

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Angiotensin Production by the Heart : A Quantitative Study in Pigs With the Use of Radiolabeled Angiotensin Infusions

Jorge P. van Kats, A. H. Jan Danser, Jan R. van Meegen, Loes M. A. Sassen, Pieter D. Verdouw and Maarten A. D. H. Schalekamp

Circulation 1998;98;73-81

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214
Copyright © 1998 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online
ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/cgi/content/full/98/1/73>

Subscriptions: Information about subscribing to *Circulation* is online at
<http://circ.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore, MD 21202-2436. Phone 410-5280-4050. Fax: 410-528-8550. Email:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/static/html/reprints.html>

Angiotensin Production by the Heart

A Quantitative Study in Pigs With the Use of Radiolabeled Angiotensin Infusions

Jorge P. van Kats, PhD; A.H. Jan Danser, PhD; Jan R. van Meegen, BSc;
Loes M.A. Sassen, MD, PhD; Pieter D. Verdouw, PhD; Maarten A.D.H. Schalekamp, MD, PhD

Background—Beneficial effects of ACE inhibitors on the heart may be mediated by decreased cardiac angiotensin II (Ang II) production.

Methods and Results—To determine whether cardiac Ang I and Ang II are produced in situ or derived from the circulation, we infused ^{125}I -labeled Ang I or II into pigs (25 to 30 kg) and measured ^{125}I -Ang I and II as well as endogenous Ang I and II in cardiac tissue and blood plasma. In untreated pigs, the tissue Ang II concentration (per gram wet weight) in different parts of the heart was 5 times the concentration (per milliliter) in plasma, and the tissue Ang I concentration was 75% of the plasma Ang I concentration. Tissue ^{125}I -Ang II during ^{125}I -Ang II infusion was 75% of ^{125}I -Ang II in arterial plasma, whereas tissue ^{125}I -Ang I during ^{125}I -Ang I infusion was <4% of ^{125}I -Ang I in arterial plasma. After treatment with the ACE inhibitor captopril (25 mg twice daily), Ang II fell in plasma but not in tissue, and Ang I and renin rose both in plasma and tissue, whereas angiotensinogen did not change in plasma and fell in tissue. Tissue ^{125}I -Ang II derived by conversion from arterially delivered ^{125}I -Ang I fell from 23% to <2% of ^{125}I -Ang I in arterial plasma.

Conclusions—Most of the cardiac Ang II appears to be produced at tissue sites by conversion of in situ-synthesized rather than blood-derived Ang I. Our study also indicates that under certain experimental conditions, the heart can maintain its Ang II production, whereas the production of circulating Ang II is effectively suppressed. (*Circulation*. 1998;98:73-81.)

Key Words: angiotensin ■ ACE inhibitors ■ renin

From experimental and clinical studies, there is evidence to suggest that the long-term beneficial effects of ACE inhibitor drugs on postinfarction cardiac failure and remodeling as well as on left ventricular hypertrophy are at least partly independent of their systemic effect on blood pressure.^{1,2} A reduction of Ang II production locally in the heart may explain the blood pressure-independent effects of ACE inhibition in cardiac patients.

Indeed, the heart contains all components required for Ang I and Ang II production, ie, renin, angiotensinogen, and ACE.³ Renin mRNA may be present in the heart in low concentrations,⁴⁻⁷ but observations on the effect of bilateral nephrectomy in pigs demonstrated that most, if not all, renin in the heart is derived from the kidney, at least under normal conditions.³ Angiotensinogen gene expression is also low in the normal heart,^{7,8} and experiments using the isolated perfused rat heart seem to indicate that the heart produces little Ang I and II when angiotensinogen is not added to the perfusion fluid.⁹ In contrast, the synthesis of ACE in the normal heart is an established fact.¹⁰⁻¹³ Angiotensinogen and

ACE gene expression may be upregulated under pathological conditions.^{8,11,13}

The study reported here focuses on the normal heart and is carried out in pigs. It addresses the following questions: (1) How much of the Ang I and II in cardiac tissue is derived from the circulation? (2) How much of the cardiac Ang II is synthesized locally by the conversion of blood-derived Ang I and how much by the conversion of Ang I that is formed locally in the heart? and (3) What is the effect of ACE inhibitor treatment on cardiac angiotensin production?

Methods

Animals

All experiments were performed under the regulations of the Animal Care Committee of the Erasmus University, Rotterdam, The Netherlands, in accordance with the "Guiding Principles in the Care and Use of Animals" as approved by the American Physiological Society. Twenty-four female pigs (crossbred Yorkshire×Landrace, Hedelse Varkens Combinatie, Hedel, The Netherlands) with a body weight of 25 to 30 kg were included in the study. Ten animals were pretreated with the ACE inhibitor captopril, 25 mg twice daily for 3

Received September 4, 1997; revision received December 1, 1997; accepted January 14, 1998.

From the Departments of Internal Medicine (J.P.v.K., M.A.D.H.S.), Experimental Cardiology (J.P.v.K., J.R.v.M., L.M.A.S., P.D.V.), and Pharmacology (A.H.J.D.), Cardiovascular Research Institute Erasmus University Rotterdam (COEUR), the Netherlands.

Correspondence to A.H.J. Danser, PhD, Department of Pharmacology, Room EE 1418 B, Erasmus University Rotterdam, Dr Molewaterplein 50, 3015 GE Rotterdam, Netherlands.

E-mail danser@farma.fgg.eur.nl

© 1998 American Heart Association, Inc.

Selected Abbreviations and Acronyms

Ang I = angiotensin I
 Ang II = angiotensin II
 AT₁ = type 1 Ang II receptor
 HPLC = high-performance liquid chromatography

days,¹⁴ for study of the effect of ACE inhibition on cardiac Ang I and II production. The experiments were carried out 3 to 4 hours after the last dose of captopril.

Instrumentation of the Animals

The animals were prepared for hemodynamic measurements, for administration of anesthetic and ¹²⁵I-labeled angiotensins, and for blood and tissue sampling as described previously.^{3,14–16} After a stabilization period of 30 to 45 minutes after completion of instrumentation, baseline measurements of systemic hemodynamic variables were made, and blood samples were collected for the determination of blood gases. The animals were then subjected to constant infusions of either ¹²⁵I-Ang I or ¹²⁵I-Ang II.

Preparation of ¹²⁵I-Labeled Angiotensins

[Ile⁵]-Ang-(1–10) decapeptide (Ang I) and [Ile⁵]-Ang-(1–8) octapeptide (Ang II) (Bachem) were used to prepare monoiodinated ¹²⁵I-Ang I and II. The chloramine-T method was used for ¹²⁵I-labeling, and the radiolabeled peptides were purified as described previously.¹⁷ The specific radioactivity of the ¹²⁵I-Ang I and ¹²⁵I-Ang II preparations was $\approx 3.6 \times 10^6$ cpm/pmol.

Infusion of ¹²⁵I-Labeled Angiotensins

¹²⁵I-Ang I or ¹²⁵I-Ang II was administered via constant infusion into the left cardiac ventricle for 15, 60, or 120 minutes. The infusion rate was $\approx 4 \times 10^6$ cpm/min for ¹²⁵I-Ang I and $\approx 3 \times 10^6$ cpm/min for ¹²⁵I-Ang II. The levels of ¹²⁵I-Ang I and ¹²⁵I-Ang II reached their steady-state maximum within 10 minutes in plasma¹⁴ and within 60 minutes in cardiac tissue.¹⁶ Seven untreated and 7 captopril-treated pigs received a ¹²⁵I-Ang I infusion for 15 minutes. Three untreated and 3 captopril-treated pigs received a ¹²⁵I-Ang I infusion for 60 minutes. Four untreated pigs received a ¹²⁵I-Ang II infusion for 60 minutes. The number of 60-minute infusion experiments was kept at a minimum to reduce the total quantity of radiolabeled angiotensin required for the infusion. Measurements of the steady-state plasma levels of ¹²⁵I-Ang I and II were made in both the 15-minute and 60-minute experiments. Measurements of the steady-state tissue levels of ¹²⁵I-Ang I and II were made in the 60-minute infusion experiments only.

Collection of Blood and Cardiac Tissue Samples

Blood samples (10 mL) were taken from the aorta and great cardiac vein during infusion of ¹²⁵I-Ang I or II to measure the plasma levels of ¹²⁵I-Ang I and II and endogenous Ang I and II. The blood was rapidly drawn with a plastic syringe containing the following inhibitors (0.5 mL inhibitor solution in 10 mL blood): 0.01 mmol/L of the renin inhibitor remikiren (a kind gift of Dr W. Fischli, Hoffmann-LaRoche, Basel, Switzerland), 6.25 mmol/L disodium EDTA (Synthalyse), and 1.25 mmol/L 1,10-ortho-phenanthroline (Merck) (final concentrations in blood). The blood was immediately transferred into prechilled polystyrene tubes and centrifuged at 3000g for 10 minutes at 4°C. Plasma was stored at –70°C and assayed within 3 days.

Aortic blood samples (5 mL) for measurements of renin and angiotensinogen were collected in polystyrene tubes containing disodium citrate (0.1 mL in 5 mL blood; final concentration, 13 mmol/L). The samples were centrifuged at 1000g for 10 minutes at room temperature, and plasma was stored at –70°C.

Cardiac tissue was collected as follows. The heart was stopped by fibrillation while the ¹²⁵I-Ang I or II infusion was still running. The heart was quickly removed from the body, and pieces weighing 1 to

2 g were quickly excised from the left and right atria and from the left and right ventricular free wall. The tissue pieces were immediately transferred into liquid nitrogen. The tissue was frozen within 15 seconds after the heart had been stopped.

To study the ex vivo metabolism of endogenous angiotensins in cardiac tissue, the remaining part of the left ventricular free wall was kept at 37°C. Pieces of left ventricular tissue were then cut off and frozen in liquid nitrogen at various time points after the heart had been stopped. The frozen tissues were stored at –70°C and assayed within 3 days.

Measurements of ¹²⁵I-Labeled and Endogenous Angiotensins in Cardiac Tissue and Blood Plasma

Frozen tissue samples were homogenized in 20 mL ice-cold 0.1 mol/L HCl/80% ethanol as previously described.^{3,16} The homogenate was centrifuged at 20 000g for 25 minutes at 4°C. Ethanol in the supernatant was evaporated under constant air flow, and the remainder of the supernatant was diluted in 20 mL 1% ortho-phosphoric acid and centrifuged again at 20 000g. The supernatant was diluted with an equal volume of 1% ortho-phosphoric acid and then concentrated on Sep-Pak cartridges. Plasma was applied directly to the Sep-Pak cartridges.

The preparation of the Sep-Pak extracts for HPLC separation of the angiotensins and the HPLC procedure have been described elsewhere.¹⁶ The concentrations of intact ¹²⁵I-Ang I and ¹²⁵I-Ang II and the concentrations of intact Ang I and Ang II in the HPLC eluate fractions were measured by gamma counting and radioimmunoassay, respectively.¹⁷ Data were not corrected for losses that occurred during extraction and separation. These losses were <10% in plasma and maximally 20% to 30% in tissue extracts.¹⁶ In some plasma samples, the angiotensin levels were below the limit of detection. These were taken to be equal to the lower limit of detection (1.0 fmol/mL for Ang I and 0.5 fmol/mL for Ang II) to allow calculation of mean values.

Measurements of Renin and Angiotensinogen in Cardiac Tissue and Blood Plasma

Frozen tissue samples were homogenized (1:3, weight:volume) in 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl as previously described.³ Homogenates used for renin measurements were pretreated with acid to remove angiotensinase activity.³ In short, 1 mL of homogenate was dialyzed for 48 hours at 4°C against 0.05 mol/L glycine buffer, pH 3.3, containing 0.095 mol/L NaCl.³ This was followed by dialysis at 4°C for 24 hours against 0.1 mol/L phosphate buffer, pH 7.4, containing 0.075 mol/L NaCl. The content of the dialysis bags was then collected, and the volume was adjusted to 1 mL with phosphate buffer.

The concentration of renin in acid-pretreated cardiac tissue extract and in non-acid-pretreated plasma was determined by the enzyme-kinetic assay, which measures the rate of Ang I generation at pH 7.4 during incubation at 37°C with a saturating amount of porcine renin substrate, in the presence of inhibitors of angiotensinases, ACE, and serine proteases.³ The Ang I-generating activity of cardiac tissue extracts measured in the absence of the renin inhibitor remikiren minus the Ang I-generating activity in the presence of remikiren (final concentration, 10^{–5} mol/L) was taken as a measure of the concentration of renin. Inhibition of porcine renin is virtually complete at this concentration of remikiren.³ Any remaining Ang I-generating activity was assumed to be caused by enzymes other than renin. Plasma had no Ang I-generating activity in the presence of 10^{–5} mol/L remikiren.

The concentration of angiotensinogen was determined in non-acid-pretreated cardiac tissue extracts and plasma. It was measured as the maximum quantity of Ang I that was generated during incubation at 37°C with a high concentration of porcine kidney renin in the presence of inhibitors of angiotensinases and ACE.³

Calculations

The possible sources of Ang I and II in cardiac tissue are shown in Figure 1 and summarized in Table 1. The level of arterially delivered

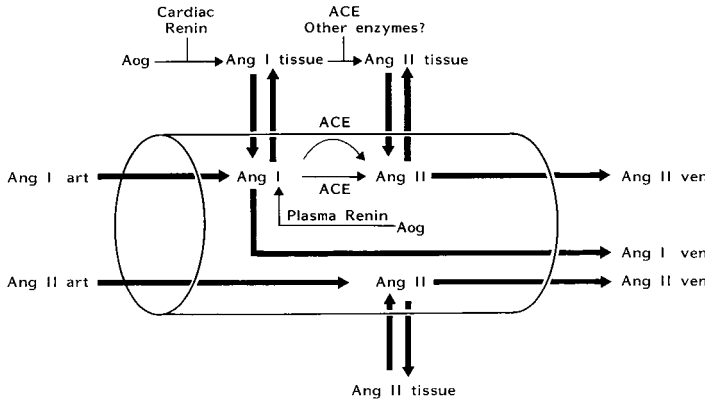


Figure 1. Schematic of sources of Ang I and II in cardiac tissue and coronary venous (ven) plasma. Ang I-to-II conversion in plasma and conversion on endothelial surface are indicated separately. Aog indicates angiotensinogen; art, arterial.

Ang II in cardiac tissue was calculated as

$$(1) \quad [\text{Ang II}_{\text{tissue}} \text{ from Ang II}_{\text{art}}] = R_1 \times [\text{Ang II}_{\text{art}}]$$

where $[\text{Ang II}_{\text{tissue}} \text{ from Ang II}_{\text{art}}]$ is the Ang II concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang II, $[\text{Ang II}_{\text{art}}]$ is the Ang II concentration in aortic blood plasma (fmol/mL), and R_1 is given by

$$(2) \quad R_1 = [^{125}\text{I-Ang II}_{\text{tissue}}] / [^{125}\text{I-Ang II}_{\text{art}}]$$

where $[^{125}\text{I-Ang II}_{\text{tissue}}]$ and $[^{125}\text{I-Ang II}_{\text{art}}]$ are the steady-state concentrations of $^{125}\text{I-Ang II}$ in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, both during the constant infusion of $^{125}\text{I-Ang II}$.

The level of arterially delivered Ang I in cardiac tissue was calculated as follows:

$$(3) \quad [\text{Ang I}_{\text{tissue}} \text{ from Ang I}_{\text{art}}] = R_2 \times [\text{Ang I}_{\text{art}}]$$

where $[\text{Ang I}_{\text{tissue}} \text{ from Ang I}_{\text{art}}]$ is the Ang I concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang I, $[\text{Ang I}_{\text{art}}]$ is the Ang I concentration in aortic blood plasma (fmol/mL), and R_2 is given by

$$(4) \quad R_2 = [^{125}\text{I-Ang I}_{\text{tissue}}] / [^{125}\text{I-Ang I}_{\text{art}}]$$

where $[^{125}\text{I-Ang I}_{\text{tissue}}]$ and $[^{125}\text{I-Ang I}_{\text{art}}]$ are the steady-state concentrations of $^{125}\text{I-Ang I}$ in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, both during the constant infusion of $^{125}\text{I-Ang I}$.

The cardiac tissue level of Ang II that is derived by conversion from arterially delivered Ang I was calculated as

$$(5) \quad [\text{Ang II}_{\text{tissue}} \text{ from Ang I}_{\text{art}}] = R_3 \times [\text{Ang I}_{\text{art}}]$$

where $[\text{Ang II}_{\text{tissue}} \text{ from Ang I}_{\text{art}}]$ is the Ang II concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang I, $[\text{Ang I}_{\text{art}}]$ is the Ang I concentration in aortic blood plasma (fmol/mL), and R_3 is given by

$$(6) \quad R_3 = ([^{125}\text{I-Ang II}_{\text{tissue}}] / [^{125}\text{I-Ang I}_{\text{art}}]) - (R_1 \times [^{125}\text{I-Ang II}_{\text{art}}] / [^{125}\text{I-Ang I}_{\text{art}}])$$

TABLE 1. Sources of Ang I and II in Cardiac Tissue

Ang I	Ang II
Ang I in coronary artery	Ang II in coronary artery
Ang I produced by the fluid-phase reaction of renin with angiotensinogen in the coronary circulation	Ang I in coronary artery Ang I produced by the fluid-phase reaction of renin with angiotensinogen in the coronary circulation
Ang I synthesized in situ in cardiac tissue	Ang I synthesized in situ in cardiac tissue

where $[^{125}\text{I-Ang II}_{\text{tissue}}]$ and $[^{125}\text{I-Ang II}_{\text{art}}]$ are the steady-state concentrations of $^{125}\text{I-Ang II}$ in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, and $[^{125}\text{I-Ang I}_{\text{art}}]$ is the steady-state concentration of $^{125}\text{I-Ang I}$ in aortic blood plasma (cpm/mL), all during the constant infusion of $^{125}\text{I-Ang I}$.

Some of the Ang I and II in cardiac tissue may originate from Ang I and II that is generated by the action of circulating renin with circulating angiotensinogen during the passage of blood from the arterial to the venous end of the coronary circulation. The Ang I-generating capacity of blood plasma was calculated as

$$(7) \quad \text{AGC}_{\text{plasma}} = [\text{Ren}] \times [\text{Aog}] / (K_m + [\text{Aog}])$$

where $\text{AGC}_{\text{plasma}}$ is the Ang I-generating capacity of plasma (fmol Ang I · mL⁻¹ · min⁻¹), $[\text{Ren}]$ is the renin concentration of plasma (fmol Ang I · mL⁻¹ · min⁻¹), $[\text{Aog}]$ is the angiotensinogen concentration of plasma (pmol/mL), and K_m is the Michaelis-Menten constant for the reaction of porcine renin with porcine angiotensinogen, which equals 420 pmol/mL.⁹ The cardiac tissue level of Ang I that originates from the Ang I-generating capacity of plasma was calculated as

$$(8) \quad [\text{Ang I}_{\text{tissue}} \text{ from AGC}_{\text{plasma}}] = R_2 / R'_2 \times \text{AGC}_{\text{plasma}} \times t$$

where $[\text{Ang I}_{\text{tissue}} \text{ from AGC}_{\text{plasma}}]$ is the Ang I concentration in cardiac tissue (fmol/g) originating from the Ang I-generating capacity of plasma, t is the coronary blood transit time, which equals 0.1 minute,¹⁸ R_2 is given by Equation 4, and R'_2 is given by

$$(9) \quad R'_2 = [^{125}\text{I-Ang I}_{\text{ven}}] / [^{125}\text{I-Ang I}_{\text{art}}]$$

where $[^{125}\text{I-Ang I}_{\text{ven}}]$ and $[^{125}\text{I-Ang I}_{\text{art}}]$ are the steady-state concentrations (cpm/mL) of $^{125}\text{I-Ang I}$ in coronary venous and aortic blood plasma, respectively, both during the constant infusion of $^{125}\text{I-Ang I}$. In Equation 8, it is assumed that the Ang I in tissue originating from the Ang I-generating capacity of blood plasma is not subject to conversion and degradation by the vascular endothelium. If, however, this Ang I is exposed to the endothelial peptidases, in the same way as arterially delivered Ang I, the factor R_2 / R'_2 in Equation 8 has to be substituted by R_2 . In reality, the fractional conversion and degradation of plasma Ang I generated in the coronary circulation are probably lower than the fractional conversion and degradation of arterially delivered Ang I. If the R_2 -to- R'_2 ratio is taken, Equation 8 gives a maximum value of $[\text{Ang I}_{\text{tissue}} \text{ from AGC}_{\text{plasma}}]$.

The cardiac tissue level of Ang II that originates from the Ang I-generating capacity of plasma was calculated as

$$(10) \quad [\text{Ang II}_{\text{tissue}} \text{ from AGC}_{\text{plasma}}] = (R'_3 \times R_2 / R'_2) \times \text{AGC}_{\text{plasma}} \times t$$

where R'_3 is given by

$$(11) \quad R'_3 = ([^{125}\text{I-Ang II}_{\text{ven}}] / [^{125}\text{I-Ang I}_{\text{art}}]) - (R'_1 \times [^{125}\text{I-Ang II}_{\text{art}}] / [^{125}\text{I-Ang I}_{\text{art}}])$$

In Equation 11, $[^{125}\text{I-Ang II}_{\text{ven}}]$ and $[^{125}\text{I-Ang II}_{\text{art}}]$ are the steady-state concentrations (cpm/mL) of $^{125}\text{I-Ang II}$ in coronary venous and aortic

TABLE 2. Baseline Hemodynamic Parameters and Steady-State Arterial Plasma Levels of ¹²⁵I-Labeled Ang I and II During Infusion of ¹²⁵I-Ang I or II in Untreated and Captopril-Treated Pigs

Treatment Group	Mean Arterial Pressure, mm Hg	Heart Rate, bpm	Cardiac Output, L/min	¹²⁵ I-Ang I Concentration, cpm/mL	¹²⁵ I-Ang II Concentration, cpm/mL	Ratio of ¹²⁵ I-Ang II to I
¹²⁵ I-Ang I infusion						
Control (n=10)	86±12	123±12	2.5±0.5	1350±380	960±390	0.71±0.22
Captopril (n=10)	85±4	112±15	2.7±0.6	1960±720*	240±210*	0.11±0.04*
¹²⁵ I-Ang II infusion						
Control (n=4)	78±13	110±15	2.3±0.7	...	1960±530	...

The plasma levels of ¹²⁵I-Ang I and II presented here were measured in blood samples taken from the aorta at the end of a 10-minute infusion period. Data are mean±SD.

**P*<0.01 for difference from results in control animals receiving ¹²⁵I-Ang I infusion (unpaired Student's *t* test).

blood plasma, respectively, and [¹²⁵I-Ang I_{art}] is the steady-state concentration (cpm/mL) of ¹²⁵I-Ang I in aortic blood plasma, all during the constant infusion of ¹²⁵I-Ang I. R'₁ is given by

$$(12) \quad R'_1 = \frac{[^{125}\text{I-Ang II}_{\text{ven}}]}{[^{125}\text{I-Ang II}_{\text{art}}]}$$

where [¹²⁵I-Ang II_{ven}] and [¹²⁵I-Ang II_{art}] are the steady-state concentrations (cpm/mL) of ¹²⁵I-Ang II in coronary venous and aortic blood plasma, respectively, both during the constant infusion of ¹²⁵I-Ang II. Equation 10 gives a maximum value of [Ang II_{tissue} from AGC_{plasma}], for the same reasons as for Equation 8.

Statistical Analysis

Data are expressed as mean±SD except when indicated otherwise. Differences in plasma levels of renin-angiotensin system components between untreated and captopril-treated pigs were tested by Student's *t* test. Differences in cardiac tissue levels of renin-angiotensin system components between untreated and captopril-treated pigs were tested by MANOVA. Statistical significance was accepted for *P*<0.05.

Results

Hemodynamic Effects of ¹²⁵I-Labeled Angiotensins

Baseline heart rate, cardiac output, and mean arterial pressure did not differ between untreated and captopril-treated pigs (Table 2). Infusions of either ¹²⁵I-Ang I or ¹²⁵I-Ang II did not

affect any of these parameters (data not shown), which is in agreement with previous studies.^{14,16,17}

¹²⁵I-Labeled Angiotensins in Cardiac Tissue and Blood Plasma

The steady-state levels of ¹²⁵I-Ang I and II in aortic plasma during constant infusion of ¹²⁵I-Ang I or II are shown in Table 2. ¹²⁵I-Ang II in plasma was lower and ¹²⁵I-Ang I was higher in the captopril-treated pigs than in the controls. The ratio of ¹²⁵I-Ang II to I in plasma, which is a measure of the degree of ACE inhibition, fell from 0.71 to 0.11 after captopril.

Table 3 gives the cardiac tissue and coronary venous plasma concentrations of Ang I and II derived from arterially delivered Ang I or II, expressed as a fraction, R, of the Ang I or II concentration in arterial blood plasma. The R values were calculated from the steady-state ¹²⁵I-Ang I and II levels in cardiac tissue (cpm/g), coronary venous plasma (cpm/mL), and aortic plasma (cpm/mL) during ¹²⁵I-Ang I or II infusions.

After captopril treatment, the coronary venous plasma concentration of ¹²⁵I-Ang II that is derived by conversion from arterially delivered ¹²⁵I-Ang I fell from 32% to 4% of the ¹²⁵I-Ang I concentration in arterial plasma. This demonstrates effective ACE inhibition in the coronary vascular bed.

TABLE 3. Cardiac Tissue and Coronary Venous Plasma Concentrations of Ang I and II Derived From Arterially Delivered Ang I or II, Expressed as a Fraction, R, of the Ang I or II Concentration in Arterial Plasma

	Cardiac Tissue			Coronary Venous Plasma		
	R ₁	R ₂	R ₃	R' ₁	R' ₂	R' ₃
Control	0.75 (0.71–0.81) (n=3)	<0.04 (n=3)	0.23 (0.12–0.37) (n=3)	0.52±0.17 (n=4)	0.41±0.14 (n=10)	0.32±0.22 (n=10)
Captopril	ND	<0.02 (n=3)	<0.02 (n=3)	ND	0.58±0.15* (n=10)	0.04±0.05* (n=10)

ND indicates not done. R₁ through R₃ are given by Equations 2, 4, and 6 and R'₁ through R'₃ are given by Equations 9, 11, and 12. The R and R' values were calculated from the ¹²⁵I-Ang I and II levels in cardiac left ventricular tissue, coronary venous blood plasma, and aortic blood plasma during ¹²⁵I-Ang I or II infusions. R₁ and R'₁ represent the concentrations of Ang II in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang II and expressed as a fraction of the concentration of Ang II in aortic plasma. R₂ and R'₂ represent the concentrations of Ang I in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang I and expressed as a fraction of the concentration of Ang I in aortic plasma. R₃ and R'₃ represent the concentrations of Ang II in cardiac left ventricular tissue and coronary venous plasma, respectively, derived by conversion from arterially delivered Ang I and expressed as a fraction of the concentration of Ang I in aortic plasma. Data are mean±SD or means and ranges.

**P*<0.01 for difference from results in control animals (unpaired Student's *t* test).

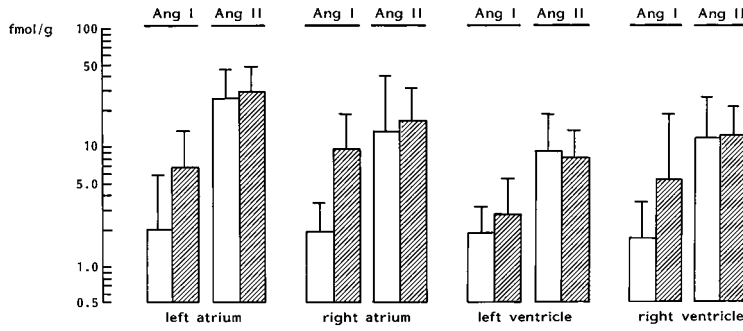


Figure 2. Ang I and II levels in cardiac tissue of untreated (open bars, n=10) and captopril-treated (hatched bars, n=10) pigs. Values are geometric means and SD. Ang I levels in the two groups of animals are significantly different ($P < 0.01$, MANOVA).

In the control group, the ^{125}I -Ang II concentration in cardiac tissue during ^{125}I -Ang II infusion was 75% of its concentration in arterial plasma. In contrast, the cardiac tissue concentration of ^{125}I -Ang I during ^{125}I -Ang I infusion was <4% of its concentration in arterial plasma. The tissue concentration of ^{125}I -Ang II that was derived by conversion from arterially delivered ^{125}I -Ang I was 23% of the ^{125}I -Ang I concentration in arterial plasma. This percentage was much lower, <2%, in the captopril-treated group, which is again an indication of effective blockade of conversion of Ang I to II.

Endogenous Angiotensins in Cardiac Tissue and Plasma

The levels of endogenous Ang I and II in cardiac tissue and blood plasma are shown in Figures 2 and 3. Plasma Ang I and II in untreated pigs were within the normal range.¹⁹ No significant differences were observed between aortic and coronary venous plasma.

The tissue levels in the various parts of the heart were not significantly different in either the captopril-treated or untreated pigs, but there were marked differences between tissue and plasma. The tissue concentration of Ang II in the untreated group was ≈ 5 times the plasma concentration of Ang II. In addition, the tissue concentration of Ang II was >5 times the concentration of Ang I, whereas in plasma, Ang II was lower than Ang I.

Ang I rose after captopril both in tissue and in plasma. Ang II in tissue did not change after captopril, whereas in plasma, Ang II fell to values close to the detection limit of the assay.

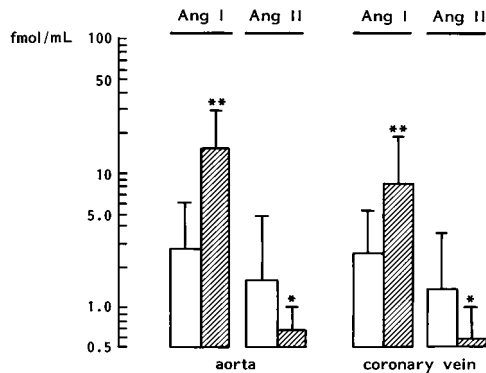


Figure 3. Ang I and II levels in blood plasma of untreated (open bars, n=14) and captopril-treated (hatched bars, n=10) pigs. Values are geometric means and SD. * $P < 0.05$, ** $P < 0.01$ for difference from control animals (unpaired Student's *t* test).

Routinely, as described in the "Methods" section, Ang I and II were measured in cardiac tissue that was frozen within 15 seconds after the heart had been stopped and removed from the body. When cardiac tissue was kept at 37°C for 1 hour after the heart had been removed from the body, the tissue levels of Ang I and II were virtually constant during this period, and they were similar to the routinely measured levels (Figure 4).

Renin and Angiotensinogen in Cardiac Tissue and Blood Plasma

The tissue levels of renin and angiotensinogen in the various parts of the heart were not significantly different in either the captopril-treated animals or in controls (Table 4). The tissue concentration of renin (expressed per gram tissue) was similar to the plasma concentration (expressed per milliliter). This suggests that the presence of renin in tissue is not restricted to the extracellular fluid compartment. In contrast, the tissue concentration of angiotensinogen (expressed per gram tissue) was 10% to 30% of the plasma concentration (expressed per milliliter).

As expected, the plasma and tissue levels of renin were higher in the captopril group than in controls. The plasma levels of angiotensinogen were not different between the two

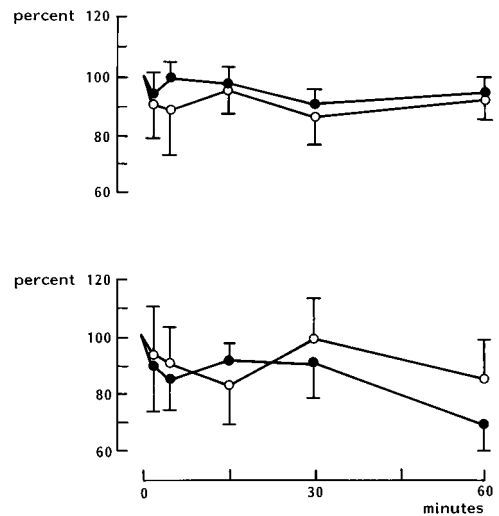


Figure 4. Ex vivo changes of Ang I (●) and II (○) at 37°C in cardiac tissue (left ventricular free wall) of untreated (top) and captopril-treated (bottom) pigs. Values (means and SD of 4 experiments) are expressed as percentage of level in tissue samples frozen within 15 seconds after removal of heart from body.

TABLE 4. Concentrations of Renin, Angiotensinogen, and the Ratio of Ang II to I Concentration in Cardiac Tissue and Arterial Blood Plasma in Untreated (n=14) and Captopril-Treated (n=10) Pigs

Treatment Group	Aortic Plasma	Left Atrium	Right Atrium	Left Ventricle	Right Ventricle
Renin, fmol Ang I · mL ⁻¹ · min ⁻¹ or fmol Ang I · g ⁻¹ · min ⁻¹					
Control	26±34	38±25	40±17	31±16	25±13
Captopril	78±39*	114±61‡	101±43‡	107±52‡	85±39‡
Angiotensinogen, pmol/mL or pmol/g					
Control	340±72	62±47	47±17	98±39	73±45
Captopril	339±103	41±25‡	30±6‡	60±42‡	44±27‡
Ang I-generating capacity, fmol Ang I · min ⁻¹ · mL					
Control	12±16				
Captopril	29±14*				
Ratio of Ang II to I					
Control	0.67±0.35	15.9±6.4	8.4±5.0	5.2±2.2	9.4±9.3
Captopril	0.06±0.07†	6.2±5.2‡	2.3±2.1‡	2.8±1.8‡	5.0±7.7‡

g indicates gram tissue wet weight. Data are mean±SD.

* $P<0.05$, † $P<0.01$ for difference from control animals (unpaired Student's *t* test).

‡Tissue concentrations of renin and angiotensinogen and the ratio of Ang II to I tissue concentration in the two groups of animals are significantly different ($P<0.01$, MANOVA).

groups, but the tissue levels of angiotensinogen were lower in the captopril group, which is an indication of increased substrate consumption due to elevated renin.

Contributions of Blood-Derived Angiotensins to the Angiotensin Levels in Cardiac Tissue

Figures 5 and 6 show the results for Ang I and II in left ventricular free wall tissue both in the captopril-treated pigs and in controls. It appears that >90% of the Ang I in tissue is synthesized in the tissue itself and is not derived from the circulation. Most of the Ang II in tissue is also synthesized in the tissue, and its source is Ang I synthesized in situ rather than Ang I from the circulation. The contribution of Ang I from the circulation to the cardiac tissue level of Ang II was

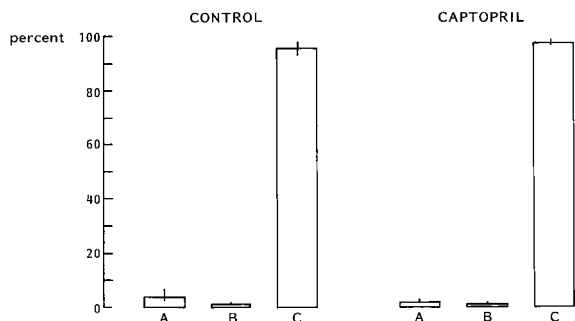


Figure 5. Contributions of various sources of Ang I to cardiac tissue (left ventricular free wall) level of this peptide in untreated and captopril-treated pigs. Values (means and ranges of 3 experiments) are percentage of total tissue level of Ang I. A, Arterially delivered Ang I; B, Ang I produced by Ang I-generating capacity of plasma in coronary circulation; C, Ang I synthesized in situ in cardiac tissue.

small in the control group and fell to nearly zero after captopril because of the blockade of Ang I-to-II conversion.

Discussion

In the present study, ¹²⁵I-Ang I and ¹²⁵I-Ang II were infused into the left cardiac ventricle to determine how much of the Ang I and II in cardiac tissue is derived from the circulation and how much is generated in situ. The results indicate that most, if not all, Ang I we measured in cardiac tissue was synthesized in situ and that most of the cardiac Ang II was produced by the conversion of this in situ-synthesized Ang I.

We used tracer doses of ¹²⁵I-Ang II to minimize the chance of a physiological effect. No hemodynamic response was detectable.^{14,16} The steady-state plasma level of ¹²⁵I-Ang II was ≈2000 cpm/mL (Table 2), which corresponded with a plasma concentration of ≈0.55 fmol/mL. The plasma level of endogenous Ang II was 2 fmol/mL. Thus, the ¹²⁵I-Ang II infusion caused an increase of the plasma Ang II concentration by only 25%. With the ¹²⁵I-Ang I infusions, the increase of plasma Ang II was even less. An important effect on AT₁ receptor density is therefore unlikely.

In our calculations, it was assumed that the fate of the radiolabeled angiotensins in cardiac tissue and the coronary circulation is comparable to that of arterially delivered nonlabeled angiotensins. Previous studies in pigs¹⁴ and humans²⁰ demonstrated that the ¹²⁵I-Ang I-to-II conversion rate, both in vitro and in vivo, is about two times the Ang I-to-II conversion rate and that the rates at which ¹²⁵I-Ang I and Ang I are degraded into peptides other than ¹²⁵I-Ang II and Ang II are not different. The ¹²⁵I-Ang II and Ang II degradation rates are also not different.²⁰ The fact that the ¹²⁵I-Ang I-to-II

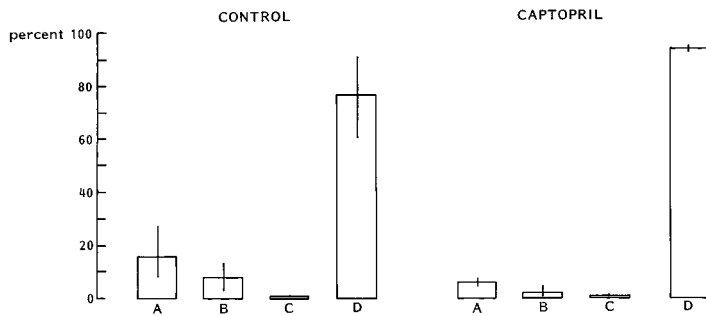


Figure 6. Contributions of various sources of Ang II to cardiac tissue (left ventricular free wall) level of this peptide in untreated and captopril-treated pigs. Values (means and ranges of 3 experiments) are percentage of total tissue level of Ang II. A, Arterially delivered Ang II; B, Ang II derived by conversion from arterially delivered Ang I; C, Ang II derived by conversion from Ang I produced by Ang I-generating capacity of plasma in coronary circulation; and D, Ang II derived by conversion from Ang I that is synthesized in situ in cardiac tissue.

conversion rate is somewhat higher than the Ang I-to-II conversion rate may have led us to overestimate the amount of Ang II in cardiac tissue that is derived from Ang I in the circulation. Therefore, this does not invalidate our conclusion that most of the Ang II in cardiac tissue is generated from in situ-synthesized rather than blood-derived Ang I.

An important methodological aspect of this study relates to the question of whether the radiolabeled and endogenous angiotensin levels we measured in cardiac tissue are representative of the levels in vivo. The finding that the ex vivo cardiac tissue levels of Ang I and II remained practically constant at 37°C lends support to the assumption that the measured levels are close to the in vivo levels. The ex vivo half life of ^{125}I -Ang II in cardiac tissue at 37°C is 30 to 40 minutes.¹⁶ Thus, the ^{125}I -Ang II level we measured in cardiac tissue that was frozen within 15 seconds after the ^{125}I -Ang I and II infusions had been stopped and the heart had been removed from the body is probably also close to the ^{125}I -Ang II level in vivo. Ex vivo production of Ang II may explain why endogenous cardiac Ang II, as opposed to radiolabeled Ang II, remained constant when cardiac tissue was kept at 37°C.

The tissue level of ^{125}I -Ang I was too low to determine the ex vivo half life of ^{125}I -Ang I. The ^{125}I -Ang I level we measured in cardiac tissue that was frozen within 15 seconds after ^{125}I -Ang I infusion had been stopped and the heart had been removed from the body was <4% of the plasma level in the aorta. The fact that the tissue level of ^{125}I -Ang I is probably only a small fraction of the level in arterial plasma in vivo as well is supported by the following considerations: (1) the rate of Ang I production in tissue ex vivo is probably not higher than in vivo, (2) the tissue concentration of Ang I we measured is close to the level in vivo, and (3) Ang I delivery by the aorta contributes to the tissue level of Ang I in vivo but not ex vivo. The third consideration implies that if the two other considerations are correct, arterially delivered Ang I will contribute little to its level in tissue.

Our finding that the tissue-to-plasma ratio of ^{125}I -Ang II during ^{125}I -Ang II infusion was much higher than the tissue-to-plasma ratio of ^{125}I -Ang I during ^{125}I -Ang I infusion may suggest that the two peptides are located in different tissue compartments. An earlier study¹⁶ in which we infused ^{125}I -Ang I and ^{125}I -Ang II into the left cardiac ventricle of pigs provided evidence that most of the ^{125}I -Ang II in cardiac tissue had been accumulated by the cells via an angiotensin AT_1 type receptor-mediated process. ^{125}I -Ang I does not bind to the AT_1 receptor and does not enter the cells via this

receptor, so the location of ^{125}I -Ang I in the tissue may be restricted to the extracellular compartment.

Studies with a modified rat Langendorff heart model, which allowed us to collect interstitial fluid transudate separately from the coronary effluent, showed that the Ang I concentration in interstitial fluid during perfusion of the heart with Ang I was only 10% to 20% of the Ang I concentration of the inflowing perfusion fluid.⁹ When this also holds for the ^{125}I -Ang I we infused into the pigs in the present experiments and when, as discussed above, ^{125}I -Ang I in cardiac tissue is restricted to the extracellular compartment, it is easy to understand why the cardiac tissue level of ^{125}I -Ang I we measured in the present study was so low.

The conclusion that ^{125}I -Ang I and therefore also the arterially delivered nonlabeled Ang I are localized in the extracellular compartment and that ^{125}I -Ang II and the arterially delivered nonlabeled Ang II are accumulated in the cells via binding to AT_1 receptors may also hold for Ang I that is synthesized at tissue sites and for Ang II that originates from in situ-synthesized Ang I. Further studies are needed to settle this issue.

Our observations in nephrectomized pigs demonstrated that at least in the healthy heart, most if not all of the cardiac Ang I and II is generated by blood-derived renin.³ The captopril-induced parallel increments in plasma renin and cardiac Ang I, as shown in the present study, support the assumption that also during captopril treatment, the cardiac production of Ang I and II depends on blood-derived renin. This, together with the evidence that most of the Ang I and II in cardiac tissue does not originate from the circulation but rather from local production, confirms the concept, already proposed by Loudon et al²¹ >10 years ago, that one of the principal functions of renin secretion by the kidney is to ensure the delivery of this enzyme to vascular tissues for the production of angiotensins in these tissues.

Recent evidence seems to indicate that binding sites for renin are present in the cell membrane fractions of rat cells from different organs, including the heart, blood vessels, and kidney.²²⁻²⁵ Also in the porcine heart, renin was found to be membrane bound.³ Cell membrane binding would be a mechanism by which renin from the circulation is accumulated at certain tissue sites, resulting in higher renin concentrations at these sites than in the circulating blood.

In view of the possibility that the beneficial effects of ACE inhibitor drugs on cardiac function and structure depend on their effect on cardiac Ang II production rather than on a decrease in circulating Ang II, it is of interest to note that in

our experiments, the cardiac tissue level of Ang II, as opposed to its level in plasma, did not fall after ACE inhibition by captopril. Other investigators, using various ACE inhibitor drugs in rats, observed a reduction in cardiac Ang II.^{26–29} However, these studies also indicated differences between the effects of ACE inhibitor treatment on Ang II production in the circulation and in tissues. Perindopril, for instance, caused a dose-dependent decrease in the Ang II-to-I concentration ratio in plasma, but at each dose, the effect on this ratio was greater in plasma than in cardiac tissue.²⁸ Quinapril lowered cardiac Ang II in rats with volume overload-induced cardiac hypertrophy but not in normal rats, whereas plasma Ang II was suppressed in both groups.²⁹ Results may therefore differ depending on the type and dose of ACE inhibitor and on whether the animals are studied under normal or pathological conditions.

In our experiments, the compensatory increase in Ang I production in the heart might have overcome the blockade of ACE. The increased tissue concentration of Ang I and the decreased tissue concentration of angiotensinogen, as observed in our study, are indications that the cardiac production of Ang I was indeed increased. It is also possible that captopril does not reach some of the tissue sites of Ang II production. Furthermore, enzymes other than ACE may be involved in the Ang I-to-II conversion in cardiac tissue, eg, chymase.³⁰ However, the role of enzymes other than ACE in cardiac Ang II production remains questionable.^{31,32}

The results of the present study, together with our recent observations on the AT₁ receptor-mediated cardiac uptake of Ang II and its long intracellular half life,¹⁶ also raise the interesting possibility that AT₁ receptor antagonism and ACE inhibition have different effects on the distribution of locally produced Ang II over the intracellular and extracellular cardiac tissue compartments. Both treatment modalities tend to reduce the number of AT₁ receptors that are occupied with Ang II, and in both cases this is counteracted by a compensatory response of stimulated renin and Ang I production. However, AT₁ receptor-mediated endocytosis of Ang II protects this peptide against the enzymes that degrade extracellular Ang II, so that during AT₁ receptor blockade a larger proportion of tissue Ang II is exposed to these enzymes. This is also supported by the finding that the ratio of cardiac Ang II to I concentration was decreased in rats by treatment with the AT₁ receptor antagonist losartan, which had no effect on cardiac ACE.³³ It is therefore possible that, for a given increment of Ang I, the tissue level of Ang II is more reduced by AT₁ receptor antagonist drugs than by ACE inhibitors. This may have clinical consequences, in light of growing evidence that the physiological responses to Ang II not only are mediated by signal transduction from cell surface-bound Ang II receptors but that intracellular Ang II also contributes to these responses.^{34–36}

Further studies of the effects of ACE inhibitors, AT₁ receptor antagonists, and renin inhibitors on the local production of Ang I and II in cardiac and vascular tissues along the lines of the present study will clarify the pathophysiological significance of Ang II production in these tissues and may

help to better define the place of these drugs in the management of heart failure and hypertension.

Acknowledgments

This study was supported by the Dutch Kidney Foundation research grant 96.1585. We thank Angelique Bouhuizen-van der Houwen and René de Bruin for their excellent technical assistance.

References

1. Linz W, Schaper J, Wiemer G, Albus U, Schölkens BA. Ramipril prevents left ventricular hypertrophy with myocardial fibrosis without blood pressure reduction: a one year study in rats. *Br J Pharmacol*. 1992; 107:970–975.
2. The SOLVD Investigators. Effects of the angiotensin-converting enzyme inhibitor enalapril on the long-term progression of left ventricular dilatation in patients with asymptomatic systolic dysfunction. *Circulation*. 1993;88:2277–2283.
3. Danser AHJ, van Kats JP, Admiraal PJJ, Derckx FHM, Lamers MJM, Verdouw PD, Saxena PR, Schalekamp MADH. Cardiac renin and angiotensins: uptake from plasma versus in situ synthesis. *Hypertension*. 1994; 24:37–48.
4. Ekker M, Tronik D, Rougeon F. Extrarenal transcription of the renin genes in multiple tissues of mice and rats. *Proc Natl Acad Sci U S A*. 1989;86:5155–5158.
5. Iwai N, Inagami T. Quantitative analysis of renin gene expression in extrarenal tissues by polymerase chain reaction method. *J Hypertens*. 1992;10:717–724.
6. Lou YK, Robinson BG, Morris BJ. Renin messenger RNA, detected by polymerase chain reaction, can be switched on in rat atrium. *J Hypertens*. 1993;11:237–243.
7. Passier RCJJ, Smits JFM, Verluyten MJA, Daemen MJAP. Expression and localization of renin and angiotensinogen in rat heart after myocardial infarction. *Am J Physiol*. 1996;271:H1040–H1048.
8. Lindpaintner K, Lu W, Niedermajer N, Schieffer B, Just H, Ganten D, Drexler H. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling of the rat. *J Mol Cell Cardiol*. 1993;25:133–143.
9. de Lannoy LM, Danser AHJ, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MADH. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart: local production of angiotensin I. *Hypertension*. 1997;29:1240–1251.
10. Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy: effects on coronary resistance, contractility, and relaxation. *J Clin Invest*. 1990;86: 1913–1920.
11. Sun Y, Cleutjens JPM, Diaz-Arias AA, Weber KT. Cardiac angiotensin-converting enzyme and myocardial fibrosis in the rat. *Cardiovasc Res*. 1994;28:1423–1432.
12. Falkenhahn M, Franke F, Bohle RM, Zhu Y-C, Stauss HM, Bachmann S, Danilov S, Unger T. Cellular distribution of angiotensin-converting enzyme after myocardial infarction. *Hypertension*. 1995;25:219–226.
13. Hokimoto S, Yasue H, Fujimoto K, Yamamoto H, Nakao K, Kaikita K, Sakata R, Miyamoto E. Expression of angiotensin-converting enzyme in remaining viable myocytes of human ventricles after myocardial infarction. *Circulation*. 1996;94:1513–1518.
14. Danser AHJ, Koning MMG, Admiraal PJJ, Derckx FHM, Verdouw PD, Schalekamp MADH. Metabolism of angiotensin I by different tissues in the intact animal. *Am J Physiol*. 1992;263:H418–H428.
15. van Kats JP, Sassen LMA, Danser AHJ, Polak MPJ, Soei LK, Derckx FHM, Schalekamp MADH, Verdouw PD. Assessment of the role of the renin-angiotensin system in cardiac contractility utilizing the renin inhibitor remikiren. *Br J Pharmacol*. 1996;117:891–901.
16. van Kats JP, de Lannoy LM, Danser AHJ, van Meegen JR, Verdouw PD, Schalekamp MADH. Angiotensin II type 1 (AT₁) receptor mediated accumulation of angiotensin II in tissues and its intracellular half life in vivo. *Hypertension*. 1997;30:42–49.
17. Admiraal PJJ, Derckx 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.
18. Serneri GGN, Boddi M, Coppo M, Chechi T, Zarone N, Moira M, Poggesi L, Margheri M, Simonetti I. Evidence for the existence of a

- functional cardiac renin-angiotensin system in humans. *Circulation*. 1996;94:1866–1893.
19. Danser AHJ, Koning MMG, Admiraal PJJ, Sassen LMA, Derkx FHM, Verdouw PD, Schalekamp MADH. Production of angiotensins I and II at tissue sites in intact pigs. *Am J Physiol*. 1992;263:H429–H437.
 20. 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.
 21. Loudon M, Bing RF, Thurston H, Swales JD. Arterial wall uptake of renal renin and blood pressure control. *Hypertension*. 1983;5:629–634.
 22. Danser AHJ, van Kesteren CAM, Bax WA, Tavenier M, Derkx FHM, Saxena PR, Schalekamp MADH. Prorenin, renin, angiotensinogen, and angiotensin-converting enzyme in normal and failing human hearts: evidence for renin binding. *Circulation*. 1997;96:220–226.
 23. Sealey JE, Catanzaro DF, Lavin TN, Gahnem F, Pittaresi T, Hu L-f, Laragh JH. Specific prorenin/renin binding (ProBP): identification and characterization of a novel membrane site. *Am J Hypertens*. 1996;9:491–502.
 24. Campbell DJ, Valentijn AJ. Identification of vascular renin-binding proteins by chemical cross-linking: inhibition of renin-binding by renin inhibitors. *J Hypertens*. 1994;12:879–890.
 25. Nguyen G, Delarue F, Berrou J, Rondeau E, Sraer J-D. Specific binding of renin to human mesangial cells in culture increases plasminogen activator-1 antigen. *Kidney Int*. 1996;50:1897–1903.
 26. Campbell DJ, Kladis A, Duncan AM. Nephrectomy, converting enzyme inhibition, and angiotensin peptides. *Hypertension*. 1993;22:513–522.
 27. Yamagishi H, Kim S, Nishikimi T, Takeuchi K, Takeda T. Contribution of cardiac renin-angiotensin system to ventricular remodeling in myocardial-infarcted rats. *J Mol Cell Cardiol*. 1993;25:1369–1380.
 28. Campbell DJ, Kladis A, Duncan AM. Effects of converting enzyme inhibitors on angiotensin and bradykinin peptides. *Hypertension*. 1994;23:439–449.
 29. Ruzicka M, Skarda V, Leenen FHH. Effects of ACE inhibitors on circulating versus cardiac angiotensin II in volume overload-induced cardiac hypertrophy in rats. *Circulation*. 1995;92:3568–3573.
 30. Urata H, Healy B, Stewart RW, Bumpus FM, Husain A. Angiotensin II-forming pathways in normal and failing human hearts. *Circ Res*. 1990;66:883–890.
 31. Zisman LS, Abraham WT, Meixel GE, Vamvakias BN, Quaife RA, Lowes BD, Roden RL, Peacock SJ, Groves BM, Reynolds MV, Bristow MR, Perryman MB. Angiotensin II formation in the intact human heart: predominance of the angiotensin-converting enzyme pathway. *J Clin Invest*. 1995;95:1490–1498.
 32. Kokkonen JO, Saarinen J, Kovanen PT. Regulation of local angiotensin II formation in the human heart in the presence of interstitial fluid: inhibition of chymase by protease inhibitors of interstitial fluid and of angiotensin-converting enzyme by Ang-(1–9) formed by heart carboxypeptidase A-like activity. *Circulation*. 1997;95:1455–1463.
 33. Campbell DJ, Kladis A, Valentijn AJ. Effects of losartan on angiotensin and bradykinin peptides and angiotensin-converting enzyme. *J Cardiovasc Pharmacol*. 1995;26:233–240.
 34. Griendling KK, Delafontaine R, Rittenhouse SE, Gimbrone MA, Alexander WA. Correlation of receptor sequestration with sustained diacylglycerol accumulation in angiotensin-II stimulated cultured vascular smooth muscle cells. *J Biol Chem*. 1987;262:14555–14562.
 35. Eggena P, Zhu JH, Sannaporn S, Giordani M, Clegg K, Andersen PC, Hyun P, Barrett JD. Hepatic angiotensin II nuclear receptors and transcription of growth-related factors. *J Hypertens*. 1996;14:961–968.
 36. Haller H, Lindschau C, Erdmann B, Quass P, Luft FC. Effects of intracellular angiotensin II in vascular smooth muscle cells. *Circ Res*. 1996;79:765–772.