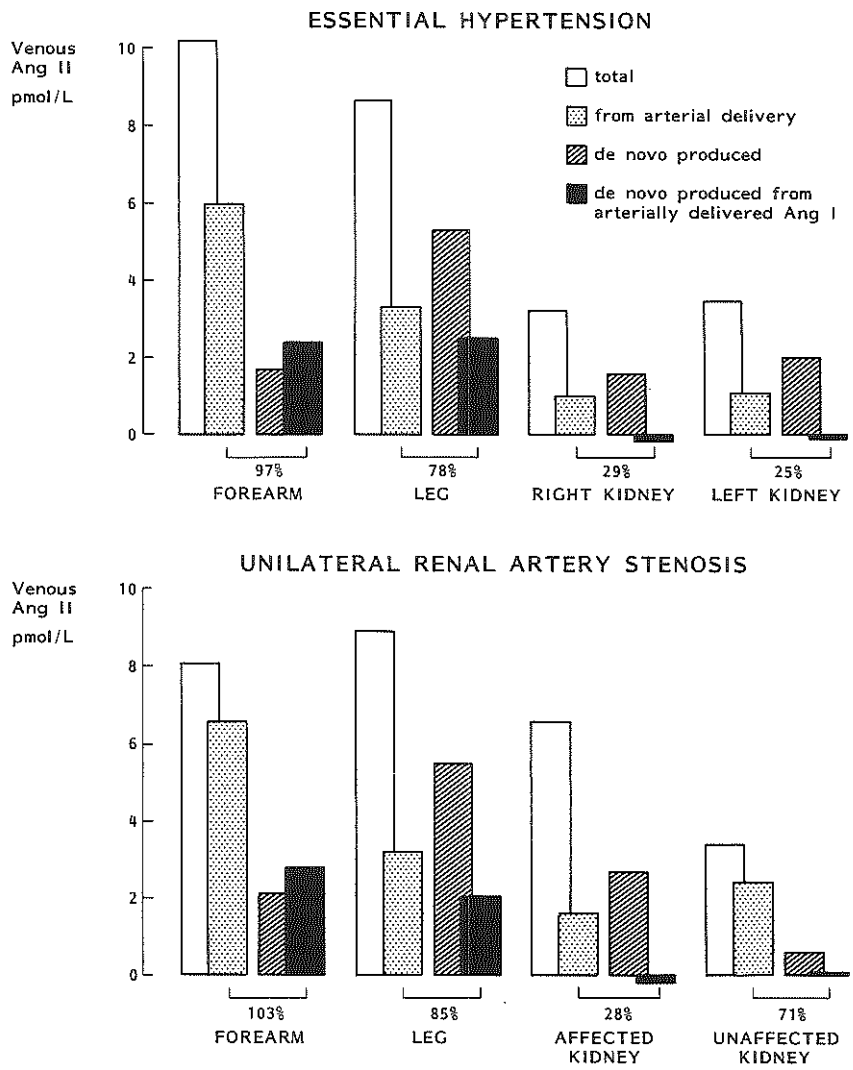

FIGURE 5

Bar graphs show venous plasma levels (median values) of angiotensin II (Ang II) in different vascular beds in subjects with essential hypertension and unilateral renal artery stenosis. Indicated are total venous Ang II (open bars) and the levels of venous Ang II originating from arterial delivery (gray bars), from total de novo production (hatched bars), and from conversion of arterially delivered angiotensin I (Ang I) (solid bars). The sum of Ang II derived from arterial delivery and Ang II derived from conversion of arterially delivered Ang I, expressed as a percentage of total venous Ang II (mean value) is also indicated. In forearm and leg, total venous Ang II was not significantly different from the level that could be accounted for by arterial delivery of Ang II and conversion of arterially delivered Ang I. The same was true for the unaffected kidney in unilateral renal artery stenosis. In the affected kidney in unilateral renal artery stenosis and in both kidneys in essential hypertension total venous Ang II was higher than could be accounted for by arterial delivery of Ang II and conversion of arterially delivered Ang I ($p<0.05$, Wilcoxon test).

assumed to be equal to the first-order rate constant of Ang II degradation, k_3. Although the results of our infusion studies indicated that k_1 and k_3 are likely to be similar, some differences may exist. In dogs, 20% of ^{125}I-Ang I given as a bolus injection into the renal artery was converted to ^{125}I-Ang II during a single passage through the kidney, and reduction of renal blood flow by renal artery constriction did not change the fractional conversion of arterially delivered ^{125}I-Ang I.[30] Given the possibility that, as we found in humans, ^{125}I-Ang I is more rapidly converted than unlabeled Ang I, the intrarenal fractional conversion of arterially delivered Ang I was probably somewhat overestimated in these dogs. In addition, the figure of 20% conversion in the dog kidney was based on the amount of ^{125}I-Ang II that was recovered in renal venous blood collected during a 1-minute period after intra-arterial injection of ^{125}I-Ang I. Because conversion in blood in vitro is not negligible, some ^{125}I-Ang II in samples from the renal vein may have been generated during the collection of blood. In the isolated perfused rat kidney, Ang I had to be infused in a 50 times higher molar dose than Ang II to induce the same vasoconstrictor response.[31] Thus, there was apparently some intrarenal conversion of arterially delivered Ang I, but only to a very limited degree.

The low degree of conversion of arterially delivered Ang I in the kidney is unexpected in view of the high concentration of Ang II found in renal tissue[32,33] and the profound renal effects of ACE inhibition. It is possible that the kidney is highly responsive even to the small quantities of Ang II that are intrarenally formed by conversion of arterially delivered Ang I. However, it seems more likely that Ang I-II conversion in the kidney occurs in a compartment that is accessible to ACE inhibitors but does not readily equilibrate with Ang I and II from the circulation.[33,34]

From previous ^{125}I-Ang I infusion studies in hypertensive subjects who were all receiving ACE inhibitor treatment, we concluded that a major part of venous plasma Ang I in forearm, leg, and kidney is produced de novo in these vascular beds, that part of this de novo production cannot be accounted for by circulating PRA, and that in the kidney in situ synthesized renin is an important determinant of local Ang I production (i.e., Ang I production not in circulating plasma).[14,15] The present study, conducted in hypertensive subjects who were not receiving ACE inhibitor treatment, confirms these conclusions. In addition, the present study shows also that a major part of venous plasma Ang II in forearm and leg originates from regional de novo production and not from arterial delivery. In the limbs, most if not all, de novo-produced venous Ang II was derived from arterially delivered Ang I. Also, in the lungs the venous level of de novo-produced Ang II could be fully accounted for by conversion of arterially delivered Ang I.[20]

In the kidney, the situation is clearly different. Whereas in forearm and leg the level of Ang II in the veins was similar to that in the artery, venous Ang II in the kidney was only half that of arterial Ang II. Moreover, whereas in forearm and leg virtually all venous Ang II could be accounted for by arterial Ang II delivery and regional conversion of arterially delivered Ang I, part of venous Ang II in the kidney appeared to come from a different source. Intrarenal Ang I production by circulating PRA followed by intrarenal conversion to Ang II could not account for this source because of the short blood transit time and the low conversion rate of blood-borne Ang I in the kidney. The calculated levels of intrarenally produced Ang II in the renal vein were, however, low, and as discussed in "Results", most of it may due to Ang I-II conversion in the samples during their collection. Thus, little intrarenally produced Ang II is released into the circulation via the renal vein. Also, little Ang II that might be produced in the hepatomesenteric vascular bed appears to be released into the circulation via the hepatic vein.

Our data, taken together, provide little evidence for the production of circulating Ang II via pathways other than conversion of blood-borne Ang I. In the vascular beds we studied, little of the Ang I that is formed in situ at tissue sites reaches the venous blood of these beds in the form of Ang II. In the renal and hepatomesenteric vascular beds, this might be due to a lack of vascular endothelial ACE activity, as indicated by the low degree of conversion of Ang I that is arterially delivered to these vascular beds. On the other hand, in the limbs there is marked conversion of arterially delivered Ang I, and the locally formed Ang I appears to enter the blood at a level distal to the site where arterially delivered Ang I is converted to Ang II, so that this conversion site is bypassed. Ang I formed at tissue sites probably enters the circulation at the level of the capillaries or venules, whereas Ang I-II conversion occurs at the level of the arterioles. This explanation would fit with the hypothesis that the vascular endothelium is the physiologically relevant production site of circulating Ang II.[35] Our experiments cannot exclude the possibility that part of the in situ synthesized Ang I is converted before it enters the circulation and that the Ang II thus formed remains in the tissues and does not easily reach the circulating blood. At any rate, the results of the present study illustrate the high degree of compartmentalization of Ang I and II production.

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Chapter 5

Effect of Stimulation of Renal Renin Secretion by Diuretic Treatment on Regional Production of Angiotensins I and II

Summary

To assess the effect of stimulation of renal renin secretion on the generation of angiotensins I and II at tissue sites, ^{125}I-angiotensin I was given via constant intravenous infusion to subjects with essential hypertension and renal artery stenosis who were treated with the diuretic furosemide (40 mg, once daily). Plasma levels of radiolabeled and endogenous angiotensins I and II were measured in the aorta, and in the antecubital, femoral, renal, and hepatic veins. The regional extraction of ^{125}I-angiotensin I was 55% across the forearm, 85% across the leg and the kidneys, and more than 95% across the hepatomesenteric vascular bed. In the forearm 15-20%, and in the leg 30% of arterially delivered angiotensin I was converted to angiotensin II in essential hypertension and in unilateral renal artery stenosis. Little or no conversion could be demonstrated in the renal and hepatomesenteric vascular beds. These results are not different from those obtained in untreated subjects. Furosemide stimulated the release of angiotensin I and II from extrarenal tissue sites into the systemic circulation. In the kidney where little conversion of arterially delivered angiotensin I occurs, furosemide stimulated only the release of angiotensin I and not angiotensin II. The stimulated release of angiotensins I and II from the extrarenal vascular beds was closely related to the increased release of renin from the kidney. However, a large fraction of the de novo produced venous angiotensin I in the extrarenal vascular beds did not originate from the action of circulating renin on circulating angiotensinogen, but appeared to originate from the catalytic action of renin that is bound to or taken up by tissues. Part of this locally produced angiotensin I enters the blood stream and is then transported to the pulmonary and extrapulmonary sites where angiotensin I-II conversion occurs.

Introduction

There is now good evidence for the local generation of angiotensins I and II (Ang I and II) in various tissues of experimental animals. Results from earlier studies indicated that the release of Ang I and II from rabbit mesenteric arteries and isolated perfused rat hindlimbs might occur independently, at least in part, of the circulating renin-angiotensin system.[1-4] In later studies however the spontaneous release of Ang I and II from perfused rat hindlimbs was found to be very low, and only after perfusion with exogenous renin did the release of these peptides increase to substantial levels.[5-7] In addition the spontaneous release of angiotensin peptides by the hindlimbs from binephrectomized rats and rabbits was extremely low.[8,9] In the earlier experiments technical problems with the angiotensin assays may have led to falsely high results.

In studies in which ^{125}I-Ang I was infused into subjects with unilateral renal artery stenosis (URAS) and essential hypertension (EHT), who were treated with the angiotensin-converting enzyme (ACE) inhibitor captopril, we showed that a major fraction of the generation of circulating Ang I in the vascular beds of the forearm and leg could not be accounted for by the action of circulating renin on circulating angiotensinogen, and appeared to occur outside the circulating plasma.[10,11] These results were confirmed in similar studies in untreated hypertensive subjects.[12] These studies also showed that most, if not all, circulating Ang II was derived from circulating Ang I.

The present study addresses the role of kidney-derived renin in local Ang I and II formation at tissue sites, by investigating the effect of stimulation of renin secretion by diuretic treatment on the local production of Ang I and II at extrarenal tissue sites. Results were compared with data we earlier obtained in untreated subjects.[12]

Methods

Blood Sampling, Separation and Assay of Angiotensins, and Measurement of Renal Plasma Flow

Blood for angiotensin measurements, 10 ml, was rapidly drawn (≈5-15 seconds) with a plastic syringe containing 0.5 ml inhibitor solution: 6.25 mM disodium EDTA, 1.25 mM 1,10-phenantroline, and 100 nM of the renin inhibitor CGP 29,287 (final concentrations in blood) and was then transferred into prechilled polystyrene tubes. The blood samples were centrifuged at 3,000*g* for 10 minutes at 4° C. Plasma was stored at -70° C, extracted within 2 days and assayed within 2 weeks. Angiotensins were extracted from plasma by reversible adsorption to octadecylsilyl-silica (Sep-Pak C18, Waters Chromatography Div., Milford, Mass., USA) and separated by reversed-phase HPLC as described previously.[10,13] The concentrations of ^{125}I-Ang I and II in HPLC fractions were measured in the gamma counter, and the concentrations of endogenous Ang I and II in the HPLC fractions were measured by radioimmunoassay (RIA).[10,11]

Effect of Diuretic Treatment

Blood for plasma renin activity measurement, 10 ml, was collected in polystyrene tubes containing 0.2 ml disodium EDTA solution (final concentration in blood, 6.25 mM). The samples were centrifuged at 3,000*g* for 10 minutes at room temperature and plasma was stored at -20° C. The levels of plasma renin activity (PRA) were determined by the enzyme-kinetic method, in which Ang I generated during incubation with endogenous plasma angiotensinogen at pH 7.4 and 37° C was measured by RIA.[10]

Total renal plasma flow (right plus left kidney) was calculated from the renal clearance and extraction of ^{131}I-iodohippurate.[14]

Subjects and Infusion Protocol

Regional metabolism and production of Ang I and II were studied in 14 hypertensive subjects, 9 male, 5 female, age 50.9±10.9 years (mean±SD, range 27-63 years) who had been treated with the diuretic furosemide (40 mg, p.o., once daily) for 2 days. Subjects were studied at the time they were undergoing renal vein renin sampling followed by renal angiography for diagnostic purposes. Seven subjects proved to have unilateral renal artery stenosis (60-90% narrowing of the arterial lumen). The remaining subjects showed no abnormalities on their angiogram and were considered to have essential hypertension.

Subjects received an intravenous infusion of ^{125}I-Ang I at a constant rate of $5.2\pm1.6\times10^6$ cpm/min (mean±SD) for 20 minutes. Blood samples were taken from the antecubital, femoral, hepatic, and both renal veins between 8 and 20 minutes after the ^{125}I-Ang I infusion was started. Plasma levels of ^{125}I-Ang I and II are constant during this period.[10] Simultaneously with each venous sample, a sample from the aorta was taken. For the determination of the elimination half-life of ^{125}I-Ang I, samples from the aorta were taken immediately after discontinuation of the infusion. Subjects received 5 ml of a 1% potassium iodide solution per day, from 2 days before to 2 days after the ^{125}I-Ang I infusion. The subjects had given their informed consent and the study was approved by the Hospital Ethics Review Committee.

Calculations

Regional Metabolism of Angiotensins I and II. The regional extraction ratio of Ang I, E_I, was calculated from the following equation[12]

$$-\ln(1-E_I) = k_1t+k_2t \qquad (1)$$

in which k_1 is the first-order rate constant of Ang I degradation and k_2 is the first-order rate constant of Ang I-II conversion (degradation refers to metabolism of Ang I to peptides other than Ang II), and t is the regional mean blood transit time. We previously found that k_1 for unlabeled Ang I is equal to $*k_1$ for ^{125}I-Ang I, and k_2 for unlabeled Ang I equals $0.65\times*k_2$ for ^{125}I-Ang I. k_1t and k_2t can then be calculated as follows

$$k_1t = *k_1t = -\ln\{(\text{Ang I}_{out}+\text{Ang II}_{out})/(\text{Ang I}_{in}+\text{Ang II}_{in})\} \qquad (2)$$

and

$$k_2t = 0.65\times*k_2t$$
$$= 0.65\times[\ln\{(\text{Ang I}_{out}+\text{Ang II}_{out})/(\text{Ang I}_{in}+\text{Ang II}_{in})\}-\ln(\text{Ang I}_{out}/\text{Ang I}_{in})] \qquad (3)$$

The fractional regional degradation of arterially delivered Ang I is defined as follows

$$\text{fractional Ang I degradation} = E_I\times k_1t/(k_1t+k_2t) \qquad (4)$$

Table 1
Levels of Plasma Renin Activity, Angiotensins I and II, ^{125}I-Angiotensins I and II Across Various Vascular Beds During ^{125}I-Angiotensin I Infusion in Seven Furosemide Treated Subjects With Essential Hypertension

		Plasma level									
Vascular bed	*n*	PRA pmol.l^{-1}.min^{-1}		Ang I pmol/l		Ang II pmol/l		^{125}I-Ang I cpm/l×10^3		^{125}I-Ang II cpm/l×10^3	
Aorta	7	43	(18-136)	29	(20-158)	18	(5.7-111)	617	(319-1,175)	1,109	(648-1,513)
Antecubital vein				44	(25-187)*	18	(5.1-111)	289	(163-507)*	890	(502-1,158)*
Aorta	6	44	(18-141)	28	(20-170)	17	(4.8-127)	621	(379-1,280)	1,268	(685-1,468)
Femoral vein				50	(29-170)*	18	(5.5-83)	88	(22-355)*	487	(313-930)*
Aorta	7	35	(17-148)	29	(20-149)	18	(6.0-103)	629	(291-1,123)	1,030	(613-1,549)
Right renal vein		43	(32-185)*	44	(28-246)*	4.5	(0.6-16)*	60	(57-122)*	111	(42-193)*
Aorta	7	50	(18-141)	31	(20-170)	18	(4.8-127)	606	(379-1,280)	1,083	(647-1,468)
Left renal vein		53	(27-260)*	44	(24-246)*	3.6	(1.3-18)*	63	(57-154)*	91	(39-207)*
Aorta	5	63	(22-120)	54	(19-153)	23	(18-102)	609	(287-703)	1,261	(1,022-1,522)
Hepatic vein		41	(20-73)	34	(12-73)	1.5	(1.1-3.0)	14	(<5-41)	18	(<5-25)

Data are median values and ranges. * $p<0.05$ for difference between aortic and venous plasma levels (Wilcoxon signed rank test).
PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II.

Effect of Diuretic Treatment

The fractional regional conversion of arterially delivered Ang I is defined as follows

$$\text{fractional Ang I conversion} = E_I \times k_2 t/(k_1 t + k_2 t) \quad (5)$$

Contribution of Regional Angiotensin Production to the Regional Venous Plasma Levels of Angiotensins I and II. Endogenous Ang I in regional venous plasma originates from arterial delivery and from de novo production. De novo production of Ang I takes place at tissue sites from where part of this locally produced Ang I reaches the circulation. In addition, some de novo production of Ang I takes place in the plasma compartment by PRA, during the passage of blood from the arterial to the venous side of the vascular bed. Regional venous Ang I derived from arterial delivery can be calculated as follows

$$\text{Ang } I_{out} \text{ from Ang } I_{in} = \text{Ang } I_{in} \times (1 - E_I) \quad (6)$$

in which Ang I_{in} is the concentration of endogenous Ang I in inflowing (arterial) plasma, and E_I is calculated with Equation 1. Regional venous Ang I derived from regional production by circulating PRA can be calculated as

$$\text{Ang } I_{out} \text{ from PRA} = \text{PRA} \times \text{blood transit time} \quad (7)$$

Equation 7 does not take into account the regional metabolism of Ang I, and therefore leads to overestimation of the level of regional venous Ang I that is regionally produced by circulating PRA.

Endogenous Ang II in regional venous plasma also originates from arterial delivery and de novo production. De novo production of Ang II originates, at least in part, from the conversion of arterially delivered Ang I. Regional venous Ang II derived from arterial delivery was calculated as follows

$$\text{Ang } II_{out} \text{ from Ang } II_{in} = \text{Ang } II_{in} \times (1 - E_{II}) \quad (8)$$

in which Ang II_{in} is the concentration of endogenous Ang II in inflowing (arterial) plasma, and E_{II} is the regional extraction ratio of Ang II. E_{II} was calculated from the following equation

$$-\ln(1 - E_{II}) = k_3 t = k_1 t = {}^{*}k_1 t \quad (9)$$

in which k_3 is the first-order rate constant of Ang II degradation.[12]

The regional venous levels of Ang II derived from conversion of arterially delivered Ang I can be calculated as

$$\text{Ang } II_{out} \text{ from conversion of Ang } I_{in} = \text{Ang } I_{in} \times (E_I - E_{II}) \quad (10)$$

Regional Production of Angiotensins I and II. In the subjects with essential hypertension it was possible to calculate the rate of production of circulating Ang I and II by the kidneys. We determined the renal plasma flow (right plus left kidney), Q, as the ^{131}I-iodohippurate clearance divided by the mean ^{131}I-iodohippurate extraction ratio of the two kidneys. Renal Ang I production is then given by the equation

$$\text{Ang I production} = \{\text{Ang } I_{out} - (1 - E_I) \times \text{Ang } I_{in}\} \times Q \quad (11)$$

and

Table 2
Levels of Plasma Renin Activity, Angiotensins I and II, ^{125}I-Angiotensins I and II Across Various Vascular Beds During ^{125}I-Angiotensin I Infusion in Seven Furosemide Treated Subjects With Unilateral Renal Artery Stenosis

		Plasma level							
Vascular bed	*n*	PRA pmol.l^{-1}.min^{-1}		Ang I pmol/l		Ang II pmol/l		^{125}I-Ang I cpm/l×10^3		^{125}I-Ang II cpm/l×10^3	
Aorta	7	65	(25-168)	49	(21-234)	30	(14-155)	470	(336-662)	1,452	(893-2,479)
Antecubital vein				56	(24-316)*	31	(9.5-132)	182	(75-258)*	807	(594-1,621)*
Aorta	7	61	(30-168)	51	(26-197)	30	(12-134)	468	(367-626)	1,547	(919-2,357)
Femoral vein				66	(31-340)*	30	(8.5-139)	41	(17-103)*	699	(363-1,105)*
Aorta	7	61	(22-170)	46	(17-225)	28	(17-156)	454	(303-626)	1,568	(864-2,357)
Renal vein *(affected kidney)*		113	(39-366)	205	(54-813)*	15	(3.7-90)*	49	(17-241)*	176	(93-258)*
Aorta	7	59	(30-166)	51	(26-243)	30	(12-154)	468	(207-698)	1,258	(919-2,601)
Renal vein *(unaffected kidney)*		58	(26-169)	35	(18-199)*	11	(2.1-25)*	77	(29-220)*	157	(118-410)*
Aorta	5	49	(25-76)	38	(21-72)	27	(14-50)	470	(338-517)	1,216	(869-1,507)
Hepatic vein		39	(25-48)	28	(11-31)	1.4	(1.0-4.7)	12	(<5-15)	21	(11-128)

Data are median values and ranges. * $p<0.05$ for difference between aortic and venous plasma levels (Wilcoxon signed rank test).
PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II.

$$\text{Ang II production} = \{\text{Ang II}_{out} - (1 - E_{II}) \times \text{Ang II}_{in}\} \times Q \quad (12)$$

in which $\{\text{Ang I}_{out} - (1 - E_I) \times \text{Ang I}_{in}\}$ and $\{\text{Ang II}_{out} - (1 - E_{II}) \times \text{Ang II}_{in}\}$ represent mean values for the two kidneys, and Q is the renal plasma flow.

Statistical Evaluation

Statistical differences were evaluated by using the paired or unpaired Student's t test. When data were not normally distributed the non-parametric Wilcoxon rank sum or Mann-Whitney U tests were used instead. Significance was assumed at the $p<0.05$ level.

Results

Plasma Levels of Angiotensins I and II during Infusion of ^{125}I-Angiotensin I

The arterial and venous plasma levels of endogenous and radiolabeled Ang I and II in furosemide-treated subjects with EHT and URAS are given in Tables 1 and 2, respectively.

In the forearm and leg the venous level of Ang I was significantly higher than the arterial level in both groups. There was no arteriovenous gradient of Ang II across the limbs. Thus there was net release of Ang I into the systemic circulation, and no net release of Ang II.

In the subjects with EHT renal venous Ang I on both sides was higher than arterial Ang I, whereas renal venous Ang II was lower than arterial Ang II. Renal venous Ang I and II levels on the right side were not different from those on the left. Thus in EHT there was net release of Ang I by the kidneys into the systemic circulation and net extraction of Ang II from the systemic circulation. In the subjects with URAS, the renal venous plasma level of Ang I on the affected side was 4 times higher than arterial Ang I, whereas on the unaffected side renal venous Ang I was lower than arterial Ang I. Thus there was a significant net release of Ang I into the systemic circulation from the affected kidney, and there was net extraction of Ang I by the unaffected kidney. Both on the affected and unaffected side in these subjects, renal venous Ang II was lower than arterial Ang II, which indicates net extraction of Ang II from the systemic circulation on both sides. On the affected side renal venous Ang II appeared somewhat higher than on the unaffected side but the difference did not reach statistical significance. The levels of Ang I and II in the hepatic vein were much lower than in the artery both in the group with EHT and in the group with renal artery stenosis.

Regional Metabolism of ^{125}I-Angiotensin I

In the subjects with EHT the extraction ratio of ^{125}I-Ang I was 0.51±0.10 across the forearm, 0.85±0.09 across the leg, 0.87±0.05 across the right kidney,

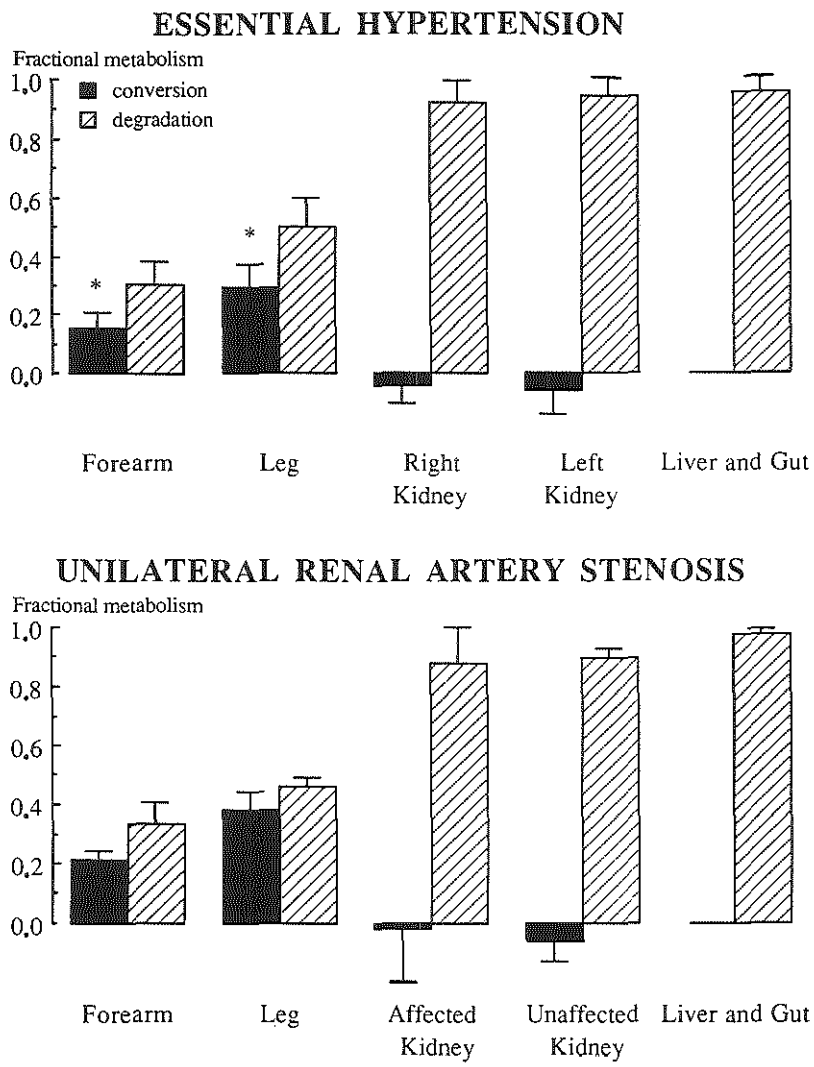

FIGURE 1
*Bar graphs show fractional conversion and degradation (mean±SD) of arterially delivered angiotensin I (Ang I) in different vascular beds in subjects with essential hypertension and subjects with unilateral renal artery stenosis who had been treated with the diuretic furosemide. Fractional conversion and degradation were calculated using Equations 5 and 4. *p<0.05 for difference between essential hypertension and renal artery stenosis; (Mann-Whitney U test)*

0.87±0.06 across the left kidney, and 0.95±0.06 across the hepatomesenteric vascular bed (mean±SD). In the subjects with URAS the extraction ratio of ^{125}I-Ang I was 0.61±0.09 across the forearm, 0.89±0.05 across the leg, 0.86±0.11 across the affected kidney, 0.84±0.07 across the unaffected kidney, and 0.98±0.01 across the hepatomesenteric vascular bed. The extraction ratios were not significantly different between the two groups of subjects. The elimination half-lives of ^{125}I-Ang I from the plasma, 0.31±0.10 minute in EHT and 0.36±0.16 minute in URAS, were also not significantly different.

In the forearm 15-20%, and in the leg 30-35% of arterially delivered Ang I was converted to Ang II (Figure 1). Percent regional conversion of arterially delivered Ang I in the limbs was lower in the subjects with EHT than in subjects with URAS. In the forearm 30%, and in the leg 50% of arterially delivered Ang I was degraded, i.e. metabolized to peptides other than Ang II. Percent regional degradation of arterially delivered Ang I in the limbs in the group with EHT was not different from percent degradation in the group with URAS.

We did not find significant conversion of arterially delivered Ang I in the kidneys neither in the group with EHT nor in the group with URAS. In the hepatic vein the levels of ^{125}I-Ang I and II were too low to calculate the degree of Ang I-II conversion in the hepatomesenteric vascular bed.

Regional Production of Angiotensin I

In the forearm and leg up to 60-85% of the production of circulating Ang I leg could not be accounted for by circulating PRA, both in the subjects with EHT and in the subjects with URAS (Figure 2). In the kidneys in EHT approximately 80% of the production of circulating Ang I could not be accounted for by this source. In the subjects with URAS the renal venous level of intrarenally produced Ang I was seven times higher on the affected side than on the unaffected side. In the affected kidney, 90%, and in the unaffected kidney, 65% of the production of Ang I could not be accounted for by circulating PRA. In the hepatomesenteric vascular bed, PRA accounted for most, 65-85%, of the regional production of Ang I in the hepatic vein. Although in the forearm, the leg, and the kidney circulating PRA could not account for the production of circulating Ang I in these vascular beds, there was a close correlation between the venous plasma level of regionally produced Ang I and the level of circulating PRA. This is shown in Figure 3, which also includes previously published data on untreated and captopril-treated subjects.[10,12] Actually, when circulating PRA was close to zero, the venous plasma level of regionally produced Ang I was also close to zero. This strongly suggests that not only in the kidney but also in the forearm and the leg, the local production of circulating Ang I is dependent mainly on kidney-derived renin.

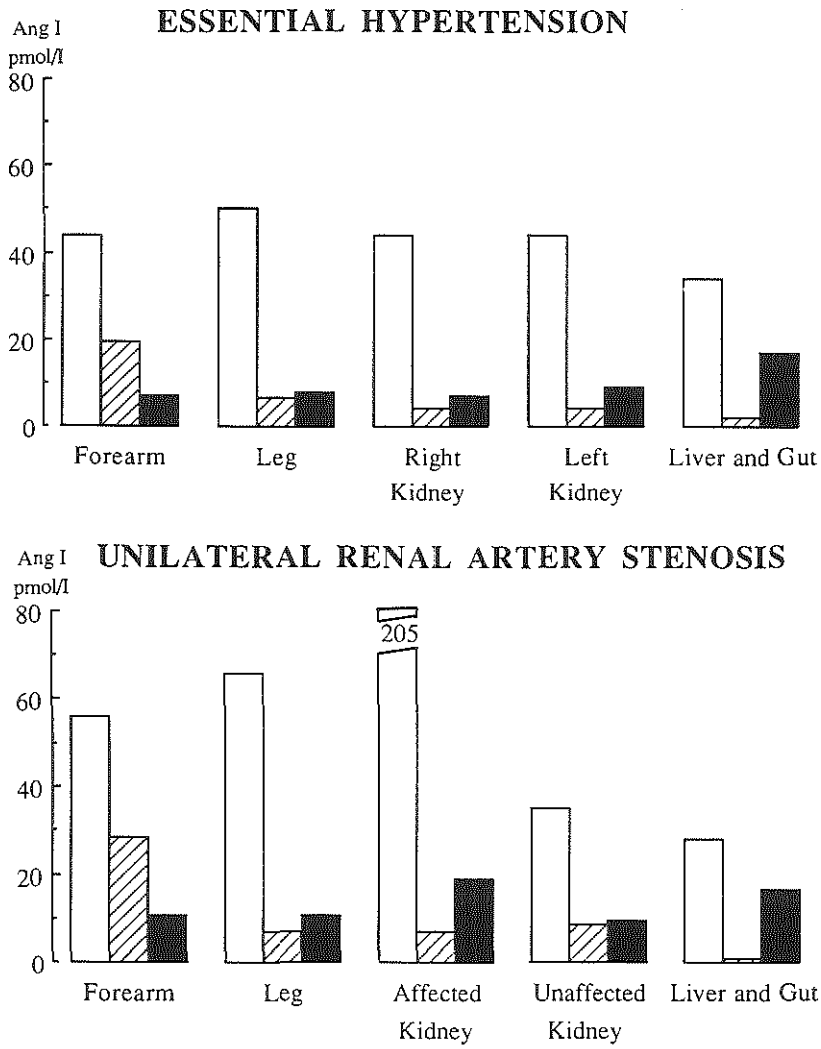

FIGURE 2

Bar graphs show the total venous plasma levels (median values) of angiotensin I (Ang I, open bars) and the venous levels of Ang I derived from arterial delivery (hatched bars) and from regional production by circulating PRA (solid bars) in different vascular beds in subjects with essential hypertension and unilateral renal artery stenosis who were treated with the diuretic furosemide. Venous Ang I derived from arterial delivery was calculated using Equation 6, and venous Ang I derived from production by circulating PRA was calculated using Equation 7 assuming a mean transit time of blood of 0.17 minute in the limbs and the kidney, and 0.42 minute in the hepatomesenteric vascular bed.[15]

Table 3
Production Rate of Circulating Angiotensins I and II by the Kidneys in Subjects with Essential Hypertension

		FUROSEMIDE n=7	UNTREATED n=7	CAPTOPRIL n=8
Renal Plasma Flow	l/min	0.478±0.051	0.522±0.094	0.572±0.039
Extraction of Ang I		0.88±0.02	0.87±0.02	0.80±0.02 *
Extraction of Ang II		0.89±0.01	0.88±0.02	0.80±0.01 *
Arterial Ang I	pmol/l	29.1	24.0	46.3
Arterial Ang II	pmol/l	18.6	12.5	2.5*
Arterial PRA	$pmol.l^{-1}.min^{-1}$	42.9	30.9	35.2
Renal venous Ang I	pmol/l	48.0	33.0	47.5
Renal venous Ang II	pmol/l	3.9	3.4	0.9*
Renal venous PRA	$pmol.l^{-1}.min^{-1}$	46.7	40.7	45.6
Ang I production †	pmol/min	26.4	11.8	20.1
Ang II production †	pmol/min	1.2	1.5	0.2*

Data are mean±SEM or median values and represent averages for the left and right kidneys. *$p<0.05$ for difference from untreated group. †Ang I and II production was calculated using Equations 11 and 12. Subjects from untreated and captopril-treated groups have been described in previous papers.[10,12] PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II.

Because we measured the renal plasma flow, we were able to estimate the production rate of circulating Ang I by the kidneys in subjects with EHT. Results from these calculations are shown in Table 3, which also shows data obtained in previously described untreated and in captopril-treated subjects with EHT.[10,12] The production rate of circulating Ang I was approximately twofold higher in the furosemide-treated and in the captopril-treated subjects than in the untreated group but both differences did not reach statistical significance ($0.05<p<0.1$). The intrarenal Ang I production by circulating PRA is too low to account for the total intrarenal production of circulating Ang I. In subjects with EHT the intravascular plasma volume in both kidneys together is approximately 80 ml,[15] and it can be calculated that within this volume Ang I production by circulating PRA

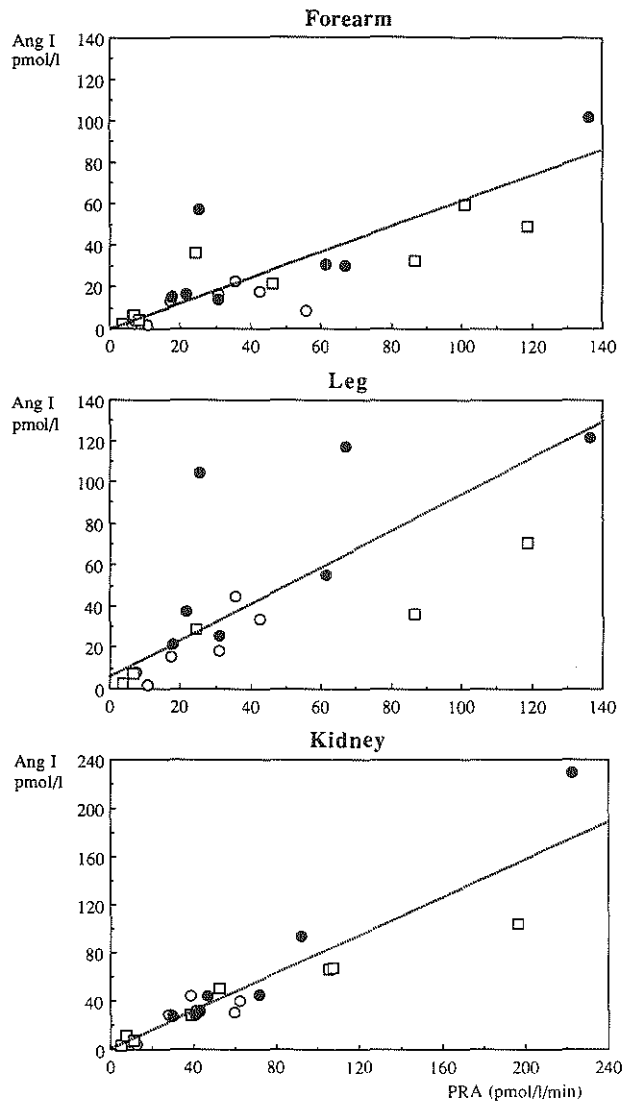

FIGURE 3

Scatter plots show the relationship between the venous level of regionally produced angiotensin I (Ang I) and circulating plasma renin activity (PRA) in various vascular beds in subjects with essential hypertension, who did not receive antihypertensive treatment (circles), or who were treated with the diuretic furosemide (dots), or with the ACE inhibitor captopril (squares). Regression lines were calculated by the method of least squares; forearm, y = 0.54 +0.53x, r=0.88; leg, y = 6.4 +0.75x, r=0.83; kidney, y = -0.31 +0.78x, r=0.90. Data from untreated subjects and captopril-treated subjects have been described in previous papers.[10,12]

contributes only for 20-28% to the total production rate of circulating Ang I. This percentage is similar to the contribution of Ang I production by circulating PRA that we calculated with Equations 6 and 7.

Regional Production of Angiotensin II

A major fraction of venous Ang II in the forearm and the leg also appeared to be derived from regional production (Figure 4). However, in the forearm and the leg most, if not all, of the production of circulating Ang II appeared to originate from the regional conversion of arterially delivered Ang I, both in subjects with EHT and in URAS. In the kidneys in subjects with EHT the renal venous plasma level of Ang II was slightly higher than expected from the arterial delivery of Ang II and from the intrarenal conversion of arterially delivered Ang I. The level of intrarenally produced venous Ang II, however, was 3 to 6 times lower than the level of venous Ang II produced by the limbs. In the subjects with URAS renal venous Ang II was also higher than expected from the arterial delivery of Ang II and from the intrarenal conversion of arterially delivered Ang I, more so on the affected side than on the unaffected side. Thus it appears that in EHT and in URAS some circulating Ang II has been produced within the renal vascular bed. However the 'true' production of circulating Ang II by the kidney may be somewhat lower than the production we actually found because, especially when the plasma level of Ang I in the renal vein is high, some Ang II is generated ex vivo during handling of the samples. Ang II in the hepatic vein was very low in both groups of subjects and most, if not all, Ang II was derived from arterial delivery.

Analogous to calculation of the rate of Ang I production by the kidneys in EHT we were also able to estimate the rate of production of circulating Ang II (Table 3). Furosemide had no significant effect on the rate of production of circulating Ang II compared with untreated subjects, whereas captopril reduced the rate of Ang II production by at least 80%. During furosemide treatment the production rate of circulating Ang I by the kidney was approximately 20 times higher than the production rate of circulating Ang II.

Thus, it appears that the kidney preferably releases Ang I into the systemic circulation but little or no Ang II. This suggests that the intrarenal production of circulating Ang I occurs in a compartment separate from the site where Ang II production occurs.

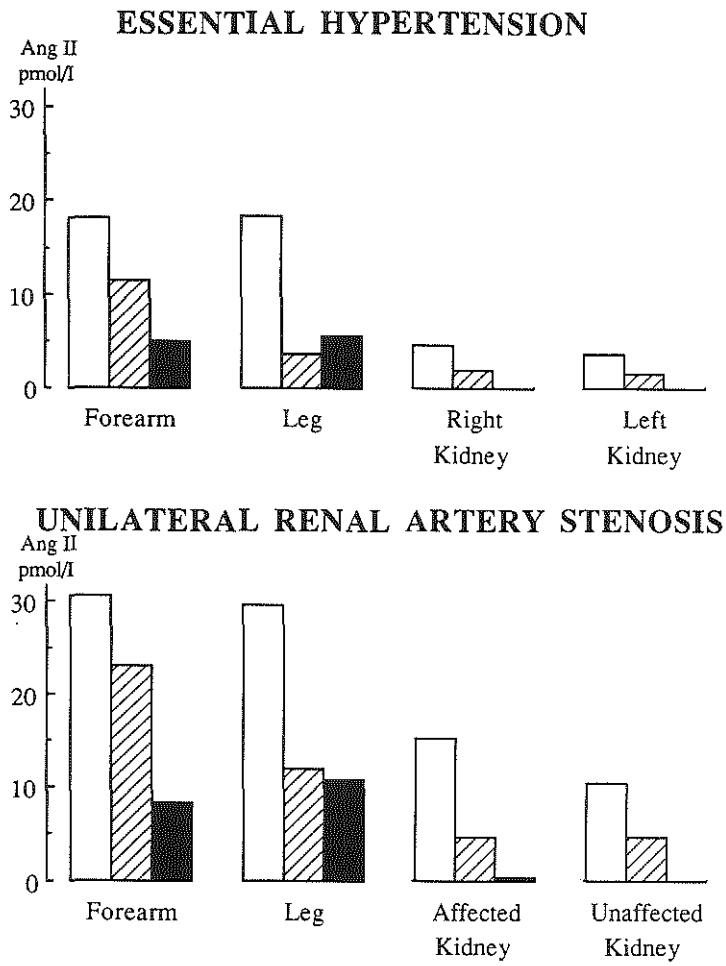

FIGURE 4
Bar graphs show total venous plasma levels (median values) of angiotensin II (Ang II, open bars) and the venous levels derived from arterial delivery (hatched bars) and from conversion of arterially delivered angiotensin I (Ang I, closed bars)in different vascular beds in subjects with essential hypertension and subjects with unilateral renal artery stenosis. Venous Ang II derived from arterial delivery was calculated using Equation 8, and venous Ang I originating from conversion of arterially delivered Ang I was calculated using Equation 10.

Discussion

In previous studies, in which ^{125}I-Ang I was infused into untreated and captopril-treated subjects with EHT and URAS we found that circulating PRA could not account for the regional production of circulating Ang I in the forearm, the leg, and the kidney. Therefore a major fraction of circulating Ang I appeared to be produced outside the circulating plasma.[10-12] In contrast, most of the Ang I in the hepatic vein that was produced in the hepatomesenteric vascular bed could be accounted for by circulating PRA. The regional production of Ang I at tissue sites in the extrarenal vascular beds, i.e. that part of the Ang I production that is not due to circulating PRA, may depend on kidney-derived renin that is taken up from the blood but may also depend on in situ synthesized renin.[16] Our present observations in furosemide-treated subjects are in agreement with these data.

The importance of kidney-derived renin is supported by our data collected in furosemide-treated, captopril-treated, and untreated subjects; the regional production of circulating Ang I in the forearm and the leg was closely correlated with the level of circulating PRA. The importance of kidney-derived renin as a determinant of local Ang I production at extrarenal tissue sites is further supported by the fact that in bilaterally nephrectomized humans, in which PRA is close to zero, the levels of Ang I and Ang II in circulating plasma are also close to zero.[17] In addition, animal experiments show that in the isolated perfused rat and rabbit hindlimb the spontaneous release of Ang I and II is very low,[5-9] but perfusion of these limbs with exogenous renin initially stimulates the release of Ang I and II in a dose-dependent manner. The stimulating effect, however, is not sustained because of depletion of a local pool of angiotensinogen.[5] These observations in humans and animals do not exclude that in extrarenal vascular beds the production at tissue sites of Ang I that does not readily equilibrate with Ang I in circulating plasma, may depend on in situ synthesized rather than kidney-derived renin.

In the limbs of furosemide-treated subjects, 20-30% of arterially delivered Ang I was converted to Ang II. This result is similar to the conversion we measured in the limbs of untreated subjects.[12] It shows that a substantial part of circulating Ang II is generated in extrapulmonary vascular beds. In the limbs of furosemide-treated subjects 35-50% of arterially delivered Ang I was degraded, which is similar to the results in captopril-treated and in untreated subjects.[10-12] Little or no renal conversion of arterially delivered Ang I could be demonstrated in subjects with URAS both on the affected and the unaffected side, and in EHT.

In the furosemide-treated subjects most, 60-100%, of the production of circulating Ang II in the limbs could be accounted for by the regional conversion

of arterially delivered Ang I. This is similar to the results we obtained in untreated subjects.[12]

In the kidneys in subjects with EHT the production rate of circulating Ang I and II was determined. In untreated subjects and in furosemide-treated subjects Ang I production by the kidney was 10 to 20 times in excess of Ang II production, and it appears that the intrarenal renin-angiotensin system almost exclusively releases Ang I into the circulation. This is remarkable because the levels of Ang II within renal tissue are much higher than the levels of Ang II in renal venous plasma,[18,19] and are even high enough to detect by immunocytochemical methods.[20] This suggests that Ang II which is present within the kidney does not reach easily the circulating blood.

In conclusion, the present study shows that the release of Ang I and II from extrarenal tissue sites into the systemic circulation is stimulated by furosemide treatment. In the kidney where little of the arterially delivered Ang I is converted to Ang II, only the release of Ang I, and not Ang II, is stimulated by furosemide. While the stimulating effect of furosemide on the release of Ang I and II from extrarenal tissue sites is quantitatively related to the increased release of renin from the kidney, a large fraction of de novo produced venous Ang I in the extrarenal vascular beds does not appear to originate from the action of circulating renin on circulating angiotensinogen. Most likely kidney-derived renin is bound to or taken up by tissues where its catalytic action leads to the production of Ang I. Part of this locally produced Ang I reaches the regional veins from where it enters the systemic circulation. It is then transported to the pulmonary and extrapulmonary sites where the Ang I-II conversion occurs.

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Chapter 6

Metabolism of Angiotensin I by Different Tissues in the Intact Animal

Summary

To quantify regional conversion of angiotensin I to angiotensin II and its degradation to peptides other than angiotensin II, monoiodinated ^{125}I-angiotensin I was given to anesthetized pigs by constant infusion into the left cardiac ventricle. At steady state, blood samples were taken from the aorta and various regional veins. Distribution volume appeared to be 24% of body weight. After angiotensin-converting enzyme inhibitor treatment, fractional angiotensin I metabolism (fraction of arterially delivered angiotensin I that was metabolized during a single passage of blood) was 10% in the lungs (conversion 4%), compared with 56% in the combined systemic vascular beds (conversion 1%). Fractional angiotensin I metabolism during angiotensin-converting enzyme inhibition was 93% in the kidney; 50-70% in myocardium, skeletal muscle, head and skin; 21% in the left cardiac cavity; and 14% in the right cardiac cavity. Without angiotensin converting enzyme inhibition, fractional angiotensin I metabolism was 29% in the lungs (conversion 25%); 49% in the combined systemic vascular beds (conversion 10%); 38% in the left cardiac cavity (conversion 11%); and 14% in the right cardiac cavity (conversion 0%). It may thus be concluded that *1*) extrapulmonary vascular beds make an important contribution to the conversion of circulating angiotensin I and *2*) there is rapid extrapulmonary angiotensin I degradation that does not depend on angiotensin I-II conversion.

Introduction

Angiotensin II (Ang II), the most important biologically active product of the renin-angiotensin system, is a powerful hypertensive agent. It acts on specific receptors in vascular smooth muscle, kidney, adrenal, brain and other tissues.[1-3] The Ang II that reaches these receptors is derived from angiotensin I (Ang I),

which is produced both in circulating blood and in the tissues.[4,5] To better understand the workings of the tissue renin-angiotensin systems, as opposed to the circulating renin-angiotensin system, it is essential to determine how Ang I is handled by the tissues. Arterially delivered Ang I is rapidly extracted from the circulation by the tissues, due to the hydrolytic attack by peptidases that degrade Ang I into smaller biologically inactive peptides or convert Ang I to Ang II. To date, however, few studies have been performed in intact animals to address this issue. In one such study ^{125}I-Ang I was infused into the renal artery of the dog to measure intrarenal Ang I degradation and Ang I-II conversion.[6,7] We administered ^{125}I-Ang I, via systemic intravenous infusion, to subjects with essential hypertension who were being treated with the angiotensin converting enzyme (ACE) inhibitor captopril. We found that the extraction of ^{125}I-Ang I by the kidney, the leg, the forearm and the liver did not differ from the extraction of unlabeled native Ang I.[4]

The aim of the present study was to examine further whether ^{125}I-Ang I is a suitable tracer to assess the distribution of endogenous Ang I and to quantify Ang I-II conversion and Ang I metabolic degradation in the intact organism. For this purpose we administered ^{125}I-Ang I, alone or in combination with unlabeled Ang I, via a constant infusion into the left cardiac ventricle of the pig and studied the whole body as well as the regional metabolism of the two peptides, both during ACE inhibitor treatment and without such treatment.

Methods

Chemicals

[Ile^5]-Ang-(1-10) decapeptide (Ang I), [Ile^5]-Ang-(1-8) octapeptide (Ang II), and [Ile^5]-Ang-(2-8) heptapeptide (Ang III) were obtained from Bachem, Bubendorf, Switzerland. [Ile^5]-Ang-(2-10) nonapeptide (Ang-(2-10)) was from Senn Chemicals, Dielsdorf, Switzerland. [Ile^5]-Ang-(3-8) hexapeptide (Ang-(3-8)), [Ile^5]-(4-8) pentapeptide (Ang-(4-8)), and [Ile^5]-Ang-(1-7) heptapeptide (Ang-(1-7)) were from Peninsula Laboratories, Belmont, CA, USA. Methanol, ortho-phosphoric acid (both analytical grade) and 1,10-phenantroline were from Merck, Darmstadt, FRG. Bovine serum albumin was from Sigma Chemical Co., St. Louis, Mo, USA. Water for high-performance liquid chromatography (HPLC) was prepared with a Milli-Q system from Waters Chromatography Div., Milford, Mass, USA. The renin inhibitor Ro 42-5892 was a kind gift of Dr. P. van Brummelen (Hoffmann-La Roche, Basel, Switzerland).

Preparation of Radiolabeled Angiotensins

Monoiodinated ^{125}I-Ang I was prepared with the chloramine-T method and purified as described previously.[4,8] The specific radioactivity of the ^{125}I-Ang I preparation was ~ 3.6×10^6 cpm/pmol (74 kBq/pmol). ^{125}I-labeled preparations of Ang II, Ang III, Ang-(3-8), Ang-(4-8), Ang-(2-10), Ang-(1-7) and tyrosine were also made.

Separation of Angiotensins by HPLC

Angiotensins and their metabolites were extracted from plasma by reversible adsorption to octadecylsilyl silica (Sep-Pak C18, Waters) and separated by reversed-phase HPLC, according to the method of Nussberger et al.[9] with some modifications.[4] Separations were performed on a reversed-phase Nucleosil C18 steel column of 250×4.6 mm and 10 μm particle size. Mobile phase A was 0.085% ortho-phosphoric acid containing 0.02% sodium azide. Mobile phase B was methanol. The flow was 1.5 ml/min and the working temperature was 45° C. Sep-Pak plasma extracts were dissolved in 100 μl of HPLC solvent and injected. Elution was performed as follows: 65% A/35% B from 0 to 6 minutes followed by a linear gradient to 45% A/55% B until 12 minutes. The eluate was collected in 20-seconds fractions into polystyrene tubes coated with bovine serum albumin. The concentrations of ^{125}I-Ang I and its metabolites in the HPLC fractions were measured in the gamma counter (80% efficiency). The fractions containing unlabeled Ang I and Ang II were neutralized with 0.5 M sodium hydroxide and vacuum dried at 4° C. Recovery after HPLC separation alone was 90-95% for both labeled and unlabeled Ang I and Ang II. The overall recovery of ^{125}I-Ang I and ^{125}I-Ang II added to plasma samples was 85±7% and 84±8% (mean±SD, n=6) respectively. Similar values were obtained for Ang I and Ang II. Results were not corrected for incomplete recovery.

Assay of Angiotensins

Ang I and II concentrations were measured by radioimmunoassay after Sep-Pak extraction and HPLC separation.[4] The Ang I antiserum cross-reacted with Ang-(2-10) (100%) but not (<0.1%) with Ang II, Ang III, Ang-(3-8), Ang- (4-8), or Ang-(1-7). The Ang II antiserum cross-reacted with Ang III (55%), Ang-(3-8) (73%) and Ang-(4-8) (100%), but not (<0.2%) with Ang I, Ang-(2-10), or Ang-(1-7). These patterns of cross-reactivity show that the antibodies in both antisera were directed against the C-terminal sequences of Ang I and II.

In Vivo Studies on Angiotensin I Metabolism

Studies were carried out in twenty female pigs (crossbred Yorkshire × Landrace, Hedelse Varkens Combinatie, Hedel, The Netherlands) with a body weight of 23-31 kg. The pigs were kept on a normal sodium diet. Twelve pigs had been treated with captopril, 25 mg twice daily for 3 days. The experiments in these pigs were performed 3-4 hours after the last captopril dose.

The pigs were sedated with azaperone (Stresnil, Janssen Pharmaceutics, Beerse, Belgium), 5 mg/kg im, 15 minutes later followed by metomidate (Hypnodil, Janssen Pharmaceutics), 3 mg/kg iv. Subsequently the pigs were intubated for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were adjusted to keep arterial blood gases within the normal range. In order to maintain an adequate anesthesia, a catheter was placed in the superior caval vein via the external jugular vein for the administration of 160 mg/kg α-chloralose (Merck). Fluid losses were corrected with saline, also via this catheter. A peripheral electrocardiogram lead was monitored throughout the experiment. An 8-F catheter inserted into the descending aorta, via a femoral artery, was used to measure central aortic pressure (50 AD pressure transducer, Spectramed, Bilthoven, The Netherlands). A 7-F catheter was manipulated into the left ventricle, via the carotid artery, under X-ray control, for the infusion of ^{125}I-Ang I and unlabeled Ang I. After the heart had been exposed by a midsternal split, electromagnetic flow probes (Skalar, Delft, The Netherlands) were placed around the ascending aorta and the left anterior descending coronary artery.

Blood for angiotensin measurements was collected from the aorta, pulmonary artery, left and right atrium, and great cardiac vein in eight captopril-treated and eight untreated pigs. An 8-F catheter was placed in the aorta, via a femoral artery. A 7-F catheter was introduced into the pulmonary artery under radio-graphic control, via a femoral vein. The left and the right atrial

appendages were catheterized each with an 8-F catheter. The vein accompanying the left anterior descending coronary artery (great cardiac vein) was cannulated with a polyethylene catheter. In 4 additional captopril-treated pigs, blood was collected from the aorta and the femoral, renal, jugular vein and ear lobe veins. A 7-F catheter was inserted into the right femoral vein and subsequently positioned in the right renal vein under fluoroscopy. An 7-F catheter was placed in the left femoral vein. The left jugular vein was cannulated with a 7-F catheter. The ear lobe vein was cannulated with a polyethylene catheter.

After a stabilization period of 45 minutes, following the completion of instrumentation, the pigs were given a constant infusion of ^{125}I-Ang I, 3.6±0.5×10^6 cpm/min (mean±SD) either alone (9 captopril-treated pigs, 5 untreated pigs) or combined with unlabeled Ang I, 0.9±0.3 nmol/min (3 captopril-treated pigs, 3 untreated pigs), into the left cardiac ventricle. During the infusion, which lasted 20 minutes, hematocrit measurements were performed in arterial blood to enable us to convert blood flow into plasma flow. Blood samples (5 ml) to measure ^{125}I-labeled and unlabeled angiotensins were taken from each sampling site at 2 minutes before and at 9, 14 and 19 minutes after the start of the infusion. To determine the half-lives of ^{125}I-Ang I and unlabeled Ang I blood samples were taken in the first 2- to 3-minute period after the infusion had been stopped.

The blood was rapidly drawn with a plastic syringe containing the following inhibitors (0.25 ml inhibitor solution in 5 ml blood): 0.01 mM Ro 42-5892, 6.25 mM disodium EDTA, and 1.25 mM 1,10-phenantroline (final concentrations in blood). Previous measurements of plasma renin activity in porcine plasma had shown that a concentration of 0.01 mM of the renin inhibitor Ro 42-5892 was sufficient to cause complete inhibition of Ang I production (unpublished results). The blood samples were immediately transferred into prechilled polystyrene tubes and centrifuged at 3,000*g* for 10 minutes at 4° C. Plasma was stored at -70° C and assayed within 2 weeks.

In Vitro Studies on Angiotensin I Metabolism

Since the results of our in vivo studies raised the possibility that the rate of of ^{125}I-Ang I-II conversion might be different from the rate of Ang I-II conversion, the in vitro elimination rate of ^{125}I-Ang I in porcine plasma was compared with that of unlabeled Ang I under standardized conditions, both in the presence and absence of captopril. In the presence of captopril the elimination of ^{125}I-Ang I and Ang I is due to degradation, whereas in the absence of captopril the elimination is due both to ^{125}I-Ang I and Ang I degradation and ^{125}I-Ang I-II and Ang I-II conversion.

Forty milliliters of blood were collected from four female pigs, with a body weight of 24-29 kg. The blood was collected into polystyrene tubes containing trisodium citrate (0.2 ml in 10 ml blood, final concentration 0.013 M). The blood samples were immediately centrifuged at 3,000*g* for 10 minutes at room temperature. Plasma was stored at -20° C.

Before the experiment, the renin inhibitor Ro 42-5892 was added to plasma (final concentration 0.01 mM), to prevent Ang I formation. Captopril (final concentration 0.4 mM) was added to study degradation separately from conversion.

After the plasma containing the inhibitors had been brought to a temperature of 37° C in a waterbath, the experiment was started by adding at t = 0 either 10^6 cpm ^{125}I-Ang I or 2 pmol Ang I (both in a volume of 100 µl) to 2 ml plasma. Plasma aliquots of 200 µl were taken at 0.25, 1, 2, 5, 10, 20, and 40 minutes and immediately mixed with an inhibitor solution containing the following inhibitors (20 µl inhibitor solution in 200 µl plasma) 1.25 mM 1,10-phenantroline and 6.25 mM disodium EDTA (final concentrations in plasma). The samples were kept on ice and Sep-Pak extraction of angiotensins was performed within 1 hour. The Sep-Pak extracts were applied to the HPLC column and ^{125}I-Ang I and Ang I were measured as described before.

Captopril, in the concentration we used, caused complete blockade of conversion. Experiments, in which ^{125}I-Ang II or Ang II was added to plasma, demonstrated that angiotensin degradation was not altered by captopril.

Calculations

Regional Extraction of Angiotensin I. The Ang I extraction ratio, E, is defined as follows

$$E = 1-(\text{Ang } I_{out}/\text{Ang } I_{in}) \quad (1)$$

in which Ang I_{in} is the steady-state concentration of exogenous Ang I in inflowing (arterial) plasma, and Ang I_{out} is the concentration in outflowing (venous) plasma during the infusion of Ang I into the left cardiac ventricle.

Extraction ratios of ^{125}I-Ang I and unlabeled Ang I were calculated for the following vascular beds: *1*) left cardiac cavity (left atrium to aorta), *2*) combined systemic vascular beds (aorta to right atrium), *3*) myocardium (aorta to great cardiac vein), *4*) right cardiac cavity (right atrium to pulmonary artery), *5*) lungs (pulmonary artery to left atrium), *6*) skeletal muscle (aorta to femoral vein), *7*) kidney (aorta to renal vein), *8*) head (aorta to jugular vein), and *9*) skin (aorta to ear lobe vein).

The clearance of Ang I from the blood during its passage in a given vascular bed is given by the following equation

$$\text{plasma clearance} = Q \times E = Q \times (\text{Ang } I_{in} - \text{Ang } I_{out})/\text{Ang } I_{in} \quad (2)$$

in which Q is the plasma flow.

The clearance rate calculated in this way is not the total clearance by a given tissue because only part of the intracardially infused and arterially delivered Ang I reaches the tissue sites where it is metabolized. Therefore the term intrinsic clearance is introduced.[10-12] Intrinsic clearance is a measure of the inherent ability of the tissues to metabolize Ang I, i.e., the maximal eliminating capacity in the absence of flow limitations. To calculate the intrinsic clearance, it is assumed that the Ang I concentration in the tissue compartment from which Ang I is eliminated equals its concentration in outflowing plasma, so that

$$\text{intrinsic clearance} = Q \times E/(1-E) = Q \times (\text{Ang } I_{in} - \text{Ang } I_{out})/\text{Ang } I_{out} \quad (3)$$

This equation is based upon the so-called "venous equilibrium" model, in which the vascular bed between the arterial and venous sampling sites is considered to be a single, well-stirred compartment.[10,11] For most vascular beds, Ang I_{in} and Ang I_{out} are the concentrations of exogenous Ang I in plasma of arterial and venous blood respectively. For the right cardiac cavity, Ang I_{in} is the concentration of exogenous Ang I in the right atrium and Ang I_{out} the concentration in the pulmonary artery. For the left cardiac cavity, Ang I_{in} was calculated as

$$\text{Ang } I_{in} = \text{Ang } I_{\text{left atrium}} + \text{infusion rate/cardiac output of plasma} \quad (4)$$

Ang I_{out} for the left cardiac cavity is the concentration of exogenous Ang I in the aorta.

Regional Degradation and Conversion of Angiotensin I. The regional Ang I extraction ratio, E, is determined by the metabolic rate constant, *k*, and the time, t, during which the arterially delivered Ang I is exposed to peptidases during the passage of the blood from the arterial to the venous side of the vascular bed.

E can be written as a function of *k* and t, as follows

$$E = 1-e^{-kt} \quad (5)$$

The metabolism of Ang I comprises both Ang I degradation and Ang I-II conversion, so that

$$k = k_1 + k_2 \quad (6)$$

in which k_1 is the first-order rate constant for Ang I degradation and k_2 the first- order rate constant for conversion. Substitution of Equation 6 into Equation 5 gives

$$E = 1 - e^{-(k1t+k2t)} \quad (7)$$

in which t is the time during which Ang I is exposed to the degrading and converting enzymes as the blood flows from the arterial side to the venous side. From Equation 7 it follows that

$$k_1t + k_2t = -\ln(1-E) = -\ln(\text{Ang } I_{out}/\text{Ang } I_{in}) \quad (8)$$

Assuming k_1 to be the same for Ang II as for Ang I, k_1t can be calculated as

$$k_1t = -\ln\{(\text{Ang } I_{out}+\text{Ang } II_{out})/(\text{Ang } I_{in}+\text{Ang } II_{in})\} \quad (9)$$

Subsequently k_2t can be calculated by subtracting k_1t from $k_1t + k_2t$ as

$$k_2t = \ln\{(\text{Ang } I_{out}+\text{Ang } II_{out})/(\text{Ang } I_{in}+\text{Ang } II_{in})\} - \ln(\text{Ang } I_{out}/\text{Ang } I_{in}) \quad (10)$$

The fractional degradation of arterially delivered Ang I was defined as follows

$$\text{Fractional degradation} = E \times k_1t/(k_1t+k_2t) \quad (11)$$

The fractional conversion of arterially delivered Ang I was defined as follows

$$\text{Fractional conversion} = E \times k_2t/(k_1t+k_2t) \quad (12)$$

Statistics

Differences in metabolism between ^{125}I-Ang I and Ang I were evaluated for statistical significance ($p<0.05$) by using Student's t test for paired observations. Differences in metabolism of Ang I between captopril-treated and untreated pigs were analyzed with Student's t test for unpaired observations.

Results

Hemodynamic Effects of ^{125}I-Angiotensin I and Angiotensin I Infusions

Heart rate and cardiac output of plasma did not change during the infusion experiments (Figure 1). Coronary plasma flow, as judged from the left anterior descending coronary artery flow, also remained constant. The mean value of cardiac output of plasma was used to calculate ^{125}I-Ang I and Ang I clearance rates in the pulmonary and combined systemic vascular beds, respectively. Arterial pressure showed a tendency to fall during the experiment, but the change

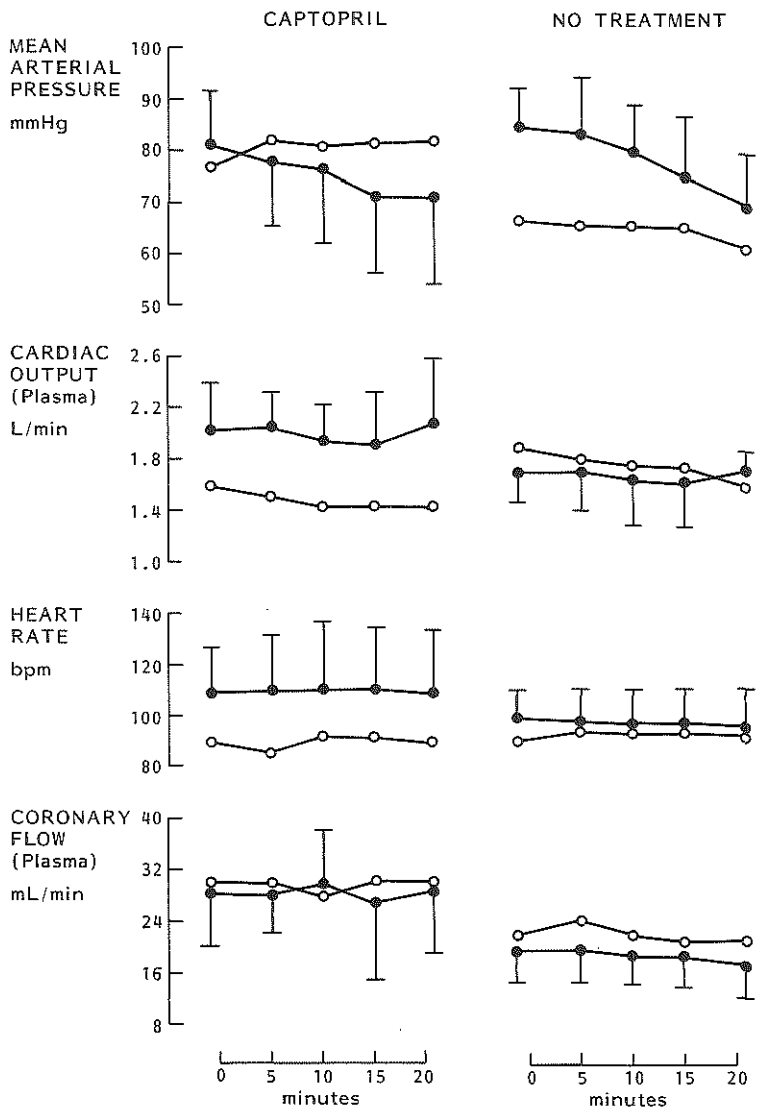

FIGURE 1

Hemodynamic parameters (mean±SD) in captopril-treated and untreated pigs before, during, and after constant infusion into left cardiac ventricle of either ^{125}I-Ang I alone (●, n=5 in both groups) or ^{125}I-Ang I in combination with Ang I (○, n=3 in both groups). Coronary flow, flow in left anterior descending coronary artery.

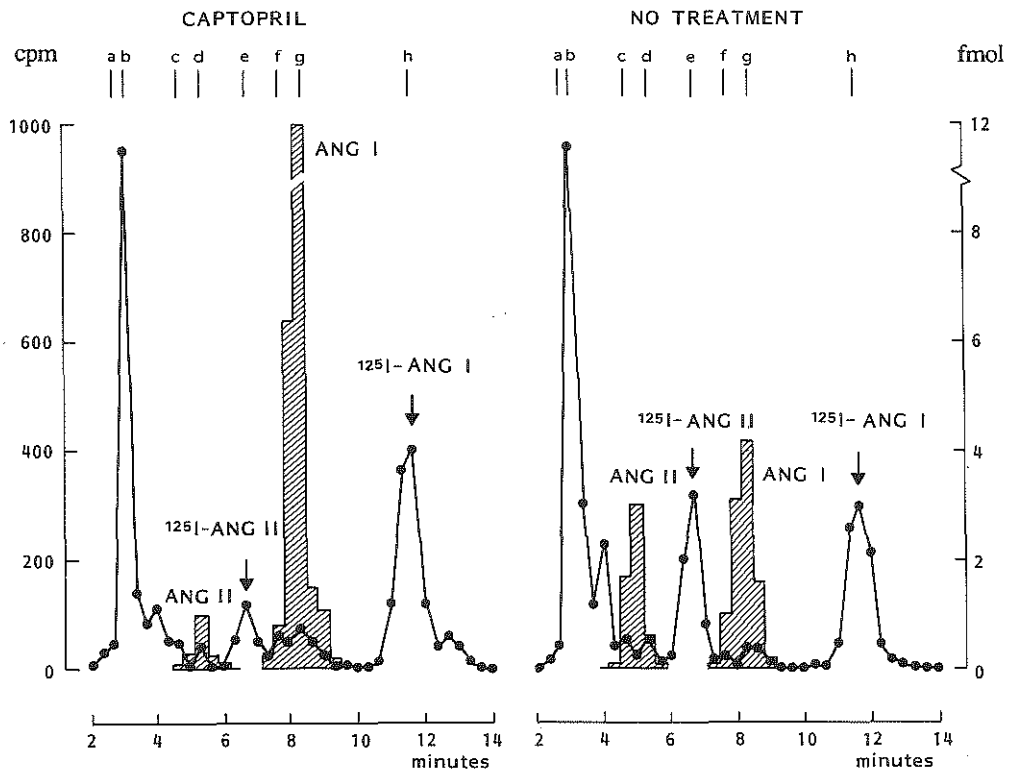

FIGURE 2

High-performance liquid chromatography (HPLC) elution profile of ^{125}I-labeled and endogenous angiotensins in aortic plasma of a captopril-treated (left panel) *and an untreated pig* (right panel), *during constant infusion of ^{125}I-Ang I into left cardiac ventricle. Samples were taken 9 minutes after the start of the infusion. Radiolabeled peptides (line) were measured by gamma counting, and endogenous angiotensins (bars) were measured by radioimmunoassay. Details on the HPLC elution program are given in the text (see "Methods"). Retention times of radiolabeled standards are indicated at the top. a, ^{125}I-Ang-(1-7); b, ^{125}I-tyrosine; c, ^{125}I-Ang III; d, ^{125}I-Ang-(4-8); e, ^{125}I-Ang II; f, ^{125}I-Ang-(2-10); g, ^{125}I-Ang-(3-8); h, ^{125}I-Ang I.*

was not statistically significant. Coronary plasma flow tended to be higher with captopril treatment than without treatment, but again the difference did not reach statistical significance.

Table 1
Steady-State Plasma Levels of ^{125}I-Angiotensin I and ^{125}I-Angiotensin II in Captopril-Treated and Untreated Pigs During Constant ^{125}I-Angiotensin I Infusion into Left Cardiac Ventricle

Sampling site	^{125}I-Ang I (cpm/l×10^3)		^{125}I-Ang II (cpm/l×10^3)	
	Captopril Treatment	No Treatment	Captopril Treatment	No Treatment
Aorta	1,861±553	1,197±267	189±166	922±145
Right atrium	769±169	534±129	128±114	710±134
Pulmonary artery	656±102	466±105	107±100	595±95
Left atrium	568±114	259±102	161±152	771±121
Great cardiac vein	959±145	585±215	166±149	993±226

Data are means±SD, n=8. ^{125}I-Ang I infusion rate was 3.1±0.3×10^6 cpm/min in captopril-treated pigs and 3.4±0.6×10^6 cpm/min in untreated pigs.

Identification of ^{125}I-Angiotensins I and II and Other ^{125}I-Labeled Peptides Present in Plasma During ^{125}I-Angiotensin I Infusion

HPLC elution profiles of ^{125}I-labeled peptides in plasma from captopril-treated and untreated pigs are shown in Figure 2. Satisfactory separations were obtained between ^{125}I-Ang I and ^{125}I-Ang II, and between these peptides and their ^{125}I-labeled metabolites. A comparison with the retention times of the various ^{125}I-labeled standards demonstrated that the ^{125}I-Ang I and ^{125}I-Ang II peaks were virtually free of ^{125}I-tyrosine, ^{125}I-Ang-(1-7), ^{125}I-Ang III, ^{125}I-Ang-(3-8), ^{125}I-Ang-(4-8), or ^{125}I-Ang-(2-10). In addition, the Ang I antiserum was able to bind 90-100% of the radioactivity in the ^{125}I-Ang I peak (and <3% in the ^{125}I-Ang II peak), whereas the Ang II antiserum bound >90% of the radioactivity in the ^{125}I-Ang II peak (and <5% in the ^{125}I-Ang I peak).

In addition to the ^{125}I-Ang I and ^{125}I-Ang II peaks, separate peaks with retention times corresponding with ^{125}I-tyrosine [and possibly also ^{125}I-Ang-(1-7)] and ^{125}I-Ang-(2-10) could be readily identified in plasma. Peaks corresponding with other metabolites, such as ^{125}I-Ang III and ^{125}I-Ang-(4-8), were very low or absent.

Not only radiolabeled Ang I and Ang II, but also unlabeled Ang I and Ang II were satisfactorily separated from each other and from either tyrosine, Ang-(1-7), Ang III, Ang-(3-8), Ang-(4-8) and Ang-(2-10). The small amounts of

Table 2
Regional Extraction Ratios of ^{125}I-Angiotensin I in Captopril-Treated and Untreated Pigs During Constant ^{125}I-Angiotensin I Infusion into Left Cardiac Ventricle

Vascular Bed	Captopril Treatment	No Treatment
Left cardiac cavity	0.22±0.16	0.46±0.13 *
Right cardiac cavity	0.14±0.09	0.14±0.09
Combined systemic vascular beds	0.57±0.11	0.55±0.06
Lungs	0.14±0.10	0.47±0.12 *
Myocardium	0.45±0.11	0.52±0.13
Head	0.58 (0.47-0.67)	ND
Skeletal muscle	0.70 (0.58-0.84)	ND
Kidney	0.94 (0.86-0.98)	ND
Skin	0.52 (0.47-0.56)	ND

Data for left and right cardiac cavities, the combined systemic vascular beds, lungs and myocardium are means±SD, n=8). Data on head, skeletal muscle, kidney and skin are means and ranges n=4). ND, not done. * p<0.05, captopril treatment versus no treatment.

radioactivity present in the HPLC fractions containing unlabeled Ang I and Ang II did not interfere with the radioimmunoassays of these peptides.

Plasma Levels of ^{125}I-Angiotensin I During and Following ^{125}I-Angiotensin I Infusion

The arterial and venous plasma levels of ^{125}I-Ang I remained constant from the 9th minute of ^{125}I-Ang I infusion (the first time blood was sampled during the infusion) until the end of the infusion, which demonstrates that a steady state was reached. The steady-state plasma levels of ^{125}I-Ang I across the various vascular beds (mean value of the 3 samples taken at 9, 14, and 19 minutes from each sampling site) were used to calculate the ^{125}I-Ang I extraction ratios of these beds. Tables 1 and 2 summarize the results obtained in eight captopril-treated and eight untreated pigs. The high extraction ratios of ^{125}I-Ang I document the rapid metabolism of ^{125}I-Ang I by the tissues. It should be noted that ^{125}I-Ang I was extracted not only in vascular beds where the arterially delivered blood has to pass the capillaries to reach the veins but also in the right and left cardiac cavities (right atrium to pulmonary artery and left atrium to aorta). The ^{125}I-Ang I extraction ratios of the combined systemic vascular beds (aorta to right atrium)

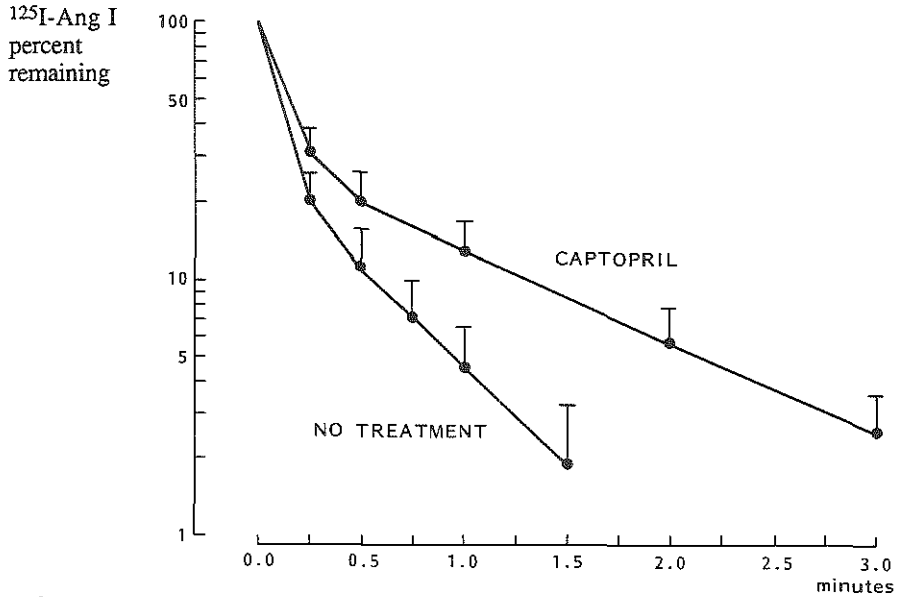

FIGURE 3
Elimination of ^{125}I-Ang I from aortic plasma in captopril-treated and untreated pigs (means±SD, n=8 in both groups), after discontinuation of constant infusion of ^{125}I-Ang I into left cardiac ventricle.

and the myocardium (aorta to great cardiac vein) were not significantly altered by captopril treatment. In contrast, the ^{125}I-Ang I extraction ratio of the lungs (pulmonary artery to left atrium) was greatly reduced by captopril ($p<0.01$), confirming that Ang I-II conversion is the main pathway by which Ang I is eliminated by the lungs.

In contrast with ^{125}I-Ang I, the total radioactivity levels in plasma kept increasing during the infusion, at all sampling sites, indicating accumulation of one or more radiolabeled metabolites. At the end of the infusion >95% of the total radioactivity of inflowing plasma was recovered in the outflowing plasma across the cardiac cavities and lungs. Across the other vascular beds we studied 90-95% of arterially delivered radioactivity was recovered in venous plasma. These findings document that the extraction of ^{125}I-Ang I by the tissues we studied is mainly, if not wholly, due to its local metabolism. There was no indication of local accumulation of unmetabolized ^{125}I-Ang I.

The disappearance of ^{125}I-Ang I from plasma after the ^{125}I-Ang I infusion had been stopped was followed in the aorta in all animals (Figure 3). In the first 0.5 minute the level fell by >50%. Thereafter it fell more gradually, in a mono

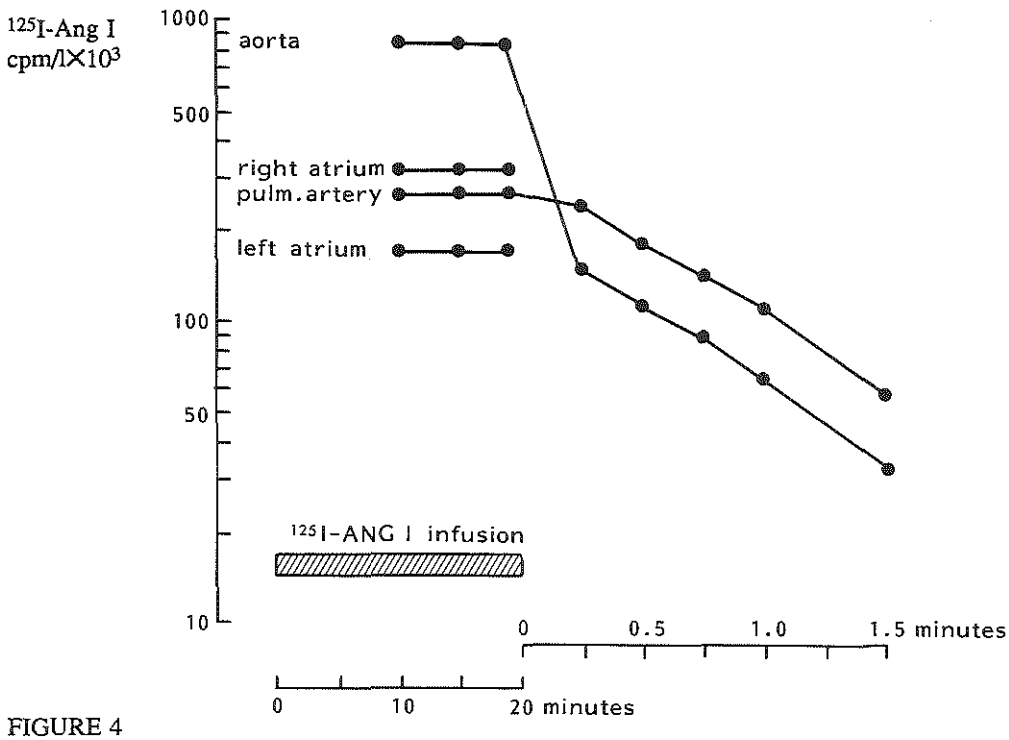

FIGURE 4

Plasma levels of ^{125}I-Ang I in the aorta, right atrium, pulmonary artery and left atrium at steady state during constant infusion of ^{125}I-Ang I into left cardiac ventricle and in the elimination phase (aorta and pulmonary artery) after discontinuation of infusion. Data are from a representative experiment in an untreated pig.

exponential way, with a half-time ($t_{1/2}$) of 0.82±0.12 minute with captopril and 0.38±0.17 minute without captopril (mean±SD, n=8 in both groups, p<0.01).

In two animals the disappearance of ^{125}I-Ang I after the ^{125}I-Ang I infusion had been stopped was followed both in the pulmonary artery and in the aorta. At steady-state during the infusion, the level of ^{125}I-Ang I in the aorta was higher than in the pulmonary artery, but within 30 seconds after the infusion had been stopped ^{125}I-Ang I in the aorta fell to a level close to the level in the pulmonary artery (Figure 4). Thereafter ^{125}I-Ang I both in the aorta and in the pulmonary artery fell in a monoexponential way, and the slopes of the two elimination curves were not different. At the moment the infusion was stopped, the level of ^{125}I-Ang I in the aorta that was derived by extrapolation of the monoexponential curve, was not different from the level in the left atrium at steady-state during the infusion. The pulmonary artery-aorta ^{125}I-Ang I concentration ratio in the

elimination phase was not different from the steady state pulmonary artery-left atrium ratio during the infusion. Thus, in contrast with the arteriovenous ^{125}I-Ang I concentration ratio across the combined systemic vascular beds, the arteriovenous ratio across the lungs during the infusion was maintained after the infusion. These results are in agreement with the assumption that in the combined systemic vascular beds Ang I is cleared from a single well-stirred compartment that is larger than the blood compartment (venous equilibrium model, see above), whereas in the pulmonary vascular bed Ang I is mainly cleared from the blood compartment.

Plasma Levels of ^{125}I-Angiotensin I and Angiotensin I During and Following Combined ^{125}I-Angiotensin I and Angiotensin I Infusion

The plasma levels of Ang I at the various sampling sites (aorta, right atrium, pulmonary artery, left atrium, great cardiac vein) before the infusion were similar, both in the captopril-treated and untreated animals (Table 3). They were 20.1-23.3 pmol/l in the former group, as compared to 2.5-4.9 pmol/l in the latter group. The Ang II levels before the infusion were 2.1-3.3 pmol/l and 1.9-3.0 pmol/l, respectively. These results show that reduced Ang I-II conversion after ACE inhibition is overcome by the elevated Ang I levels.

Ang I and II rose by a factor of 7 or more during the infusion in the captopril-treated group, and by >100 in the untreated group. From the steady-state plasma levels of Ang I during the infusion, the Ang I extraction ratios across the various vascular beds were calculated and compared with the ^{125}I-Ang I extraction ratios. The results are summarized in Table 4. With captopril treatment no difference in extraction by the various vascular beds was observed between ^{125}I-Ang I and Ang I. Without captopril treatment, however, the extraction by the lungs, the combined systemic vascular beds, and the left cardiac cavity was higher for ^{125}I-Ang I than for Ang I. The elimination half-lives of ^{125}I-Ang I and Ang I were not significantly different in the captopril-treated or in the untreated animals (Figure 5).

Regional ^{125}I-Ang I Degradation and ^{125}I-Ang I-II Conversion Compared With Regional Angiotensin I Degradation and Angiotensin I-II Conversion

The metabolism of Ang I can be divided into two enzymatic processes, Ang I degradation and Ang I-II conversion. The reaction kinetics of these two processes are characterized by the first-order rate constants k_1 and k_2 for degradation and conversion, respectively. As can be seen from Table 5, k_1 for ^{125}I-Ang I and k_1 for Ang I were similar. In contrast, k_2 for ^{125}I-Ang I was 1.3–2.7 times k_2 for Ang I, which indicates that the ^{125}I-Ang I-II conversion rate was approximately two times the Ang I-II conversion rate. These findings explain why in the

Table 3
Steady-State Plasma Levels of Angiotensin I and Angiotensin II in Captopril-Treated and Untreated Pigs Before and During Constant Angiotensin I Infusion into Left Cardiac Ventricle

	Angiotensin I (pmol/l)		Angiotensin II (pmol/l)	
Sampling site	Captopril Treatment	No Treatment	Captopril Treatment	No Treatment
Aorta	21.4 --> 376	3.6 --> 541	3.3 --> 36.6	2.6 --> 229
Right atrium	23.3 --> 201	4.9 --> 285	2.3 --> 24.8	2.2 --> 175
Pulmonary artery	21.9 --> 170	3.6 --> 249	2.1 --> 21.5	1.9 --> 149
Left atrium	20.1 --> 146	2.5 --> 187	3.0 --> 32.1	3.0 --> 218
Great cardiac vein	21.1 --> 175	3.4 --> 330	3.2 --> 29.5	2.3 --> 272

Data (mean values, n=3) before and during infusion are given. Angiotensin I infusion rate was 0.77 nmol/min in captopril-treated pigs and 1.07 nmol/min in untreated pigs.

untreated pigs the ^{125}I-Ang I extraction ratios were higher than the Ang I extraction ratios.

In vitro experiments, in which ^{125}I-Ang I and Ang I were added to plasma and the elimination of the two peptides was followed, also showed a higher conversion rate for ^{125}I-Ang I than for Ang I. Elimination followed first-order kinetics, with a rate constant k of 0.68±0.04 min^{-1} for ^{125}I-Ang I and 0.41±0.02 min^{-1} for Ang I (mean±SD, n=5). After complete blockade of the conversion by the addition of captopril, k was reduced to 0.17±0.02 min^{-1} for ^{125}I-Ang I and 0.14±0.01 min^{-1} for Ang I (n=5). The slow elimination of ^{125}I-Ang I and Ang I in plasma containing captopril compared with plasma without captopril shows that the in vitro elimination of ^{125}I-Ang I and Ang I from plasma in the absence of captopril was mainly caused by ^{125}I-Ang I-II conversion and Ang I-II conversion, respectively. In the presence of captopril k equals the first-order rate constant for degradation, k_1. By subtracting k_1 from the value of k measured in the absence of captopril, one obtains the first-order rate constant for conversion, k_2. For ^{125}I-Ang I, k_2 was 0.51±0.03 min^{-1}, and for Ang I it was 0.27±0.02 min^{-1}. It appeared that k_2 was 1.9±0.3 times higher for ^{125}I-Ang I than for Ang I. These in vitro findings are in good agreement with the in vivo results described above.

Table 6 gives the calculated regional degradation and conversion constants, k_1t and k_2t, respectively, for Ang I. Calculations were based on Equations 8-10,

Table 4
Regional Extraction Ratios of ^{125}I-Angiotensin I and Angiotensin I in Captopril-Treated and Untreated Pigs During Constant Combined Infusion of ^{125}I-Angiotensin I and Angiotensin I into Left Cardiac Ventricle

	Captopril Treatment		No Treatment	
Vascular bed	^{125}I-Ang I	Ang I	^{125}I-Ang I	Ang I
Left cardiac cavity	0.35	0.37	0.45	0.33*
Right cardiac cavity	0.20	0.15	0.07	0.12
Combined systemic vascular beds	0.49	0.46	0.55	0.46*
Lungs	0.17	0.14	0.38	0.27*
Myocardium	0.54	0.51	0.44	0.40

Data are mean values (n=3). * The extraction ratio for ^{125}I-Ang I was higher than for Ang I in all three experiments. Ang I, angiotensin I.

which give the k_1t and k_2t values for ^{125}I-Ang I. Because of the results of the above experiments in which the conversion rates of ^{125}I-Ang I and Ang I were compared, k_2t for ^{125}I-Ang I was multiplied by 0.5 to give k_2t for Ang I. In the combined systemic vascular beds but not in the lungs and the cardiac cavities, blockade of Ang I-II conversion by captopril treatment was associated with a rise in the Ang I degradation constant.

From the data presented in Table 6, the fractional degradation and conversion of arterially delivered Ang I in the various vascular beds can be calculated by using Equations 11 and 12. The results are shown in Figure 6. In the untreated animals, 25±7% of the arterially delivered Ang I was converted to Ang II during a single passage of blood in the lungs and 10±2% in the combined systemic vascular beds (mean±SD, n=8). Fractional Ang I degradation in these animals was only 4±3% in the lungs compared with 39±6% in the combined systemic vascular beds. In the captopril-treated animals fractional Ang I-II conversion was reduced to very low levels, 4±4% in the lungs and 1±1% in the combined systemic vascular beds. Fractional degradation during captopril treatment remained low in the lungs, 6±8%, but rose to 55±11% in the combined systemic vascular beds. Thus the captopril-induced reduction in Ang I-II conversion in the combined systemic vascular beds was associated with a compensatory rise in Ang I degradation.

Table 5
Regional ^{125}I-Angiotensin I and Angiotensin I Degradation and Conversion Constants, in Captopril-Treated and Untreated Pigs During Constant Combined Infusion of ^{125}I-Angiotensin I and Angiotensin I into Left Cardiac Ventricle

	Captopril Treatment				No Treatment			
	^{125}I-Ang I		Ang I		^{125}I-Ang I		Ang I	
Vascular bed	k_1t	k_2t	k_1t	k_2t	k_1t	k_2t	k_1t	k_2t
Left cardiac cavity	0.36	0.09	0.47	0.05*	0.37	0.26	0.29	0.12*
Right cardiac cavity	0.23	0.01	0.17	0.00	0.11	0.00	0.15	0.01
Combined systemic vascular beds	0.66	0.05	0.62	0.02*	0.53	0.29	0.51	0.12*
Lungs	0.02	0.17	0.07	0.08*	0.06	0.45	0.01	0.35*
Myocardium	0.72	0.11	0.74	0.04*	0.24	0.36	0.27	0.25*

Data are mean values, n=3. k_1t is regional ^{125}I-Ang I or Ang I degradation constant; k_2t is regional ^{125}I-Ang I or Ang I conversion constant. Ang I, angiotensin I.
* k_2t for Ang I was smaller than for ^{125}I-Ang I in all three experiments.

Angiotensin I Clearance Rates and Distribution Volume

Clearance calculations included a correction for the higher conversion rate of ^{125}I-Ang I compared with Ang I (see above). Pulmonary clearance of Ang I was 0.15±0.16 l/min in the captopril-treated animals and 0.49±0.16 l/min in the untreated animals (n=8 in both groups, p<0.01). Intrinsic clearance of Ang I in the combined systemic vascular beds was 2.51±0.94 l/min with captopril and 1.70±0.61 l/min without captopril (n=8 in both groups, difference not significant). Thus, with captopril treatment, the contribution of the lungs to the whole body clearance of Ang I appears to be negligible. Under these circumstances the whole body clearance of Ang I can be calculated, according to the single compartment model, as (infusion rate of ^{125}I-Ang I)/(^{125}I-Ang $I_{\text{left atrium}}$), in which ^{125}I-Ang $I_{\text{left atrium}}$ is the steady-state concentration of ^{125}I-Ang I in the left atrium during the infusion of ^{125}I-Ang I into the left cardiac ventricle. Whole body clearance was 5.60±1.07 l/min (n=8). Distribution volume, calculated as (whole body clearance$\times t_{1/2}$)/ln2, was 6.47±0.93 liter. This value represents 18-32% (mean 24%) of total body weight. These results show that Ang I is not limited to the intravascular compartment, because the plasma volume is only 4-5% of total body weight.[13]

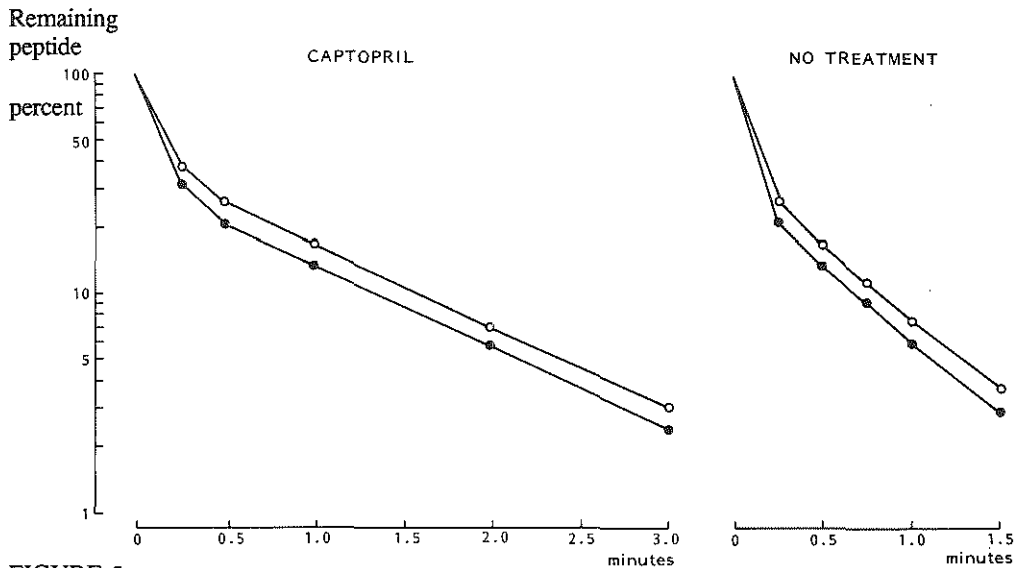

FIGURE 5

Plasma levels (mean values, n=3) of ^{125}I-Ang I (● ●) and Ang I (○ ○) in the aorta in the elimination phase after discontinuation of constant combined infusion of ^{125}I-Ang I and Ang I into left cardiac ventricle.

Discussion

Earlier reports have raised some doubt as to whether ^{125}I-labeled angiotensins can be used as a tracer for in vivo metabolic studies.[14,15] The aim of the present work was to address this point specifically with regard to Ang I, because this peptide is the direct product of renin's enzymatic action and because little quantitative data are available on Ang I metabolism by the tissues in vivo. Such data are relevant particularly in the light of recent evidence of renin's action at tissue sites as opposed to renin in circulating plasma.[4,5]

We used highly purified monoiodinated ^{125}I-Ang I in our experiments. It was infused directly into the left cardiac ventricle to obtain immediate mixing of the tracer with the arterial blood delivered to the tissues. We performed our experiments in pigs, because in these animals it is possible to collect enough blood from multiple sampling sites and to collect these samples quickly enough to prevent metabolism ex vivo. The arterial and venous plasma levels of ^{125}I-Ang I at steady state were used to quantify the metabolism of endogenous Ang I by the various vascular beds. Total radioactivity, which includes ^{125}I-Ang I as well as its radiolabeled metabolites, was also measured in arterial and venous plasma. Our

Table 6
Regional Angiotensin I Degradation and Conversion Constants Calculated from the Steady-State Plasma Levels of ^{125}I-Angiotensin I in Captopril-Treated and Untreated Pigs During Constant Infusion of ^{125}I-Angiotensin I into Left Cardiac Ventricle

	Captopril Treatment k_1t	No Treatment k_1t	Captopril Treatment k_2t	No Treatment k_2t
Left cardiac cavity	0.23±0.17	0.35±0.15	0.02±0.02	0.15±0.06*
Right cardiac cavity	0.15±0.11	0.15±0.12	0.00±0.00	0.00±0.01
Combined systemic vascular beds	0.84±0.23	0.54±0.11*	0.02±0.01	0.14±0.03
Lungs	0.07±0.10	0.04±0.04	0.05±0.04	0.30±0.10*
Myocardium	0.59±0.26	0.32±0.21*	0.03±0.02	0.23±0.05*
Head	0.77 (0.56-1.07)		0.05 (0.02-0.10)	
Skeletal muscle	1.04 (0.68-1.65)		0.12 (0.08-0.19)	
Kidney	2.58 (1.51-3.49)		0.24 (0.20-0.26)	
Skin	0.69 (0.64-0.74)		0.02 (0.00-0.04)	

Data on the left and right cardiac cavities, the combined systemic vascular beds, lungs and myocardium are mean±SD, n=8. k_1t, regional ^{125}I-Ang I or Ang I degradation constant; k_2t, regional ^{125}I-Ang I or Ang I conversion constant. Data on head, skeletal muscle, kidney and skin are mean values and ranges, n=4. * p<0.05, captopril treatment versus no treatment.

results demonstrated that the low ^{125}I-Ang I vein-artery ratios as measured across the various vascular beds are indeed due to rapid metabolism and not to local accumulation of ^{125}I-Ang I.

Our results in pigs in which ^{125}I-Ang I and Ang I were infused simultaneously demonstrate that the metabolism of ^{125}I-Ang I was different from that of Ang I. It appeared that the extraction ratios across the pulmonary and the combined extrapulmonary vascular beds were higher for ^{125}I-Ang I than for Ang I, a phenomenon that was observed in untreated pigs, but not in pigs treated with the ACE inhibitor captopril. The metabolism of Ang I comprises Ang I-II conversion and Ang I degradation. By the latter process peptides other than Ang II are formed. The above results indicate that the rate of ^{125}I-Ang I-II conversion was higher than the rate of Ang I-II conversion but that the rate of degradation was the same for ^{125}I-Ang I and Ang I. This was further confirmed by expressing the extraction ratios, E, as a function of k_1t and k_2t, k_1 and k_2 being the first-order rate constants for degradation and conversion respectively, and t the time ^{125}I-Ang I or Ang I is exposed to the degrading and converting enzymes. By doing so

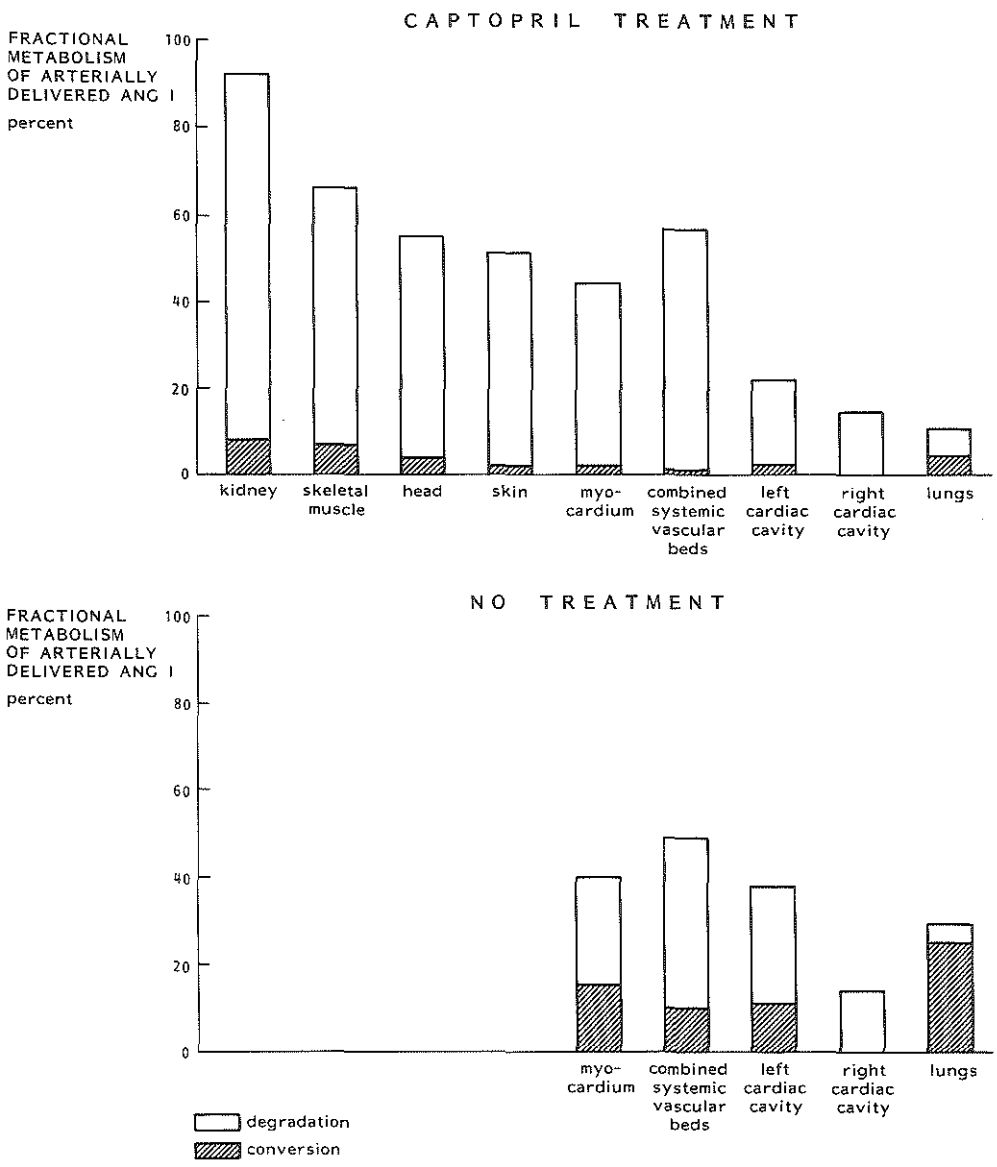

FIGURE 6

Fractional degradation (open bars) and fractional conversion (hatched bars) of arterially delivered Ang I in different vascular beds. Data (mean values) on myocardium, combined systemic vascular beds, lungs, and right and left cardiac cavity were obtained in 8 captopril-treated and 8 untreated pigs. Data on other vascular beds were obtained in 4 captopril-treated pigs.

it was found that k_2t for ^{125}I-Ang I-II conversion in the pulmonary and the combined extrapulmonary vascular beds was indeed higher than k_2t for Ang I-II conversion, and that k_1t for ^{125}I-Ang I degradation was not different from k_1t for Ang I degradation. As expected, it was conversion and not degradation that was reduced by captopril treatment. The first-order rate constant for ^{125}I-Ang I-II conversion in vivo appeared to be approximately two times that of Ang I-II conversion. In vitro experiments with plasma demonstrated that the maximal velocity-Michaelis constant ratio of ACE with ^{125}I-Ang I as the substrate was also two times higher than with Ang I as the substrate. This is probably the explanation for our findings in vivo. To calculate Ang I extraction by the vascular beds, the measurements of ^{125}I-Ang I extraction were corrected for the difference between ^{125}I-Ang I-II and Ang I-II conversion rates. This was done by multiplying the first order rate constant for ^{125}I-Ang I-II conversion by a factor of 0.5. In these calculations it was assumed that k_1t for ^{125}I-Ang I degradation was not different from k_1t for ^{125}I-Ang II degradation. Preliminary experiments in which we infused ^{125}I-Ang II instead of ^{125}I-Ang I, showed indeed little difference in degradation rate between ^{125}I-Ang I and ^{125}I-Ang II, both across the pulmonary and the combined systemic vascular beds (unpublished results). In our calculations it was further assumed that k_1t for Ang I degradation was not different from k_1t for Ang II degradation.

The intrinsic clearance of Ang I in the combined systemic vascular beds was calculated to be approximately one to two times the cardiac output of plasma. This is much too high to be explained by metabolism in the circulating blood and elimination via glomerular filtration. The high clearance rate is caused by rapid metabolism in the tissues. It is interesting to note that in the heart the rapid metabolism of plasma Ang I does not seem to depend on the passage of blood through the capillaries. Probably the endocardium, like the vascular endothelium, is a site of Ang I metabolism. The presence of ACE on the surface of the cardiac valves has indeed been demonstrated.[16,17]

In the combined systemic vascular beds ~20% of the metabolism of arterially delivered Ang I was due to Ang I-II conversion, whereas in the pulmonary vascular bed ~90% of the metabolism was due to Ang I-II conversion. It is well known that the lungs are not the only site of Ang I-II conversion.[18-20] Our study provides quantitative information on the extrapulmonary Ang I-II conversion and shows in fact that about one-half of the conversion of circulating Ang I occurs outside the lungs. There was not only considerable conversion in the combined systemic vascular beds but also in the left cardiac cavity.

After ACE inhibitor treatment there was little Ang II generation from infused Ang I. The ACE inhibitor-induced decrease in k_2t for Ang I-II conversion was associated with an increase in k_1t for Ang I degradation in the

combined systemic vascular beds, but not in the cardiac cavities and the lungs. The mechanism of this increase in k_1t is unknown. Our study indicates that systemically delivered Ang I is rapidly distributed over a compartment with a size corresponding to 24% of total body weight, which is also the size of the extracellular fluid volume. There may be some degree of compartmentalization of the processes of conversion and degradation in the sense that it is mainly the blood-borne Ang I that is subject to conversion, whereas interstitial fluid Ang I is mainly subject to degradation. Our calculations of k_1t and k_2t do not account for this, but it is conceivable that such compartmentalization may explain why, in contrast with the combined systemic vascular beds, in the cardiac cavities and the lungs no rise in k_1t was observed after ACE inhibitor treatment. In the cardiac cavities and the lungs the Ang I delivered by the inflowing blood is primarily exposed to the endocardium and the endothelial surface of the lung vessels. The lung is known to contain relatively little interstitial fluid compared with other tissues,[21] so that in the lung the arterially delivered Ang I is mainly restricted to the blood compartment. Our results are supported by experiments, in which isolated rabbit aorta was incubated with Ang I. Also in these experiments inhibition of ACE was associated with increased degradation.[22] Anyhow, our results show that decreased conversion during captopril treatment was associated with increased degradation. Thus there was little or no Ang I accumulation during ACE inhibitor treatment. Renin stimulation is therefore by far the most important, if not the only, cause for the rise in plasma Ang I that is usually observed after ACE inhibitor treatment.

We conclude that monoiodinated ^{125}I-Ang I is a suitable tracer for the type of metabolic studies we did, with the proviso that one has to account for the fact that ^{125}I-Ang I is converted by ACE at a higher rate than Ang I. Our study indicates that Ang I infused into the circulation is rapidly distributed over a compartment corresponding in size with the extracellular fluid volume. The large arteriovenous concentration gradients of ^{125}I-Ang I that were observed across the various vascular beds during ^{125}I-Ang I infusion are caused by rapid metabolism in the tissues. Ang II formation depends on ACE, but Ang I conversion is not a critical intermediary step in Ang I metabolism. The present results may be used as a basis for further studies aimed at quantifying in the intact animal Ang I production at tissue sites versus Ang I production in the circulation.

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Chapter 7

Production of Angiotensins I and II at Tissue Sites in the Intact Pig

Summary

To estimate the contribution of angiotensin I and II production at tissue sites to the circulating levels, angiotensin I and II and their radiolabeled counterparts were measured in arterial and venous plasma across various vascular beds during constant infusion of ^{125}I-angiotensin I into the left cardiac ventricle of anesthetized pigs. In the combined systemic vascular beds, angiotensin I production was closely correlated with plasma renin activity and angiotensin II production was greater than in the lungs. In the lungs virtually no angiotensin I but 31% of angiotensin II in venous plasma was derived from de novo production, which could be fully accounted for by conversion of circulating angiotensin I. In myocardium, head, skin, skeletal muscle, and kidney, respectively, 40, 58, 55, 67, and 94% of venous angiotensin I, and 32, 49, 40, 59, and 85% of venous angiotensin II were derived from de novo production. In these extrapulmonary beds part of de novo produced angiotensin I and II appeared not to be generated, respectively, by plasma renin activity and by conversion of circulating angiotensin I. These results indicate that production of angiotensin I at tissue sites contributes to its circulating level, and that some circulating angiotensin II may not be derived from circulating angiotensin I.

Introduction

The classic view of renin and angiotensins as circulating hormones has been challenged by a number of investigators in recent years. It is now recognized that many tissues contain a complete renin-angiotensin system, and there is increasing evidence that part of the angiotensin I (Ang I) in circulating plasma does not originate from the catalytic action of circulating renin on circulating angiotensinogen.[1-4] Some plasma Ang I is probably produced by renin that is present in vascular tissue.[5] Vascular renin may be plasma-derived[6,7] or is perhaps synthesized in situ.[1,8] It is not known how much Ang I is produced outside the

circulating blood and how much of this extracirculatory Ang I enters the circulation.

Angiotensin II (Ang II), the most important biologically active product of the renin-angiotensin system in circulating plasma, is commonly thought to be derived from the conversion of circulating Ang I by membrane-bound angiotensin-converting enzyme (ACE) of the vascular endothelium. ACE, however, is not only present in the vascular endothelium but also elsewhere in vascular tissue,[9-11] and it is therefore conceivable that part of circulating Ang II is derived from the conversion of Ang I that is produced at tissue sites outside the circulating blood.

Both Ang I and Ang II are rapidly eliminated by the tissues because of degradation by peptidases into peptides with little or no biological activity. Ang I is also eliminated by its conversion to Ang II. In a previous study we measured degradation and conversion in different vascular beds in intact pigs.[12] This was done by administering ^{125}I-Ang I via constant infusion into the left cardiac ventricle and by measuring the steady-state plasma levels of ^{125}I-Ang I and ^{125}I-Ang II in the inflowing (arterial) and outflowing (venous) blood of different vascular beds. Here we report on the results of simultaneous measurements of the plasma levels of endogenous Ang I and II and radiolabeled Ang I and II, during the constant infusion of ^{125}I-Ang I. From these results combined with data presented in the previous paper we were able to estimate what proportion of Ang I is produced in circulating plasma and what at tissue sites. It was also possible to estimate the proportion of Ang II produced by the conversion of arterially delivered Ang I and the proportion that is not.

Methods

Assay of Angiotensins

The materials used and the methods for measuring the plasma levels of labeled and unlabeled Ang I and II are described in the accompanying paper.[12]

Measurement of Plasma Renin Activity

Plasma renin activity (PRA) was measured by incubating plasma for 10, 20, 30, and 60 minutes at pH 7.4 and 37° C in the presence of a mixture of inhibitors to block Ang I degradation, Ang I-II conversion, and prorenin-renin conversion, and to prevent bacterial growth.[13] Preliminary experiments showed that Ang I generation was linear in the first 30 minutes of incubation. Thereafter Ang I generation tended to decrease slightly. Only the first linear part of Ang I generation was used for measuring PRA.

Infusion Protocol

Twenty-four female pigs (crossbred Yorkshire × Landrace, Hedelse Varkens Combinatie, Hedel, The Netherlands) with a body weight of 23-31 kg were included in the study. The pigs

were kept on a normal sodium diet. In 12 pigs the renin production was stimulated by treatment with captopril (25 mg twice daily, for 3 days), and in 4 pigs by treatment with furosemide (40 mg twice daily, for 3 days). The experiments in the captopril-treated and furosemide-treated pigs were performed 3-4 hours after dosing.

The pigs were anesthetized, and arterial and venous catheters were placed as described before.[12] Blood pressure, heart rate, cardiac output and left anterior descending coronary artery blood flow were monitored continuously during the infusion. Hematocrit measurements were performed in arterial blood to enable us to convert blood flow measurements into plasma flow values. Sampling sites were aorta, pulmonary artery, left and right atrium, great cardiac vein, and renal, femoral, jugular and ear lobe veins.

After a stabilization period of 45 minutes following the completion of instrumentation, the pigs were given a constant infusion of ^{125}I-Ang I, $3.6\pm0.9\times10^6$ cpm/min (mean±SD), into the left cardiac ventricle during 20 minutes. The infusion did not influence blood pressure, heart rate, cardiac output or coronary blood flow.

Blood samples for measuring the plasma levels of ^{125}I-labeled and unlabeled angiotensins were taken from the various sampling sites at 2 minutes before and at steady state during the infusion of ^{125}I-Ang I as described before.[12]

Blood samples for measuring PRA were also taken during the infusion. The blood was collected into polystyrene tubes containing disodium EDTA (0.1 ml EDTA solution in 3 ml blood, final concentration in blood 10 mM). The samples were centrifuged at 3,000*g* for 10 minutes at room temperature. Plasma was stored at –20° C.

Calculations

Regional Production of Angiotensin I. Regional Ang I production was calculated for the following vascular beds: *1*) lung (pulmonary artery to left atrium), *2*) combined systemic vascular beds (aorta to right atrium), *3*) myocardium (aorta to great cardiac vein), *4*) head (aorta to jugular vein), *5*) skin (aorta to ear lobe vein), *6*) skeletal muscle (aorta to femoral vein) and *7*) kidney (aorta to renal vein).

Regional Ang I production will be referred to in two ways: *1*) total regional Ang I production and *2*) regional Ang I production by circulating PRA. Total regional Ang I production in the systemic vascular beds was calculated as the product of the regional intrinsic clearance and the plasma level of Ang I in outflowing (venous) blood

$$\text{total regional Ang I production} = Q\times[E_I/(1-E_I)]\times\text{Ang } I_{out} \quad (1)$$

in which Ang I_{out} is the concentration of endogenous Ang I in outflowing (venous) plasma. Q is the regional plasma flow. For the combined systemic vascular beds Q equals the cardiac output of plasma. E_I is the extraction ratio of Ang I, which according to our previous paper[12] is given by the following formula:

$$E_I = 1-e^{-(*k1t+0.5*k2t)} \quad (2)$$

in which $*k_1$ and $*k_2$ are the first-order rate constants of ^{125}I-Ang I degradation (to peptides other than ^{125}I-Ang II) and ^{125}I-Ang I-II conversion, respectively, and t is the time during which ^{125}I-Ang I is exposed to the degrading and converting enzymes as the blood flows from the arterial to the venous side. Equation 2 accounts for the difference in conversion rate between labeled and unlabeled Ang I.[12]

Equation 1 is based on the "venous equilibrium" model. In this model the vascular bed between the arterial and venous sampling sites is considered to be a single, well-stirred

compartment with an Ang I concentration equal to that in outflowing (venous) plasma.[14]

As shown previously,[12] the venous equilibrium model can be applied to the systemic vascular beds but not to the pulmonary vascular bed. For the lungs it can be assumed that the clearance of Ang I equals its clearance from plasma, so that

$$\text{total pulmonary Ang I production} = Q \times [\text{Ang } I_{out} - (1 - E_I) \times \text{Ang } I_{in}] \quad (3)$$

in which Ang I_{out} and Ang I_{in} are the plasma concentrations of endogenous Ang I in the left atrium and the pulmonary artery, respectively. Q equals the cardiac output of plasma.

The regional Ang I production by PRA was calculated as follows

$$\text{regional Ang I production by PRA} = Q \times \text{PRA} \times \text{transit time} \quad (4)$$

in which transit time is the transit time of plasma as it flows from the arterial to the venous side of the vascular bed.

Regional venous Ang I originates in part from arterially delivered Ang I and is in part derived from de novo production. Regional venous Ang I from arterial delivery is given by the equation

$$\text{Ang } I_{out} \text{ from Ang } I_{in} = (1 - E_I) \times \text{Ang } I_{in} \quad (5)$$

Regional Production of Angiotensin II. Regional production of Ang II was calculated for the same vascular beds as for Ang I. In analogy with regional Ang I production, the regional production of Ang II will be referred to as *1*) total regional Ang II production, and *2*) regional Ang II production by conversion of arterially delivered Ang I. Total regional Ang II production in the systemic vascular beds was calculated as the product of the regional intrinsic clearance of Ang II and the plasma level of Ang II in outflowing (venous) blood

$$\text{total regional Ang II production} = Q \times [E_{II}/(1 - E_{II})] \times \text{Ang } II_{out} \quad (6)$$

in which Ang II_{out} is the concentration of endogenous Ang II in outflowing (venous) plasma. E_{II} is the extraction ratio of Ang II, which is given by the following formula

$$E_{II} = 1 - e^{-*k1t} \quad (7)$$

This equation is analogous with Equation 2 for E_I, the extraction ratio of Ang I. The difference between the two equations is related to the fact that Ang I is subject to both conversion and degradation, whereas Ang II is only subject to degradation. Equation 7 is based upon the assumption that the first-order rate constants for ^{125}I-Ang II degradation and ^{125}I-Ang I degradation are equal. It further accounts for the fact that the degradation rates for labeled and unlabeled Ang I are not different.[12]

In analogy with pulmonary Ang I production, the total pulmonary Ang II production was calculated as follows

$$\text{total pulmonary Ang II production} = Q \times [\text{Ang } II_{out} - (1 - E_{II}) \times \text{Ang } II_{in}] \quad (8)$$

The regional Ang II production from arterially delivered Ang I was calculated as the product of regional plasma flow, the plasma level of Ang I in inflowing (arterial) blood, and the fractional conversion of arterially delivered Ang I. Fractional conversion of arterially delivered Ang I is given, according to our previous paper,[12] by the following equation

Production of Angiotensins I and II

$$\text{fractional conversion} = E_I \times 0.5 \times {}^*k_2 t / ({}^*k_1 t + 0.5 \times {}^*k_2 t) \quad (9)$$

so that

regional Ang II production from arterially delivered Ang I =

$$Q \times \text{Ang } I_{in} \times E_I \times 0.5 \times {}^*k_2 t / ({}^*k_1 t + 0.5 \times {}^*k_2 t) \quad (10)$$

Regional venous Ang II originates in part from arterially delivered Ang II and is in part derived from regional de novo production. Regional conversion of arterially delivered Ang I is, at least partly, responsible for this de novo production. Regional venous Ang II from arterial delivery is given by the following equation

$$\text{Ang } II_{out} \text{ from Ang } II_{in} = (1 - E_{II}) \times \text{Ang } II_{in} \quad (11)$$

If, as mentioned above, it is assumed that the first-order rate constants for ^{125}I-Ang II degradation and ^{125}I-Ang I degradation are equal and that the degradation rates for labeled and unlabeled Ang I are also equal,[12] the regional venous Ang II produced by regional conversion of arterially delivered Ang I is given by the following equation

$$\text{Ang } II_{out} \text{ from Ang } I_{in} = (E_I - E_{II}) \times \text{Ang } I_{in} \quad (12)$$

Statistics

Differences in Ang I and Ang II concentration between the various sampling sites were evaluated for statistical significance ($p<0.05$) by using Scheffé test for multiple comparison. Differences between total regional Ang I production and regional Ang I production by PRA, and differences between total regional Ang II production and regional Ang II production from arterially delivered Ang I were evaluated for statistical significance ($p<0.05$) by using Student's t test. Differences between the various groups of pigs were evaluated for statistical significance ($p<0.05$) by using either Student's t test or Mann-Whitney's U test for unpaired observations.

Results

Plasma Levels of Angiotensins I and II and Plasma Renin Activity

The plasma levels of Ang I and II, and PRA were stable over the experimental period the blood samples were collected. As expected, the levels of PRA and Ang I were increased in the captopril-treated and furosemide-treated pigs compared with the untreated animals (Table 1). The levels of Ang I and PRA were linearly correlated, and the regression line in the captopril-treated group was not significantly different from that in the other two groups (Figure 1). The levels of Ang I and Ang II were also correlated, but for a given level of Ang I, Ang II was lower in the captopril-treated group than in the other two groups (Figure 2).

Plasma Ang I in the untreated and the furosemide-treated pigs was higher in

Table 1
Plasma Levels of Angiotensin I, Angiotensin II and Plasma Renin Activity

	NO TREATMENT (n=8)	CAPTOPRIL Group 1 (n=8)	CAPTOPRIL Group 2 (n=4)	FUROSEMIDE (n=4)
Angiotensin I (pmol/l)				
Aorta	2.9±1.4	22±15	30±17	14±8
Right atrium	3.5±2.0*	23±15	-	19±11*
Pulmonary artery	3.4±1.7*	22±15	-	18±11*
Left atrium	2.1±0.9	21±16	-	13±8
Great cardiac vein	2.9±1.2	21±14	-	13±7
Jugular vein	-	-	30±14	18±11**
Ear lobe vein	-	-	34±18	-
Femoral vein	-	-	32±18	-
Renal vein	-	-	46±29**	-
Angiotensin II (pmol/l)				
Aorta	2.0±1.3	2.2±2.2	3.1±1.9	12±7
Right atrium	1.7±1.1*	1.8±1.3	-	10±5*
Pulmonary artery	1.4±0.9*	1.5±1.4	-	8.9±4.7*
Left atrium	2.2±1.8	2.2±2.0	-	13±8
Great cardiac vein	1.8±0.8	2.3±2.1	-	13±7
Jugular vein	-	-	3.3±2.5	12±7
Ear lobe vein	-	-	2.8±1.2	-
Femoral vein	-	-	2.8±1.5	-
Renal vein	-	-	2.0±1.2***	-
Plasma Renin Activity ($pmol.l^{-1}.min^{-1}$)				
Aorta	7.4±5.7	49±40	38±19	60±32
Right atrium	7.7±6.5	50±39	-	56±26
Pulmonary artery	7.8±6.7	50±38	-	60±30
Left atrium	7.4±5.8	47±33	-	56±27
Great cardiac vein	7.9±7.5	50±38	-	60±31
Jugular vein	-	-	38±20	51±26
Ear lobe vein	-	-	39±18	-
Femoral vein	-	-	34±17	-
Renal vein	-	-	76±50**	-

Data are mean±SD. PRA, plasma renin activity. * In untreated and furosemide-treated pigs, angiotensin plasma levels in right atrium and pulmonary artery were different from plasma levels in aorta and left atrium (p<0.05, Scheffé test for multiple comparison). ** levels were higher than in aorta in all 4 experiments. *** levels were lower than in aorta in all 4 experiments.

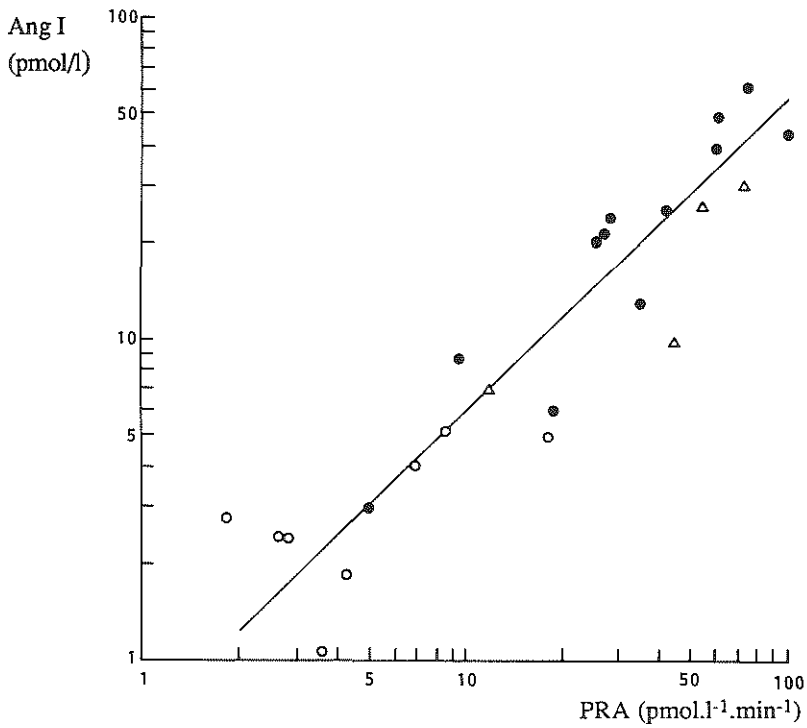


FIGURE 1

Relationship between the aortic plasma levels of plasma renin activity (PRA) and Ang I in untreated (○), captopril-treated (●), and furosemide-treated (Δ) pigs (log Ang I =0.89 ×log PRA –0.12, r=0.92).

the right atrium than in the aorta, and it was lower in the left atrium than in the pulmonary artery (Table 1). Thus there was net Ang I delivery to the systemic circulation by the combined systemic vascular beds and net Ang I extraction from the systemic circulation by the lungs. These arteriovenous differences were not seen in the captopril-treated pigs.

In contrast with Ang I, Ang II was lower in the right atrium than in the aorta, and it was higher in the left atrium than in the pulmonary artery (Table 1). Again, these arteriovenous differences were seen in the untreated and furosemide-treated animals but not in the captopril-treated pigs. Thus, in the absence of ACE inhibition, there was net removal of Ang II from the systemic circulation by the combined systemic vascular beds, and net delivery of Ang II to the systemic circulation by the lungs. Across the lungs, the venoarterial difference in Ang II was close to the arteriovenous difference in Ang I. This is in accordance with our

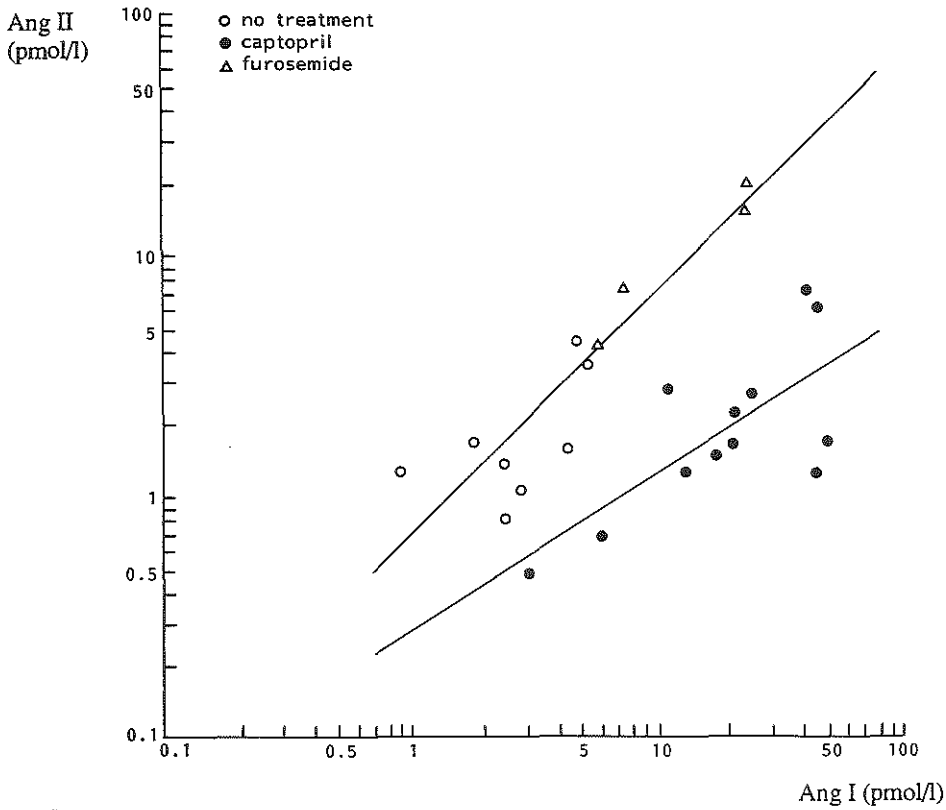


FIGURE 2

Relationship between the aortic plasma levels of Ang I and II in untreated (○) and furosemide-treated (Δ) pigs (upper line; log Ang II = 1.03 ×log Ang I –0.17, r=0.91) and in captopril-treated pigs (●) (lower line; log Ang II = 0.63 ×log Ang I –0.53, r=0.70).

previous work, showing little degradation of both Ang I and Ang II in the lungs,[12] so that most of the arterial Ang I delivered to the lungs and converted in this organ is recovered as Ang II in the blood of the left atrium.

Regional Production of Angiotensin I

As demonstrated by the data in Figure 3, the regional production rates in the systemic vascular beds were high enough to compensate for the rapid elimination of this peptide. In the lungs all Ang I in venous plasma appears to be derived from arterial delivery and not from de novo production. In the combined systemic vascular beds, and in myocardium, head, skin, skeletal muscle and kidney, respectively 59±14, 40±13, 58±6, 55±11, 67±11, and 94±5% of venous

Table 2
Angiotensin I and Angiotensin II Production Rates in Combined Systemic Vascular Beds, Lungs, and Myocardium

		NO TREATMENT	CAPTOPRIL	FUROSEMIDE
Combined systemic vascular beds				
Ang I production	(pmol/min)	3.5 (2.2-13)	43 (8.6-180)*	50 (27-85)*
Ang II production	(pmol/min)	1.6 (0.8-5.1)	3.6 (0.5-7.3)	22 (12-31)*
Lungs				
Ang I production	(pmol/min)	0.2 (0.0-0.6)	0.3 (0.0-3.6)	0.7 (0.0-1.5)
Ang II production	(pmol/min)	0.8 (0.4-4.7)	1.0 (0.2-3.4)	7.4 (2.2-17)*
Myocardium				
Ang I production	(fmol/min)	39 (12-72)	319 (95-1,035)*	252 (79-741)*
Ang II production	(fmol/min)	9.1 (2.9-23)	19 (7.3-157)	151 (50-397)*

Data are median values and ranges. Calculations for combined systemic vascular beds and the myocardium were based on Equations 1 and 6, and for the lungs on Equations 3 and 8. Cardiac output of plasma was 1.71±0.28 l/min in the no treatment group, 1.79±0.43 l/min in captopril group, and 2.30±0.52 l/min in furosemide group (mean±SD). Left anterior descending coronary artery plasma flow, which was used for the calculations of Ang I and II production by myocardium, was 20.3±5.7 ml/min in no treatment group, 29.2±8.5 ml/min in captopril group, and 24.7±8.9 ml/min in furosemide group. Because total coronary plasma flow is larger than the left anterior descending coronary artery plasma flow, actual myocardial production rates will be larger than figures presented here. * Significantly different from no treatment group ($p<0.005$, Mann-Whitney U test for unpaired observations).

Ang I was derived from de novo production (mean±SD). In Table 2, the Ang I production rates in the combined systemic vascular beds, the lungs and the myocardium are given. Ang I production was increased about 10-fold in the captopril- and furosemide-treated pigs compared with the untreated pigs.

The Ang I extraction ratios across the combined systemic vascular beds in these groups of animals were not significantly different (Figure 3). As mentioned above, the arterial Ang I-to-PRA ratios in the various animal groups were also not different. The Ang I production rates were therefore linearly correlated with the circulating levels of PRA, and for a given level of PRA, the regional Ang I

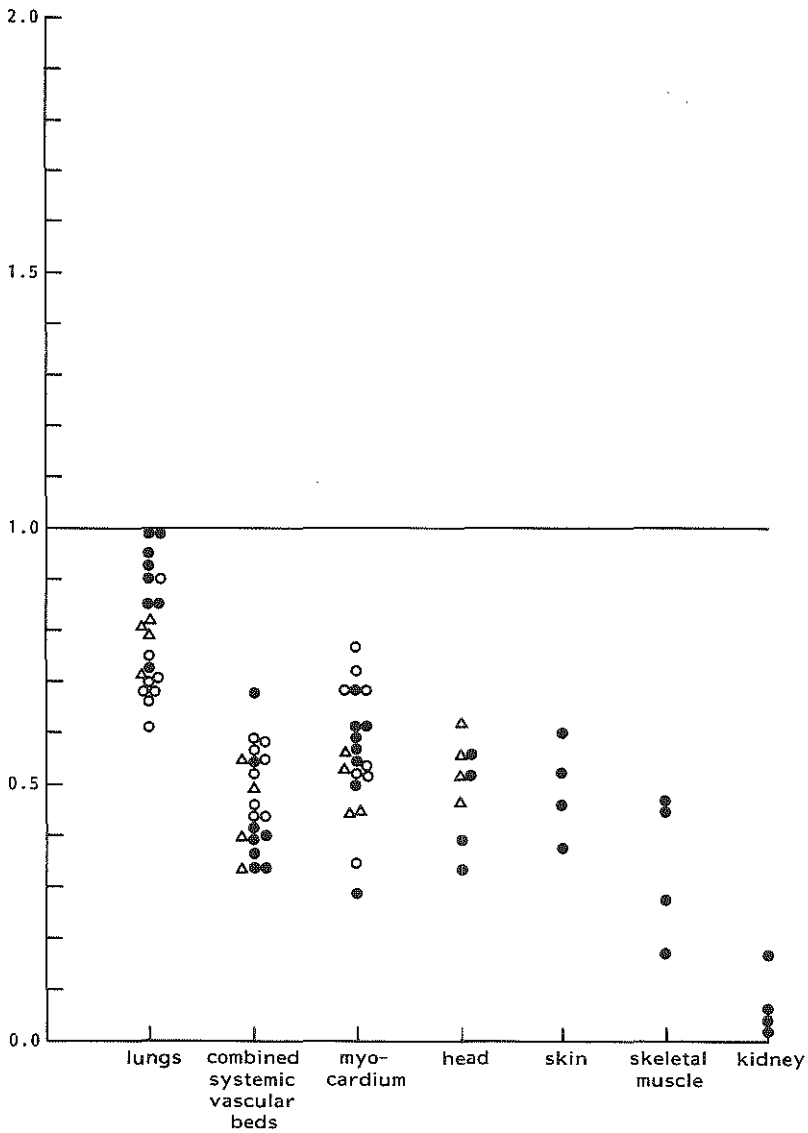
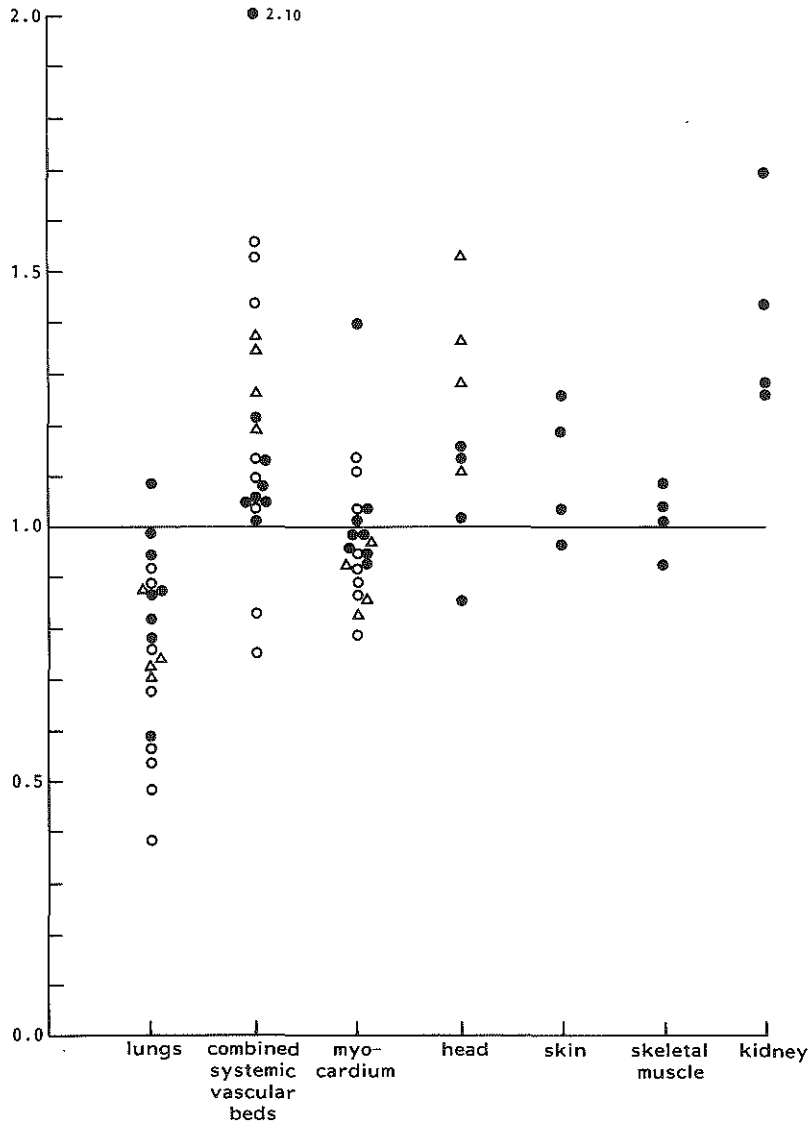


FIGURE 3
Calculated venous plasma levels of Ang I Ang I_{ven}) derived from arterially delivered Ang I (Ang I_{art}) (page 134) *vs. actually measured venous plasma levels of Ang I* (page 135) *in different*

Production of Angiotensins I and II

$(Ang\ I_{ven})/(Ang\ I_{art})$



vascular beds in untreated (○), captopril-treated (●) and furosemide-treated (Δ) pigs. Calculations were made on the basis of Equation 5. Venous Ang I levels are expressed as a fraction of the arterial level of Ang I.

Table 3
Percentage of the Total Regional Angiotensin I Production in Different Vascular Beds That Can Be Attributed to Circulating Plasma Renin Activity

Vascular bed	Ang I production by circulating PRA (% of total regional production)	
	NO CAPTOPRIL	CAPTOPRIL
Lungs	119±70	109±67
Combined systemic vascular beds	38±18*	31±17*
Myocardium	76±24*	64±40*
Head	44±14*	18±7*
Skin	-	16±4*
Skeletal muscle	-	11±5*
Kidney	-	2±2*

Data are mean±SD. No captopril group comprises both untreated and furosemide-treated pigs. Calculations were based on Equations 1, 3, and 4, assuming a blood transit time of 10 seconds in the systemic vascular beds and 3 seconds in the lungs (see "Results"). * Significantly different from 100 ($p<0.05$, Student's t test).

production rates in the untreated and furosemide-treated groups were not different from the production rate in the captopril-treated group.

When the Ang I production in the systemic vascular beds by circulating PRA (Equation 4) is expressed as a percentage of the total regional Ang I production (Equation 1), one does not need to know the regional plasma flow but one needs to know the blood transit time. Table 3 gives for a number of systemic vascular beds the percent contribution of circulating PRA to total regional Ang I production, assuming a blood transit time of 10 seconds.[15-17] Probably, the capillaries are the main site where Ang I is removed from the circulation by diffusion into the interstitium and peptidase attack. This is also the site where Ang I from the interstitium equilibrates with circulating Ang I. The blood transit times in the capillary beds is 1-3 seconds.[18] Our estimate of 10 seconds is therefore probably too high, but this overestimation strengthens the conclusion that a substantial part of Ang I production in the systemic vascular beds we studied could not be accounted for by circulating PRA. There was little Ang I production in the pulmonary vascular bed; the small amount that was produced could be accounted for by PRA assuming a pulmonary blood transit time of 3 seconds.

Regional Production of Angiotensin II

The rapid elimination of Ang II in the systemic vascular beds was compensated for by regional production of this peptide, with the kidney as an exception (Figure 4). The Ang II level in the renal vein was 60-70% of that in the artery. In the present study the renal extraction of Ang II and the arterial and venous levels of Ang II across the kidney were only measured in pigs on captopril treatment, but studies in humans also showed that the levels of Ang II in the renal vein are lower than those in the renal artery, both during captopril treatment and without such treatment.[2,19] There is also net Ang II extraction from the systemic circulation by the liver.[2] Since about 40% of the cardiac output flows through kidneys and liver, the net extraction by these organs of systemically delivered Ang II explains why the Ang II level in the right atrium was lower than in the aorta, despite the absence of an arteriovenous Ang II gradient across the other vascular beds.

In the lungs, in the combined systemic vascular beds, and in myocardium, head, skin, skeletal muscle and kidney, respectively 31±22, 39±19, 32±20, 49±8, 40±11, 59±15, and 85±8% of Ang II in venous plasma was derived from de novo production (mean±SD). In Table 2, the Ang II production rates in the combined systemic vascular beds, the lungs and the myocardium are given. The Ang II production rate in the combined systemic vascular beds was about 2 times higher than that in the lungs, which may illustrate the important contribution of extrapulmonary Ang I-II conversion to the whole body Ang II production.

Data on fractional Ang I-II conversion in the various systemic vascular beds we studied are presented in Table 4. On the basis of these data, the percent contribution of conversion of arterially delivered Ang I to the total regional production of Ang II was calculated. The results are shown in Table 5. They indicate that a substantial part of Ang II production in the systemic vascular beds could not be accounted for by conversion of arterially delivered Ang I. Ang II production in the lungs could fully be accounted for by the conversion of Ang I from the circulation.

Conversion in a vascular bed of Ang I generated by PRA during the transit of blood through that bed would also contribute to the regional de novo production of Ang II. Assuming that in a vascular bed the fractional conversion of this regionally produced Ang I is the same as the fractional conversion of Ang I that is arterially delivered to that bed, one can calculate the contribution of PRA to the total regional production of Ang II.

As shown in Table 5, this contribution of PRA is small and does not make up for the difference between total regional Ang II production and regional Ang II production from arterially delivered Ang I. Thus part of Ang II production in the circulation does not appear to originate from circulating Ang I; it may

(Ang II_{ven} derived from Ang II_{art} and from regional conversion of Ang I_{art})/(Ang II_{art})

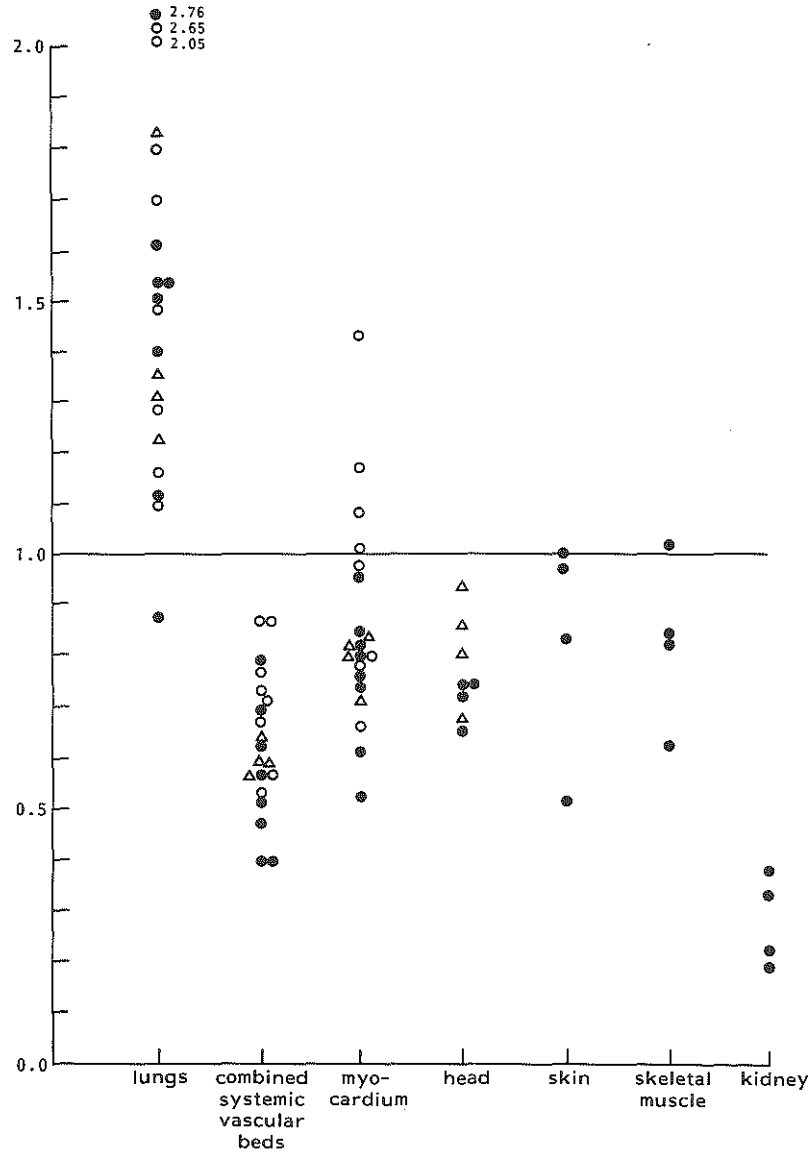
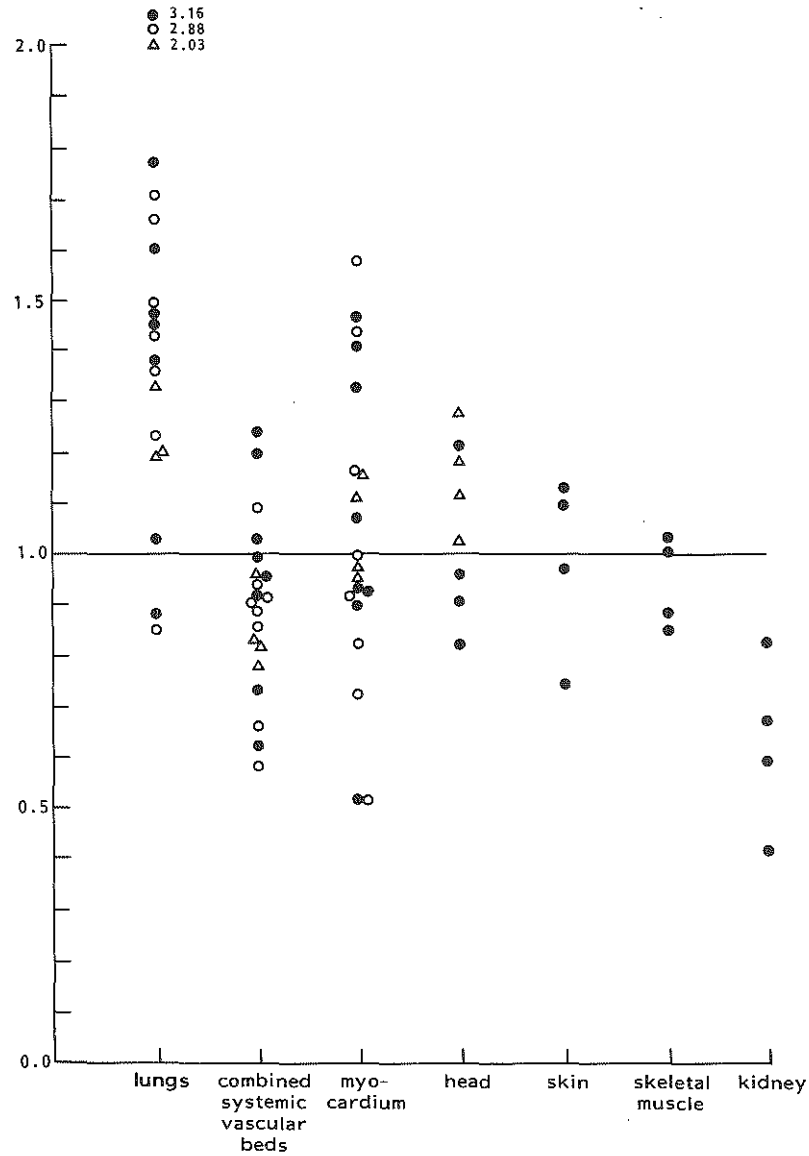


FIGURE 4
Calculated regional venous plasma levels of Ang II derived from arterially delivered Ang II and from conversion of arterially delivered Ang I (page 138) *vs. actually measured venous plasma levels of Ang II* (page 139) *in different vascular beds in untreated* (○), *captopril-treated* (●) *and*



furosemide-treated (Δ) pigs. Venous Ang II derived from arterially delivered Ang II and from regional conversion of arterially delivered Ang I was calculated on the basis of Equations 11 and 12. Venous Ang II levels are expressed as a fraction of arterial level of Ang II.

Table 4
Fractional Conversion of Arterially Delivered Angiotensin I in Different Vascular Beds

Vascular bed	NO TREATMENT	CAPTOPRIL	FUROSEMIDE
Lungs	0.25±0.07	0.04±0.04*	0.22±0.04
Combined systemic vascular beds	0.10±0.02	0.01±0.01*	0.12±0.04
Myocardium	0.17±0.02	0.02±0.02*	0.18±0.02
Head	-	0.04±0.02**	0.16±0.01
Skin	-	0.02±0.02	-
Skeletal muscle	-	0.07±0.02	-
Kidney	-	0.08±0.02	-

Data are mean±SD. Fractional conversion was calculated by using Equation 9.
* Significantly different from no treatment group and ** significantly different from furosemide group ($p<0.01$, Student's t test for unpaired observations).

originate from the conversion of Ang I produced at tissue sites.

Discussion

Estimates of Ang I and II production rates were made from measurements of the arterial and venous plasma levels of labeled Ang I and II as well as endogenous Ang I and II during constant infusion of ^{125}I-Ang I. ^{125}I-Ang I was infused directly into the left cardiac ventricle. We assumed that by this way of administration the route followed by exogenous Ang I resembled that of endogenous Ang I as closely as possible. ^{125}I-Ang I was extensively metabolized in all systemic vascular beds we studied.[12] However, the venous levels of endogenous Ang I were similar to or even higher than the arterial levels of Ang I. Venous Ang I is therefore largely derived from regional de novo Ang I production. By measuring the circulating levels of plasma renin activity (PRA) in conjunction with those of radiolabeled and endogenous Ang I we were able to calculate how much of the regional de novo production of Ang I was due to circulating PRA and how much was not. Our results indicated that a major part of the de novo production of Ang I in the systemic vascular beds we studied did not originate from the action of circulating renin on circulating angiotensinogen. These

Table 5
Percentage of Total Regional Angiotensin II Production in Different Vascular Beds That Can Be Attributed to Conversion of Circulating Angiotensin I

Vascular bed	Ang II production by conversion of circulating Ang I (% of total regional production)			
	NO CAPTOPRIL		CAPTOPRIL	
	production by conversion of arterially delivered Ang I	production by conversion of Ang I from PRA	production by conversion of arterially delivered Ang I	production by conversion of Ang I from PRA
Lungs	116±79	13±13*	117±73	10±16*
Combined systemic vascular beds	26±18*	10±4*	16±14*	6±5*
Myocardium	57±27*	17±22*	41±30*	18±13*
Head	35±3*	18±2*	33±7*	7±2*
Skin	-	-	38±27*	8±6*
Skeletal muscle	-	-	42±16*	9±5*
Kidney	-	-	12±4*	4±1*

Data are mean±SD in %. No captopril group comprises both untreated and furosemide-treated pigs. Ang II production by conversion of arterially delivered Ang I was calculated with Equation 10. Ang II production by conversion of Ang I from PRA was calculated by multiplying regional Ang I production by PRA (Equation 4) with fractional conversion of arterially delivered Ang I (data presented in Table 4). Blood transit times were assumed to be 10 seconds in systemic vascular beds and 3 seconds in lungs (see "Results"). * Significantly different from 100 ($p<0.05$, Student's t test).

calculations were based on a blood transit time of 10 seconds. As mentioned under "Results", this leads to overestimation of the contribution of PRA, but it only strengthens our conclusion that PRA could not account for the de novo Ang I production. This appeared to be the case not only in the kidneys but also in a number of other vascular beds.

The evidence for local Ang I production, i.e. Ang I production not in circulating blood, which was obtained in pigs treated with the ACE inhibitor captopril as well as in untreated animals, confirms our earlier observations in humans, all on captopril.[2] Some untreated pigs had plasma Ang I levels as low as

two to three times the detection limit of the assay. We therefore also studied a group of furosemide-treated animals. Results in these animals confirmed the conclusions drawn from the measurements in the untreated group.

The local production of Ang I in extrarenal tissues, i.e. Ang I production not due to circulating PRA, may still depend on the presence of kidney-derived renin in vascular tissue rather than on in situ synthesized renin.[6,7] In bilaterally nephrectomized patients the plasma levels of renin and Ang I are known to be very low.[20-22] The amount of Ang I entering plasma from tissue sites in these patients may therefore be expected to be low as well. Thus kidney-derived renin may indeed be responsible for most of the extrarenal production of Ang I at tissue sites, at least for that fraction of Ang I in the tissues that equilibrates with circulating Ang I. In accordance with this conclusion is the fact that the spontaneous release of angiotensins by isolated perfused organs is very low (rat hindquarter)[23] or even undetectable (rat heart).[24] Perfusion of rat hindquarters and heart with renin results in a dose-dependent release of Ang I and II.[23,24]

Our calculations are based on the so-called venous equilibrium model for the intrinsic clearance of exogenous and endogenous substances by tissues.[14] This model considers the distribution volume of these substances to be a single, well-stirred compartment; their concentration in this compartment equals that in the venous effluent. The volume of blood present in the conduit vessels is not taken into consideration. The implicit assumption in our calculations is therefore that the arterial and venous plasma levels of the radiolabeled and endogenous angiotensins we measured are representative for the levels at the arterial and venous side of the microcirculation. This assumption is probably valid, because there is little metabolism of angiotensins in the large vessels and because PRA may be expected to cause only small gradients in Ang I and Ang II along the large vessels. Indeed, in a study in humans we found no differences in the levels of ^{125}I-Ang I, ^{125}I-Ang II, and Ang I and Ang II between blood samples taken deeply from the femoral vein and from the iliac vein and inferior caval vein just under the entrance of the renal veins (unpublished results). We also found no differences in plasma levels between the ascending aorta and the femoral artery in captopril-treated pigs (data not shown).

From our data it could be calculated that not only ^{125}I-Ang I but also ^{125}I-Ang II was rapidly metabolized in the systemic vascular beds we studied. Again, as for Ang I, the venous levels of Ang II were similar to the arterial levels, indicating that Ang II is regionally produced. The lungs are an important site of Ang II production, but it is of some interest that the rate of de novo production of Ang II in the combined systemic vascular beds was higher than in the lungs. Ang II production in the pulmonary and the combined systemic vascular beds in the captopril-treated pigs was not lower than in the untreated pigs, despite the fact

that Ang I-II conversion was greatly reduced. Thus, the inhibition of Ang I-II conversion by captopril was overcome by increased Ang I production.

Regional de novo production of Ang II may be due to conversion of circulating Ang I, i.e. Ang I that is arterially delivered or Ang I that is regionally produced by PRA. De novo production of Ang II in the lungs could be fully attributed to conversion of circulating Ang I. In contrast, a substantial part of the de novo production of Ang II in the systemic vascular beds we studied did not originate from the conversion of circulating Ang I. In the kidneys by far the most of the de novo produced Ang II does not seem to originate from circulating Ang I. Ang I-II conversion is probably the main pathway of Ang II production. In the systemic vascular beds some of the Ang II production, and in the kidneys probably most of it, may therefore originate from the conversion of Ang I produced at tissue sites. However, alternative pathways for Ang II generation (e.g. direct cleavage from angiotensinogen) have been described.[25]

Whereas our results do provide evidence for the local formation of Ang I and Ang II in a number of tissues and for the release of these locally formed angiotensins into the circulation, the results do not militate against the fact that most of the circulating Ang I is formed by the action of circulating renin on circulating angiotensinogen and that most of the circulating Ang II is derived from circulating Ang I. Our calculations of Ang I and II production at tissue sites did not include the production of these peptides in the blood of the large arteries and veins. The volume of blood in the microcirculation of the tissues is only a small fraction of the total blood volume in the body. The total amount of circulating renin in the large arteries and veins is therefore much greater than in the microcirculation, and its contribution to the whole body production of Ang I is therefore also much greater. It should also be stressed that our calculations of Ang I and II production in tissues only accounts for that fraction of these peptides that rapidly equilibrates between tissue and plasma. Thus in fact the total Ang I and II production may be higher than our calculations would indicate. The local formation of both Ang I and II in tissues may have important physiological implications, in that it may serve to generate sufficiently high angiotensin concentrations at tissue receptor sites, concentrations that could well be higher than in the circulation.

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Chapter 8

Summary and Conclusion

This thesis describes a number of studies which were intended to contribute to our knowledge and understanding of the renin-angiotensin system. The studies were an attempt to assess the metabolism and production of Ang I and Ang II in various regional vascular beds of intact humans and animals. This was accomplished by using Iodine-125-labeled Ang I as a tracer of endogenous Ang I.

Studies in humans

In the first study, ^{125}I-Ang I was infused intravenously into subjects with hypertension. This experimental set-up allows the measurement of angiotensin metabolism (conversion and degradation) in various vascular beds, and the determination of the overflow of locally synthesized angiotensins into the systemic circulation.

In chapter 2 the methodology for the simultaneous determination of endogenous Ang I and II and of ^{125}I-Ang I and II is described. In order to validate the use of ^{125}I-Ang I as a tracer for endogenous Ang I, combined infusions of ^{125}I-Ang I and unlabeled Ang I were given to captopril-treated subjects with essential hypertension. The regional extraction ratios across the forearm, the leg, the kidney, and the liver and gut, and the elimination half-time of the two peptides were similar. Therefore the tracer appears to be suited to study the in vivo metabolism of endogenous Ang I, at least during ACE inhibition. Chapter 2 then deals with the regional metabolism of Ang I (mainly degradation), and the production of Ang I in captopril-treated subjects with essential hypertension. Ang I is extensively metabolized in the various vascular beds, and it could be shown that the rate of Ang I generation within the plasma, that is PRA, is too low to make up for the rapid metabolism of Ang I in the tissues. Thus, it appears that a major fraction of Ang I in venous plasma is formed outside the bloodstream by local Ang I-generating mechanisms.

Chapter 3 deals with the metabolism and production of Ang I in each of the two kidneys of captopril-treated subjects with unilateral renal artery stenosis. The most important finding from this study is that in the kidney affected by renal artery stenosis not only the secretion of renin into the circulation is stimulated but also the releases of Ang I. In contrast, in the unaffected kidney both the secretion

of renin into the circulation and the release of Ang I are suppressed. The data show that a major fraction of the Ang I in the renal veins is produced intrarenally at tissue sites and that the release of this locally produced Ang I into the circulation is mainly determined by the amount of in situ synthesized renin.

In chapter 4 experiments are described which were performed in untreated subjects with unilateral renal artery stenosis or essential hypertension. These experiments were intended to quantify Ang I as well as Ang II generation, and Ang I and II degradation, and Ang I-II conversion in the forearm, the leg, the kidney, and the liver and gut. The metabolism (conversion and degradation) of ^{125}I-Ang I and unlabeled Ang I was compared, by giving combined infusions of ^{125}I-Ang I and unlabeled Ang I. Results showed that the rate of Ang I-II conversion was lower than the rate of ^{125}I-Ang I-II conversion. The degradation rates of ^{125}I-Ang I and unlabeled Ang I were similar. In vitro experiments with tissue ACE, purified from human cadaver kidney, also showed a lower conversion rate for unlabeled Ang I than for ^{125}I-Ang I. To calculate the in vivo metabolism of endogenous Ang I, the lower rate of conversion of unlabeled Ang I had to be taken into account. In untreated subjects a major fraction of the regional Ang I production in the limbs and in the kidney could not be accounted for by circulating PRA alone, and appears to occur outside the circulating blood. In the forearm and in the leg extensive Ang I-II conversion was found, and our data emphasize that Ang I-II conversion in extrapulmonary vascular beds is an important source of circulating Ang II. The regional Ang II production in the limbs could be accounted for almost fully, by the regional conversion of arterially delivered Ang I. In contrast, little or no conversion of arterially delivered Ang I was detected in the renal and in the hepatomesenteric vascular beds. It was further shown that the kidneys release little or no Ang II into the circulation, which is in sharp contrast with Ang I. This was true for kidneys affected by renal artery stenosis and for unaffected kidneys. These results are a clear illustration of the high degree of compartmentalization of Ang I and Ang II production in renal tissue.

Chapter 5 deals with ^{125}I-Ang I infusion experiments in subjects with unilateral renal artery stenosis and essential hypertension, who were treated with the diuretic furosemide. These experiments were carried out to assess the effect of stimulation of renal renin secretion on the regional metabolism and production of Ang I and II. The regional extraction, and the fractional conversion and degradation of ^{125}I-Ang I were not altered by furosemide. In the limbs the release of regionally produced Ang I and II into the circulation was stimulated almost twofold by furosemide. A major portion of this release could not be accounted for by circulating PRA. The Ang I release from the limbs however was strictly proportional to the level of circulating PRA; the regression line of

the correlation between the two parameters intersects both the X- and Y-axis close to the origin. Thus, it appears that the release of Ang I from extrarenal vascular beds depends mainly on kidney-derived renin. For a major part regional Ang II production in the limbs could be accounted for by the conversion of arterially delivered Ang I. In the kidney Ang I production was also stimulated during furosemide treatment, but Ang II production remained invariably low. This again illustrates the high degree of compartmentalization of Ang I and II production in the kidney.

Studies in pigs

In these studies ^{125}I-Ang I was infused directly into the left cardiac ventricle. Because the cardiac output was measured during the infusion period, it was possible not only to determine the overflow of locally synthesized angiotensins into the circulation, but also to estimate the clearance rate and the production rate of Ang I and II in the combined systemic vascular beds and in the pulmonary vascular bed.

In the first part of chapter 6 the validation of the use of the radiolabeled tracer is described. In pigs the conversion rate of unlabeled Ang I was also slower than the conversion rate of ^{125}I-Ang I, whereas the degradation rates of unlabeled Ang I and ^{125}I-Ang I were similar. In the second part of chapter 6 the choice of an appropriate model is discussed that fits our results and can be used to calculate the clearance of Ang I in the various vascular beds. Our results indicate that in the combined systemic vascular beds Ang I is cleared from a single well-stirred compartment that is larger than the blood compartment and that the clearance of Ang I is best described by the 'venous equilibrium' model, whereas in the pulmonary vascular bed Ang I is cleared mainly from the blood compartment.

In chapter 7 experiments on the regional production of Ang I and II in pigs with non-stimulated and with stimulated renin production are described. Renin production was stimulated by treatment with the ACE inhibitor captopril or with the diuretic furosemide. As compared to untreated pigs, the regional production of Ang I in the combined systemic vascular beds was increased by both types of treatment. Ang I production could not be accounted for by Ang I generation by circulating PRA alone, and Ang I production appeared not to be confined to the blood compartment. Ang II production in the combined systemic vascular beds was higher than in the lungs, demonstrating the importance of Ang I-II conversion in extrapulmonary vascular beds. In the lungs there was virtually no production of Ang I, but considerable production of Ang II, which could be fully accounted for by the conversion of arterially delivered Ang I.

Conclusion

Finally, considering the results of our studies together, we conclude that in humans as well as in animals generation of Ang I and II occurs not only within the blood stream but at tissue sites as well. The formation of Ang I at tissue sites in extrarenal vascular beds appears to depend mainly on kidney-derived renin. This kidney-derived renin might be bound to the blood vessel wall or might have been taken up by the tissues. In the limbs conversion of arterially delivered Ang I contributes significantly to the level of Ang II in the regional veins. In the kidney in situ synthesized renin is an important determinant of the release into the circulation of Ang I that is locally formed at tissue sites. Little or no of this locally produced Ang I reaches the renal veins in the form of Ang II. Considering the high levels of Ang II in renal tissue, it may be concluded from our results that the intrarenal production of Ang I and Ang II is highly compartmentalized. Whereas a substantial fraction of circulating Ang I appears not to be derived from circulating PRA, most if not all, circulating Ang II appears to be derived from circulating Ang I. Thus, the function of the circulating renin-angiotensin system is not only to deliver Ang I to the sites of Ang I-II conversion and to transport the so-formed Ang II via the bloodstream to the target tissues but also to deliver renin to the tissues for local Ang I formation. Part of this locally formed Ang I is released into the circulation and is then transported to sites of Ang I-II conversion. It is possible that part of the locally formed Ang I is converted in situ in the tissues but, if so, little of the so-formed Ang II appears to reach the circulation.

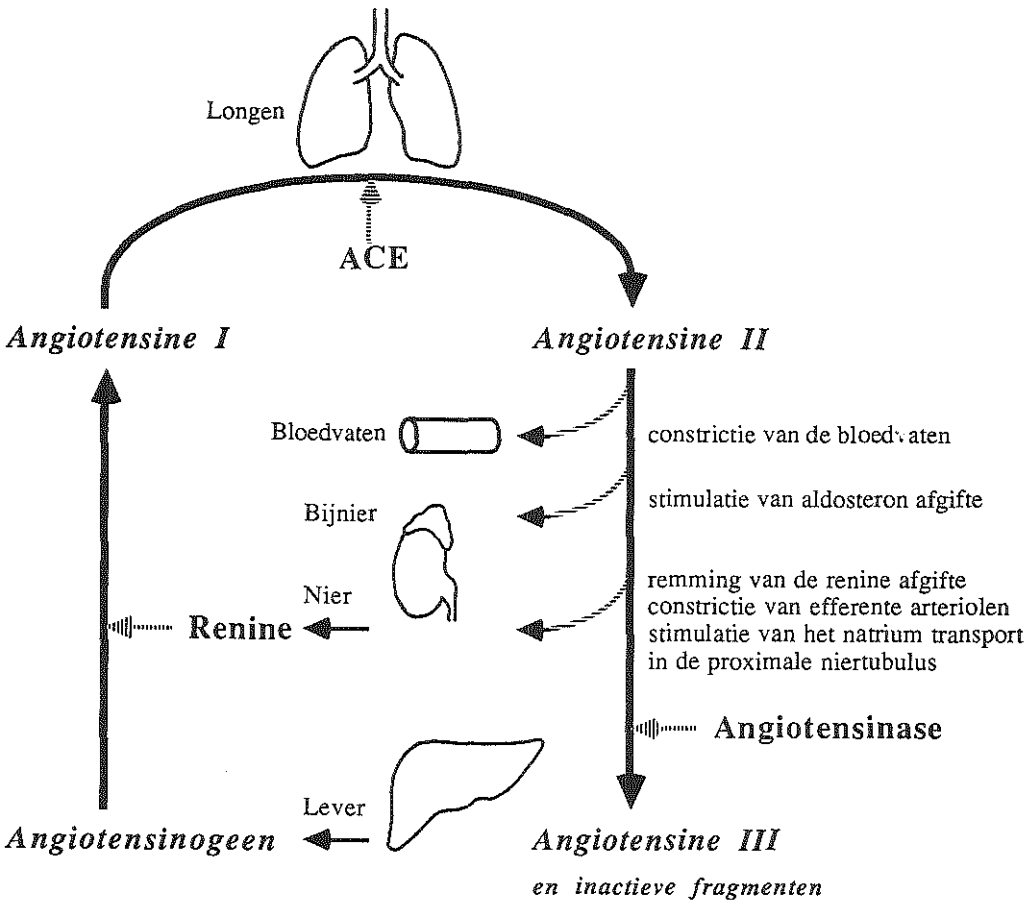
Chapter 9

Uitleg voor de leek

In dit proefschrift worden een aantal experimenten beschreven die zijn bedoeld om onze kennis van het renine-angiotensine systeem te vergroten. Dit systeem vervult een belangrijke rol bij de regulatie van de bloeddruk. Het renine-angiotensine systeem bestaat uit een aantal componenten (Figuur 1). Door de nier kan *renine* aan het bloed worden afgegeven. Renine is een enzym, een biologische katalysator. Renine knipt van *angiotensinogeen*, een door de lever gemaakt eiwit dat in het bloed circuleert, een stukje af. Dit stukje, *angiotensine I (Ang I)*, bezit geen biologische activiteit, maar het kan door het enzym *angiotensine I-converterend enzym* (ACE) worden omgezet in *angiotensine II* (Ang II). Deze omzetting, die voornamelijk in de longen en in mindere mate aan de wand van bloedvaten plaatsvindt, wordt conversie genoemd. Ang II bezit wel biologische activiteit. Het verhoogt de bloeddruk door vernauwing van de bloedvaten en het is nauw betrokken bij de water- en zouthuishouding van het lichaam. Ang II is een heel potente stof, d.w.z. al in een heel lage concentratie Ang II heeft een bloeddrukverhogend effect. De concentratie van zo'n sterk werkzame stof in het bloed mag niet te hoog worden. Daarom wordt Ang II (en Ang I) snel weer door andere enzymen, *angiotensinases*, afgebroken. Deze afbraak wordt degradatie genoemd. De conversie van Ang I in Ang II kan met bepaalde bloeddrukverlagende medicijnen, de zogenaamde ACE remmers, worden geblokkeerd. De door ons gebruikte stof captopril is zo'n ACE remmer.

Na de ontdekking van het renine-angiotensine systeem heeft men een tijd lang gedacht dat Ang I en Ang II alleen in het circulerende bloed werden gevormd. De laatste jaren zijn er steeds meer aanwijzingen gevonden dat deze gedachte niet geheel juist is. De in dit proefschrift beschreven experimenten zijn uitgevoerd om te kijken of ook buiten het circulerende bloed, in de weefsels, angiotensines worden gevormd. De experimenten, die deels bij mensen en deels bij varkens zijn uitgevoerd, hebben gemeen dat de afbraak en de productie van Ang I en Ang II wordt bestudeerd. Hiertoe is gebruik gemaakt van radioactieve merkstoffen; Jodium-125-gelabeld angiotensine I en II (^{125}I-Ang I en ^{125}I-Ang II).

In de hoofdstukken 2 tot en met 5 worden de experimenten beschreven, die zijn uitgevoerd bij patiënten met essentiële hypertensie (d.w.z. een verhoogde bloeddruk waarvoor geen eenduidige oorzaak kan worden gevonden) en bij



FIGUUR 1
Schematisch overzicht van het renine-angiotensine systeem. ACE, angiotensine I-converterend enzym.

patiënten met een verhoogde bloeddruk die wordt veroorzaakt door een vernauwing van één van de nierslagaders. In de hoofdstukken 6 en 7 worden de experimenten bij varkens beschreven.

Experimenten bij mensen

Bij de patiënten met hoge bloeddruk is de merkstof ^{125}I-Ang I toegediend door middel van infusie in de ader (vene) van de rechter onderarm. Tijdens de infusie van ^{125}I-Ang I zijn bloedmonsters afgenomen uit de grote lichaamsslag-

ader (aorta) en uit de aders van de linker onderarm, van het been, van de lever, en van de beide nieren. De concentratie van het lichaamseigen Ang I en de concentratie van ^{125}I-Ang I in de verschillende monsters is gemeten. Door het meten van de concentratie van ^{125}I-Ang I in zowel arterieel (uit de slagader) als veneus bloed (uit de ader), kan de mate worden bepaald waarin ^{125}I-Ang I (en dus ook Ang I) wordt afgebroken tijdens de passage van het bloed door een lichaamsdeel. Hieruit, en uit de gemeten concentraties van het lichaamseigen Ang I, kan de totale hoeveelheid Ang I worden berekend die in een bepaald lichaamsdeel wordt geproduceerd. De Ang I productiesnelheid in het bloedplasma per se, de "plasma renine activiteit" (PRA), is ook gemeten. Met een eenvoudig aftreksommetje kan nu worden berekend welk deel van de totale Ang I productie in het circulerende bloed en welk deel buiten de bloedbaan, in de weefsels, plaatsvindt.

In hoofdstuk 2 wordt de scheidings- en de bepalingsmethode van het lichaamseigen Ang I en Ang II en van het radioactief gemerkte ^{125}I-Ang I en ^{125}I-Ang II beschreven. Verder zijn experimenten uitgevoerd om de degradatie (afbraak) van het lichaamseigen Ang I en van ^{125}I-Ang I te vergelijken. Het blijkt dat beide stoffen even snel worden afgebroken. De merkstof ^{125}I-Ang I kan dus woden gebruikt om de degradatie van het lichaamseigen Ang I te meten. Vervolgens wordt in hoofdstuk 2 een eerste studie beschreven naar de vorming van Ang I bij patiënten met essentiële hoge bloeddruk die zijn behandeld met de ACE remmer captopril. De resultaten laten zien dat Ang I niet alleen in het circulerende bloed, maar gedeeltelijk ook buiten de bloedbaan wordt gevormd.

In hoofdstuk 3 wordt een soortgelijke studie beschreven bij patiënten die lijden aan een vernauwing van één nierslagader. Deze patiënten zijn ook behandeld met captopril. Het belangrijkste resultaat van deze experimenten is dat een nier die is aangedaan door een vernauwing van de nierslagader niet alleen renine, maar ook Ang I in verhoogde mate produceert en aan het bloed afgeeft. In de andere nier, die niet is aangedaan door een vernauwing van de nierslagader, is niet alleen de afgifte van renine aan het bloed onderdrukt, maar is ook de productie en de afgifte van Ang I sterk verminderd. De resultaten suggereren ten eerste dat een groot deel van de Ang I productie in de nier plaatsvindt in een compartiment dat zich buiten de bloedbaan bevindt, en ten tweede dat de nier de productie van Ang I in dit compartiment en/of de mate van afgifte van Ang I vanuit dit compartiment kan beïnvloeden.

In hoofdstuk 4 worden experimenten beschreven die zijn uitgevoerd bij patiënten met essentiële hoge bloeddruk en bij patiënten met een vernauwing van één nierslagader. Deze patiënten zijn niet behandeld met een ACE remmer. De productie van Ang I en Ang II, de degradatie van Ang I en Ang II, en de conversie van Ang I naar Ang II, is bestudeerd. Ook bij de niet behandelde patiënten is het metabolisme van de radioactieve merkstof ^{125}I-Ang I vergeleken

met het metabolisme van het lichaamseigen Ang I. Het blijkt dat de degradatie van het lichaamseigen Ang I en van ^{125}I-Ang I even snel verloopt, maar dat de conversiesnelheid van het lichaamseigen Ang I naar Ang II lager is dan de conversiesnelheid van ^{125}I-Ang I naar ^{125}I-Ang II. Bij de berekening van de degradatie en de conversie van het lichaamseigen Ang I is met dit verschil rekening gehouden. Net als bij de patiënten die zijn behandeld met een ACE remmer, wordt ook bij de niet behandelde patiënten een groot deel van het Ang I geproduceerd buiten het circulerende bloed. Verder blijkt dat in de onderarm en in het been 20-30% van het door de slagader aangevoerde Ang I ter plaatse wordt omgezet in Ang II, terwijl in de nieren en in de lever en de darmen weinig tot geen conversie van Ang I naar Ang II wordt gevonden. Dus in de ledematen worden zowel Ang I èn Ang II lokaal gevormd. Door de nieren daarentegen wordt wèl Ang I geproduceerd en wordt het gevormde Ang I afgegeven aan het bloed, maar wordt vrijwel géén Ang II uitgescheiden.

In hoofdstuk 5 worden experimenten beschreven die een vervolg zijn op de studie uit hoofdstuk 4. In deze vervolgstudie zijn twee soortgelijke groepen patiënten bestudeerd die voorafgaande het experiment zijn behandeld met het diureticum (plasmiddel) furosemide. Het diureticum stimuleert de afgifte van renine uit de nier. De mate van conversie en degradatie van angiotensines in met de furosemide behandelde patiënten blijkt niet door het diureticum te worden veranderd. De regionale Ang I productie in de ledematen en in de nieren verschilt wel, en is ongeveer tweemaal groter dan in de onbehandelde patiënten. De regionale Ang I productie in de met furosemide behandelde patiënten, is net als in de onbehandelde of met captopril behandelde patiënten veel groter dan de Ang I productie in het bloedplasma (PRA), maar wel strikt gecorreleerd met de PRA. Dit is een duidelijke aanwijzing dat de regionale Ang I productie in de ledematen practisch geheel afhankelijk is van renine, dat door de nier is geproduceerd en dat door de weefsels vanuit het bloed wordt opgenomen. De regionale Ang II productie in de ledematen van de met furosemide behandelde patiënten is ook verhoogd. Daarentegen is de Ang II afgifte door de nieren, net als in de onbehandelde patiënten, zeer laag.

Experimenten bij varkens

Bij de varkens is ^{125}I-Ang I toegediend door middel van infusie in de linker hartkamer. In de linker hartkamer wordt ^{125}I-Ang I direct goed met het bloed gemengd. Tijdens de infusie is bloed afgenomen uit de aorta, uit de beide hartboezems, uit de longslagader, en uit de aders van de nieren, van het oor, van de achterpoot, van het hoofd, en van de centrale ader van het hart. Gedurende de infusieperiode is ook het debiet van het hart, de "cardiac output", gemeten. Hierdoor is het mogelijk om de productie- en de klaringssnelheid van Ang I en

Ang II te berekenen zowel voor het longvaatbed als voor de rest van het lichaam.

In het eerste deel van hoofdstuk 6 wordt de validering van de geïnfundeerde merkstof, ^{125}I-Ang I, bij varkens beschreven. Net als bij de mens verloopt de degradatie van het lichaamseigen Ang I en van ^{125}I-Ang I bij de varkens even snel, maar de conversie van het lichaamseigen Ang I naar Ang II is aanmerkelijk langzamer dan de conversie van ^{125}I-Ang I naar ^{125}I-Ang II. In het tweede deel van hoofdstuk 6 wordt aandacht besteed aan de keuze van een model waarmee de klaring van Ang I vanuit het bloed kan worden beschreven. De zeer hoge klaringssnelheid, en het verloop van de Ang I concentratie na stopzetten van het ^{125}I-Ang I infuus, duiden erop dat Ang I zich in het lichaam verdeelt over een volume (compartiment) dat groter is dan het bloedvolume. De klaring van Ang I in de longen geschiedt praktisch alleen vanuit het circulerende bloed. De klaring van Ang I in de rest van het lichaam, waar Ang I zich verdeelt over een groot compartiment, beter kan worden beschreven met het zogenaamde "venous equilibrium" model.

In hoofdstuk 7 wordt de productie van Ang I en Ang II beschreven bij niet-behandelde varkens en bij varkens waarbij de renine secretie door de nier is gestimuleerd, òf door behandeling met de ACE remmer captopril òf door behandeling met het diureticum furosemide. De productie van Ang I in de behandelde varkens is duidelijk hoger dan in de niet-behandelde dieren. De productie van Ang II daarentegen wordt geremd door captopril en wordt gestimuleerd door furosemide. In de longen wordt weinig Ang I, maar relatief veel Ang II gevormd. De Ang I productie in het longvaatbed kan geheel worden verklaard door Ang I generatie in het bloed (PRA). In de rest van het lichaam wordt Ang I vooral buiten de bloedbaan geproduceerd. De Ang II productie in de longen kan worden verklaard door conversie van circulerend Ang I. De Ang II productie in de rest van het lichaam kan niet geheel worden verklaard door de conversie van circulerend Ang I. Een deel van deze Ang II productie moet dan ook worden toegeschreven aan de conversie van Ang I dat lokaal in de weefsels is geproduceerd.

Samenvattend kan worden vastgesteld dat angiotensine I en II niet alleen in het circulerende bloed maar ook in de weefsels worden geproduceerd, zowel bij de mens als bij proefdieren.

Wat is nu het nut van dit onderzoek voor de behandeling van de zieke medemens?

Op de korte termijn zullen de resultaten, zoals ze zijn beschreven in dit proefschrift, mijns inziens niet leiden tot een verbetering van de behandeling van hoge bloeddruk. De verdienste van het werk lijkt te zijn dat het onze kennis van

het renine-angiotensine systeem een klein beetje heeft vergroot. Hierdoor kunnen in de toekomst misschien geneesmiddelen worden ontwikkeld die selectief de lokale angiotensine productie in de nier, in de wand van de bloedvaten, of in het hart, kunnen beïnvloeden. Wellicht dat met zulke geneesmiddelen de behandeling van hoge bloeddruk of hartfalen kan worden verbeterd.

Verantwoording

Ik wil graag iedereen bedanken die heeft bijgedragen aan het tot stand komen van dit proefschrift. Op de omslag van een proefschrift staat alleen de naam van de promovendus vermeld, dat is fraai, maar het mag in de volgende regels duidelijk worden dat zonder de infrastructuur van de afdeling Inwendige Geneeskunde I en zonder de inzet en de steun van vele mensen dit proefschrift niet voltooid had kunnen worden.

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Curriculum Vitae

De schrijver van dit proefschrift werd geboren op 21 december 1954 te Bergen (NH). Na het behalen van het diploma Atheneum-ß aan de Rijksscholengemeenschap "Noord-Kennemerland" in Alkmaar in 1973, begon hij in datzelfde jaar de studie farmacie aan de Universiteit van Amsterdam. Na het afronden van deze studie in 1982 werkte hij twee jaar als apotheker in Apotheek "de Groote Gaper" in Hoorn (hoofd: Drs. P.G.N. Rood). In 1984 werd begonnen met de studie scheikunde aan de Universiteit van Amsterdam. Het doktoraal examen, met biochemie als hoofdvak en microbiologie als bijvak, werd afgelegd in november 1986. Van maart 1987 tot heden is hij verbonden aan de afdeling Inwendige Geneeskunde I (hoofd: Prof. Dr. M.A.D.H. Schalekamp) van het Academisch Ziekenhuis Dijkzigt in Rotterdam.

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