# Localization and Production of Angiotensin II in the Isolated Perfused Rat Heart

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# Localization and Production of Angiotensin II in the Isolated Perfused Rat Heart

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***Abstract***—We used a modification of the isolated perfused rat heart, in which coronary effluent and interstitial transudate were separately collected, to investigate the localization and production of angiotensin II (Ang II) in the heart. During combined renin (0.7 to 1.5 pmol Ang I/mL per minute) and angiotensinogen (6 to 12 pmol/mL) perfusion (4 to 8 mL/min) for 60 minutes (n=3), the steady-state levels of Ang II in interstitial transudate in two consecutive 10-minute periods were 4.3±1.5 and 3.6±1.5 fmol/mL compared with 1.1±0.4 and 1.1±0.6 fmol/mL in coronary effluent (mean±half range). During perfusion with Ang II (n=5), steady-state Ang II in interstitial transudate was 32±19% of arterial Ang II compared with 65±16% in coronary effluent (mean±SD, $P<.02$). During perfusion with Ang I (n=5), Ang II in interstitial transudate was 5.1±0.6% of arterial Ang I compared with 2.2±0.3% in coronary effluent ($P<.05$). The tissue concentration of Ang II in the combined renin/angiotensinogen perfusions (per gram) was as high as the concentration in interstitial transudate (per milliliter). Addition of losartan ($10^{-6}$ mol/L) to the renin/angiotensinogen perfusion (n=3) had no significant effect on the tissue level of Ang II, whereas losartan in the perfusions with Ang I (n=5) or Ang II (n=5) decreased tissue Ang II to undetectably low levels. The results indicate that the heart is capable of producing Ang II and that this can lead to higher levels in tissue than in blood plasma. Cardiac Ang II does not appear to be restricted to the extracellular fluid. This is in part due to $AT_1$-receptor–mediated cellular uptake of extracellular Ang II, but our results also raise the possibility of intracellular Ang II production. **(*Hypertension*. 1998;31:1111-1117.)**

**Key Words:** angiotensin II ■ heart ■ receptors, angiotensin

Angiotensin II is the primary mediator of the physiological actions of the RAS and has an important function in cardiovascular homeostasis. Ang II in the circulation is produced by the conversion of Ang I by ACE of the vascular endothelium. Ang I is generated in the circulation by the action of renin from the kidney on its substrate, angiotensinogen, produced by the liver. The various RAS components have all been identified in tissues,[1–4] which suggests the existence of a locally acting RAS in the tissues, apart from the RAS in the circulation.

Local Ang II production in the heart is often invoked to explain the results of heart failure trials evaluating the effects of drugs that block angiotensin formation.[5,6] Knowledge of cardiac Ang I and II production, however, is still fragmentary and little is known about its regulation and physiological significance.[3]

In a previous study we used a modified Langendorff rat heart preparation to investigate the production of Ang I in cardiac tissue.[7] This isolated heart preparation enabled us to collect IST separately from the CE. During perfusion with renin and angiotensinogen, Ang I appeared in the transudate. This Ang I was produced in cardiac tissue and was not derived from Ang I in the perfusate. There was no indication that the heart was capable of producing Ang I independent of arterially delivered renin and angiotensinogen. Observations in nephrectomized pigs also indicated that the cardiac tissue levels of Ang I and II are determined by kidney-derived renin.[4]

Here we report on a study in which we used the same modified Langendorff rat heart preparation to investigate the production of Ang II. The presence of Ang II in tissue depends on arterial delivery of this peptide and on its local generation. Ang II may be formed from arterially delivered Ang I or from in situ synthesized Ang I. Extracellular Ang II is known to be taken up into cells by $AT_1$-receptor endocytosis.[8–11] The adrenals, the kidneys, and the heart accumulate Ang II from the circulation by an $AT_1$-receptor–mediated process, probably endocytosis.[12]

The questions addressed by the present study are: In which tissue compartments is Ang II localized in the heart, where is it produced, and how are its levels in cardiac tissue influenced by $AT_1$-receptor–mediated cellular uptake?

## Methods

### Chemicals and Reagents

[Ile$^5$]-Ang-(1–10) decapeptide (Ang I) and [Ile$^5$]-Ang-(1–8) octapeptide (Ang II) were obtained from Bachem. BSA was from Sigma,

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**Selected Abbreviations and Acronyms**

ACE = angiotensin-converting enzyme
Ang I, II = angiotensin I, II
$AT_1$ = angiotensin II type 1 receptor
BSA = bovine serum albumin
CE = coronary effluent
HPLC = high-performance liquid chromatography
HSA = human serum albumin
ISF = interstitial fluid
IST = interstitial transudate
RAS = renin-angiotensin system

1,10-phenanthroline was from Merck, and sodium pentobarbital was obtained from Apharma. The $AT_1$-receptor antagonist losartan was a kind gift of Dr R.D. Smith, Du Pont Merck, Wilmington, Del. The renin inhibitor remikiren was a kind gift of Dr P. van Brummelen, Hoffmann-La Roche, Basel, Switzerland. Renin was prepared from porcine kidneys as described before.[7] Angiotensinogen was prepared from plasma of nephrectomized pigs.[7] All other reagents were of standard laboratory grade.

## Preparation of the Modified Langendorff Heart

All experiments were performed under the regulation 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.

Male Wistar rats (Harlan, Zeist, The Netherlands; 280 to 400 g) were anesthetized with pentobarbital (60 mg/kg IP) and heparinized (5000 U/kg IV). The hearts (1.0 to 1.4 g) were rapidly excised, cooled in ice-cold Tyrode's buffer (125 mmol/L NaCl, 4.7 mmol/L KCl, 1.4 mmol/L $CaCl_2$, 20 mmol/L $NaHCO_3$, 0.4 mmol/L $NaH_2PO_4$, 1.0 mmol/L $MgCl_2$, 10 mmol/L D-glucose, pH 7.4) until contractions stopped, and prepared for Langendorff perfusion. Carbogen-gassed (95% $O_2$/5% $CO_2$) Tyrode's buffer at 37°C was continuously perfused immediately after cannulation of the aorta at a constant perfusion pressure of 80 mm Hg. Subsequently the pulmonary artery was cannulated and the caval and pulmonary veins were carefully ligated. After the ligation procedure, which took 30 to 45 minutes, the hearts were allowed to stabilize for 30 minutes.

With this modified Langendorff heart preparation it is possible to collect separately CE and IST.[7,13] CE, ejected by the right ventricle, was collected through the cannulated pulmonary artery. IST, which drips from the heart, was collected at the apex. IST flow was 0.03 to 0.16 mL/min, corresponding with 0.7% to 2% of the coronary flow. An IST flow >2% of the coronary flow, which occurred in one out of four to five heart preparations, was considered to be an indication of leakage, for example, from veins that were not properly ligated.[7] Hearts with such high IST flow were therefore not used. IST is derived from the ISF, and the Ang I and II concentrations in IST are considered to be representative for the Ang I and II concentrations in ISF.[7]

## Perfusions With RAS Components and Collection of CE and IST

The Langendorff hearts were perfused with Tyrode's buffer through the cannulated aorta. After a 30-minute stabilization period, RAS components were infused through a T-connection into the cannulated aorta.

CE and IST were collected during and after the infusions. One-minute (4 to 8 mL), 4-minute (16 to 40 mL), or 10-minute samples (40 to 80 mL) of CE were collected into BSA-coated 10- or 50-mL polystyrene tubes. Individual drops of IST (≈50 μL each minute) and 4-minute (≈200 μL) or 9- to 10-minute (≈500 μL) samples of IST were collected into BSA-coated 1.5-mL Eppendorf cups. The Eppendorf cups and polystyrene tubes contained a mixture of inhibitors, 5, 10, or 25 μL in the Eppendorf cups (for the individual drops of IST and the 4-minute and 10-minute IST samples, respectively), and 250, 2500, and 5000 μL in the polystyrene tubes (for the 1-minute, 4-minute, and 10-minute CE samples), to prevent the ongoing formation of Ang I, the conversion of Ang I to Ang II, and the degradation of Ang I and II. The mixture consisted of 0.2 mmol/L of the renin inhibitor remikiren, 125 mmol/L disodium EDTA, and 25 mmol/L 1,10-phenanthroline.[7] Remikiren is an inhibitor of human renin ($IC_{50}$ $7\times10^{-10}$ mol/L). It also inhibits porcine renin ($IC_{50}$ $5\times10^{-8}$ mol/L).[4]

The Eppendorf cups and polystyrene tubes were kept on ice during the perfusions so that the samples were rapidly cooled during their collection and remained cold (0° to 4°C) during the experiment. After the experiment was finished, the samples for Ang I and II measurements were frozen at −80°C.

## Perfusion With Ang II to Study the Localization of Arterially Delivered Ang II

Langendorff hearts were perfused for 15 minutes with Ang II. Ang II diluted with Tyrode's buffer to a concentration of 400 pmol/mL was infused into the perfusion system at a rate of 0.1 mL/min. Ang II caused a transient fall in coronary flow that stabilized in 2 to 3 minutes at 4 to 9 mL/min. After the Ang II infusion had been switched off, the heart was either quickly removed from the perfusion apparatus and transferred into liquid nitrogen or subjected to a 10-minute washout period. The tissue was frozen within 45 seconds after the Ang II infusion had been stopped. One-minute samples of CE and individual drops of IST were collected during the infusion and washout periods in order to study the uptake and washout kinetics of Ang II. A steady state was reached within 5 minutes. Samples of CE and IST that had been collected from 7 to 10 minutes and from 11 to 14 minutes were used to determine the steady-state levels of Ang II. The frozen hearts that were not subjected to a washout period were used to measure the steady-state tissue levels of Ang II.

In a separate series of experiments, the steady-state levels of Ang II in CE, IST, and cardiac tissue were measured during perfusions with Ang II in the presence of the $AT_1$-receptor antagonist losartan ($10^{-6}$ mol/L) in the perfusion fluid. Coronary flow in these experiments was 4 to 11 mL/min.

## Perfusion With Ang I to Study the Localization of Arterially Delivered Ang I and Locally Generated Ang II

Langendorff hearts were perfused for 15 minutes with Ang I. Ang I diluted with Tyrode's buffer to a concentration of 400 pmol/mL was infused into the perfusion system at a rate of 0.1 mL/min. Coronary flow in these experiments was 5 to 10 mL/min. The uptake and washout kinetics of Ang I are known from our previous study.[7] After the Ang I infusion had been switched off, the heart was removed from the perfusion apparatus and immediately transferred into liquid nitrogen. Samples of CE and IST collected from 7 to 10 minutes and from 11 to 14 minutes were used to determine the steady-state levels of Ang I and II. The frozen hearts were used to measure the steady-state tissue levels of Ang I and II.

In a separate series of experiments, the steady-state levels of Ang I and II in CE, IST, and cardiac tissue were measured during perfusions with Ang I in the presence of the $AT_1$-receptor antagonist losartan ($10^{-6}$ mol/L) in the perfusion fluid. Coronary flow in these experiments was 4 to 9 mL/min.

## Perfusions With Renin Combined With Angiotensinogen to Study the Localization of Locally Generated Ang I and II

Langendorff hearts were perfused for 60 minutes with renin and angiotensinogen. Porcine renin diluted with Tyrode's buffer to a concentration of ≈60 pmol Ang I/min per milliliter and undiluted porcine angiotensinogen (500 pmol/mL) were infused into the perfusion system both at a rate of 0.1 mL/min. Coronary flow was 4 to 8 mL/min. The final concentrations of renin and angiotensinogen in the perfusion fluid ranged from 0.7 to 1.5 pmol Ang I/mL per minute and from 6 to 12 pmol/mL, respectively. These experiments

were performed in the presence or absence of the $AT_1$-receptor antagonist losartan ($10^{-6}$ mol/L) in the perfusion fluid. The renin and angiotensinogen solutions were kept at 4°C until they reached the aorta, in order to prevent angiotensin generation outside the heart.[7] After the perfusion had been switched off, the hearts were frozen in liquid nitrogen.

Samples of CE and IST collected from 31 to 40 minutes and from 46 to 55 minutes were used to determine the steady-state levels of Ang I and II. Collections from 41 to 45 and from 56 to 60 minutes had been used for other measurements, reported in our previous study.[7] The frozen hearts were used to measure the steady-state tissue levels of Ang I and II.

## Measurements of Ang I and II

All angiotensin measurements were performed by a technician who did not know which experiments had been carried out with losartan and which without this drug. The Ang I and II concentrations in CE and IST samples, collected during Ang I and II perfusions, were measured directly with sensitive radioimmunoassays.[14,15] Measurements were made in 50 $\mu$L of undiluted CE and in 50 $\mu$L IST diluted with 25 $\mu$L of 0.25 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. The lowest measurable Ang I concentration was 15 fmol/mL in CE and 40 fmol/mL in IST. The lowest measurable Ang II concentration was 10 fmol/mL in CE and 25 fmol/mL in IST.

The Ang I and II concentration of CE and IST samples obtained during infusion with renin combined with angiotensinogen, as well as the cardiac tissue concentrations of Ang I and II both during Ang I and II perfusion and during combined renin and angiotensinogen perfusion, were measured by radioimmunoassay after SepPak extraction and reversed-phase HPLC separation.[4,14,15] To measure tissue Ang I and II, the frozen hearts were minced and homogenized (1:10, wt/vol) in an iced solution of 0.1 mol/L HCl/80% ethanol. Homogenates were centrifuged at 20 000*g* for 25 minutes at 4°C. Ethanol in the supernatant was evaporated under constant air flow. The remainder of the supernatant was diluted in 20 mL 1% ortho-phosphoric acid and centrifuged again at 20 000*g*. The supernatant was diluted with 1% ortho-phosphoric acid 1:1 (vol/vol). The tissue homogenate supernatants and CE and IST samples were concentrated over SepPak columns (SepPak C18, Waters), and the concentrated extracts were subjected to HPLC followed by radioimmunoassay.

$^{125}$I-labeled Ang I had been added as an internal standard to the CE and IST samples before SepPak extraction and to tissue before its homogenization. Recovery was better than 70%, and the Ang I and II results were corrected for incomplete recovery. The lowest level of Ang I that could be measured with the Ang I radioimmunoassay after HPLC separation was 2.0 fmol/g in cardiac tissue, 0.05 fmol/mL in CE, and 2.5 fmol/mL in IST. The lowest level of Ang II that could be measured with the Ang II radioimmunoassay after HPLC separation was 1.0 fmol/g in cardiac tissue, 0.05 fmol/mL in CE, and 1.5 fmol/mL in IST.

## Calculations

To determine in which tissue compartments Ang I and II are localized in the heart, the measured tissue levels of Ang I and II were compared with the levels predicted on the basis of the presence of these peptides in the cardiac extracellular fluid compartments. If the measured tissue levels are higher than predicted, angiotensin is not restricted to the extracellular fluid compartments. If the measured tissue levels are lower than predicted, angiotensin has disappeared from the extracellular fluid compartments during the period between the moment the heart has been removed from the perfusion equipment and the moment the tissue is transferred into liquid nitrogen. The predicted tissue level (fmol/g) was calculated as follows:

$$\text{Predicted tissue Ang concentration}$$

$$=[\text{Ang}_{\text{IST}}]\times\text{ISF volume}+[\text{Ang}_{\text{CE}}]\times\text{IVF volume}$$

in which $[\text{Ang}_{\text{IST}}]$ is the steady-state Ang I or II concentration in IST and $[\text{Ang}_{\text{CE}}]$ is the steady-state Ang I or II concentration in CE. ISF volume is the cardiac interstitial fluid volume (0.23 mL/g), determined previously as the difference between the distribution volume of $^{51}$Cr-EDTA and $^{125}$I-HSA,[7] and IVF volume is the intravascular fluid volume (0.38 mL/g), determined previously as the distribution volume of $^{125}$I-HSA.[7] In this calculation, it is assumed that $[\text{Ang}_{\text{CE}}]$ is representative for the angiotensin concentration in most of the intravascular fluid compartment (coronary venules and veins and right ventricle).

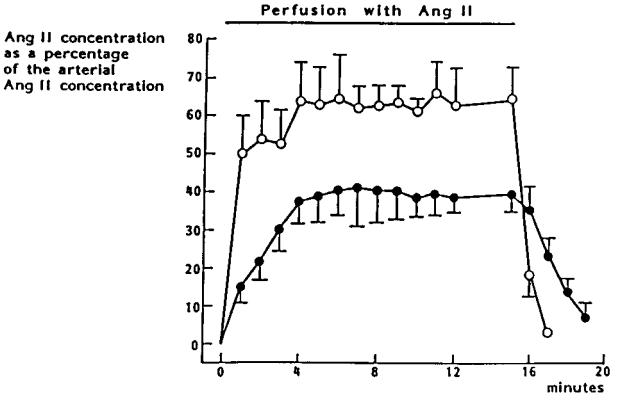


**Figure 1.** Ang II concentration in IST (●) and CE (○) during and after perfusion with Ang II (n=5). Data (mean±SEM) are presented as a percentage of the arterial Ang II concentration.

## Statistical Analysis

The Ang I and II levels in CE, IST, and cardiac tissue during Ang I or II perfusion are expressed as a percentage of the arterial levels of Ang I (Ang I perfusion) or Ang II (Ang II perfusion). Ang I and II levels in CE, IST, and cardiac tissue during renin combined with angiotensinogen perfusion are given as absolute values. Differences between the experimental groups were evaluated for statistical significance by Student's unpaired *t* test. Differences within groups were evaluated for statistical significance by Student's paired *t* test. Bonferroni's correction was applied to adjust for multiple comparisons. Differences were assigned to be significant for values of $P<.05$.

# Results

## Localization of Arterially Delivered Ang II During Perfusion With Ang II

During perfusion with Ang II (n=10), the steady-state Ang II level in CE was 65% of the arterial level (Fig 1 and Fig 2).

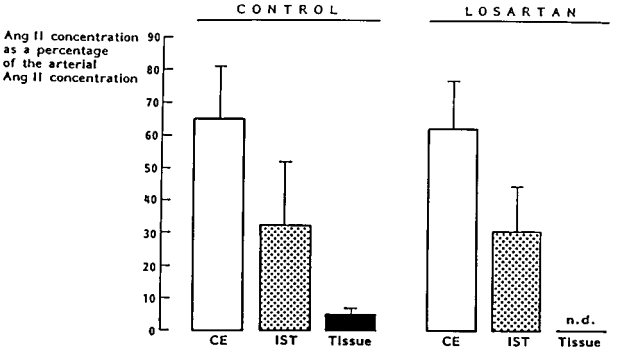


**Figure 2.** Steady-state Ang II concentration in CE and IST during perfusion with Ang II for 15 minutes and the Ang II concentration in cardiac tissue shortly after the perfusion. Perfusions were carried out in the absence (n=5) or presence (n=5) of losartan ($10^{-6}$ mol/L). The mean of the levels of the 7- to 10-minute and 11- to 14-minute perfusion periods was taken as the steady-state concentration. Data (mean±SD) are presented as a percentage of the arterial Ang II concentration. Ang II in IST was lower than in CE ($P<.02$) and higher than in cardiac tissue ($P<.05$) both with and without losartan. n.d. indicates not detectable.

Thus 35% of the arterially delivered Ang II was removed by the heart during a single passage of the perfusate. The intravascular fluid volume of the Langendorff heart was measured in our previous study of this model and was found to be 0.38 mL/g. Heart weight was 1.0 to 1.4 g, thus the intravascular fluid volume per heart was 0.38 to 0.53 mL. The perfusate flow was 4 to 9 mL/min; therefore the perfusate transit time of the isolated heart preparation was 0.04 to 0.13 minutes. It can be concluded, therefore, that 35% of the arterially delivered Ang II was removed in 0.04 to 0.13 minutes, which corresponds with a t½ of 0.06 to 0.21 minute. A steady-state level in IST was reached within 5 minutes. It was ≈35% of the arterial level (39% in the experiments in which also the Ang II washout was measured, Fig 1; 32% in the experiments in which also the tissue Ang II concentration was measured, Fig 2). The steady-state Ang II concentration in IST was significantly lower than in CE.

After discontinuation of the Ang II perfusion, Ang II disappeared from IST, in a monophasic way, with a t½ of 1.4±0.3 minutes (mean±SD, n=5).

The cardiac tissue level of Ang II (per gram of tissue), shortly after the Ang II perfusion had been switched off, was ≈5% of the arterial level (per milliliter of perfusate) (Fig 2). This is lower than predicted on the basis of the presence of Ang II the cardiac extracellular fluid compartments (see "Methods"). The predicted tissue concentration of Ang II would be 30% to 35% of the arterial concentration. This discrepancy suggests that extracellular Ang II was rapidly degraded in the short period (maximally 45 seconds) between the moment the Ang II infusion had been stopped and the moment the tissue was transferred into liquid nitrogen.

During perfusion with Ang II in the presence of the $AT_1$-receptor antagonist losartan (n=5), the steady-state Ang II levels in CE and IST were not significantly different from the levels during Ang II perfusion in the control situation (Fig 2). Ang II, which was above the detection limit in all cardiac tissue samples in the control Ang II perfusion experiments, could not be detected in the tissue samples in the Ang II/losartan perfusion experiments. This is likely to be due to blockade of the $AT_1$-receptor–mediated cellular uptake of extracellular Ang II.

## Localization of Arterially Delivered Ang I and Locally Generated Ang II During Perfusion With Ang I

During perfusion with Ang I (n=5), the steady-state Ang I level in CE was 48% of the arterial level (Fig 3). Thus 52% of arterially delivered Ang I was removed by the heart during a single passage of perfusate. Part of the Ang I removal was caused by conversion to Ang II (Fig 4).

The steady-state Ang II level in IST was higher than in CE, whereas the steady-state Ang I level in IST was lower than in CE. This, together with the finding that during perfusion with Ang II the Ang II level in IST was also lower than in CE (see above), indicates that part of the Ang II present in IST during Ang I perfusion was not derived from Ang II in the perfusate.

The cardiac tissue level of Ang I (per gram of tissue), shortly after the Ang I perfusion had been switched off, was ≈5% of the arterial level (per milliliter of perfusate) (Fig 3).

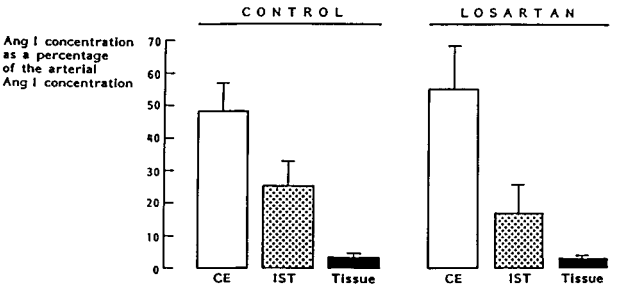


**Figure 3.** Steady-state Ang I concentration in CE and IST during perfusion with Ang I for 15 minutes and the Ang I concentration in cardiac tissue shortly after the perfusion. Perfusions were carried out in the absence (n=5) or presence (n=5) of losartan ($10^{-6}$ mol/L). The mean of the levels of the 7- to 10-minute and 11- to 14-minute perfusion periods was taken as the steady-state concentration. Data (mean±SD) are presented as a percentage of the arterial Ang I concentration. Ang I in IST was lower than in CE ($P<.02$) and higher than in cardiac tissue ($P<.02$) both with and without losartan.

This is lower than predicted on the basis of the presence of Ang I in the cardiac extracellular fluid compartments (see "Methods"). The predicted tissue concentration of Ang I would be 22% of the arterial concentration. This discrepancy suggests that extracellular Ang I, like extracellular Ang II, was rapidly degraded in the short period between the moment the Ang I infusion had been stopped and the moment the tissue was transferred into liquid nitrogen.

During perfusion with Ang I in the presence of the $AT_1$-receptor antagonist losartan (n=5), the steady-state Ang I and II levels in CE and IST were not significantly different from those during Ang I perfusion in the control situation (Fig 3 and Fig 4). Ang II, which was above the detection limit in all cardiac tissue samples in the control Ang I perfusion experiments, could not be detected in the tissue samples in the Ang I/losartan perfusion experiments, which is probably due to blockade of the $AT_1$-receptor–mediated cellular uptake of extracellular Ang II.

## Localization of Locally Generated Ang I and II During Combined Renin/Angiotensinogen Perfusion

Results of the combined renin/angiotensinogen perfusions are shown in Fig 5 and Fig 6. During combined renin/angioten-

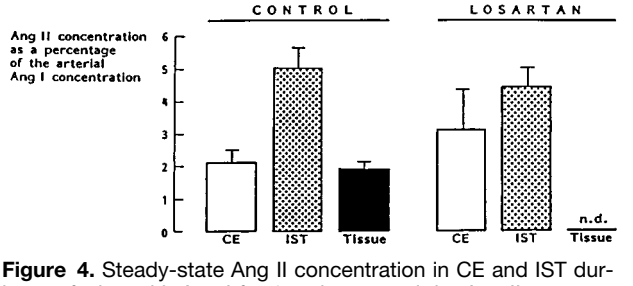


**Figure 4.** Steady-state Ang II concentration in CE and IST during perfusion with Ang I for 15 minutes and the Ang II concentration in cardiac tissue shortly after the perfusion. Perfusions were carried out in the absence (n=5) or presence (n=5) of losartan ($10^{-6}$ mol/L). The mean of the levels of the 7- to 10-minute and 11- to 14-minute perfusion periods was taken as the steady-state concentration. Data (mean±SD) are presented as a percentage of the arterial Ang I concentration. Ang II in IST was higher than in CE ($P<.05$) and cardiac tissue ($P<.05$) both with and without losartan. n.d. indicates not detectable.

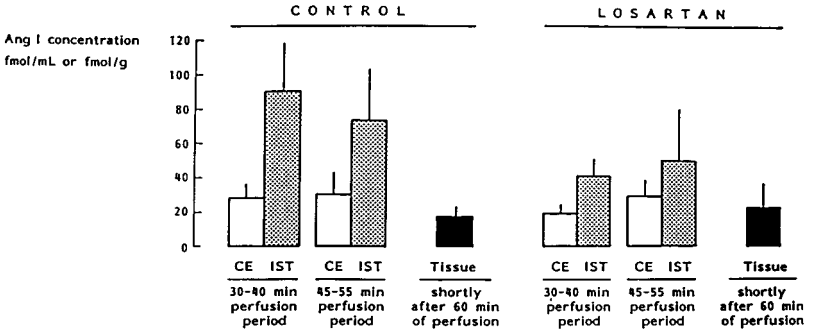


**Figure 5.** Steady-state Ang I concentration in CE and IST during combined renin/angiotensinogen perfusion for 60 minutes and the Ang I concentration in cardiac tissue shortly after the perfusion. Perfusions were carried out in the absence (n=3) or presence (n=3) of losartan ($10^{-6}$ mol/L). Data are presented as mean and half range.

sinogen perfusion in the absence of losartan (n=3), the Ang I levels in IST in the 31- to 40-minute and 46- to 55-minute perfusion periods were 4.2 (1.5 to 8.2) and 3.2 (0.9 to 6.5) times higher than in CE, respectively (mean and range). The Ang II levels in IST in these perfusion periods were 4.8 (2.7 to 6.8) and 3.8 (2.2 to 5.7) times the levels in CE.

The cardiac tissue level of Ang I, 18.2 (11.4 to 22.8) fmol/g, shortly after the combined renin/angiotensinogen perfusion had been switched off, was close to the level predicted on the basis of the presence of Ang I in the cardiac extracellular fluid compartments (see "Methods"), ≈20 fmol/g.

The cardiac tissue level of Ang II, 4.8 (2.8 to 6.4) fmol/g, was higher than the level predicted on the basis of the presence of Ang II in the cardiac extracellular fluid compartments, ≈1.5 fmol/g. This suggests that the presence of the locally formed Ang II was not limited to the intravascular and interstitial fluid.

During combined renin/angiotensinogen perfusion in the presence of the $AT_1$-receptor antagonist losartan (n=3), the Ang I and II levels measured in CE, IST, and cardiac tissue were similar to the levels measured in the control experiments without losartan. Thus blockade of the $AT_1$-receptor–mediated uptake of extracellular Ang II in these experiments did not decrease tissue Ang II to an undetectably low level as it did in the Ang I and II perfusion experiments.

## Discussion

In a previous series of experiments we studied the production of Ang I by the modified rat Langendorff heart, in which the ISF transudate could be collected separately from the CE.[7] Our present study uses the same isolated heart model and focuses on the production of Ang II.

As expected, part of the Ang I present in the perfusate during the combined renin/angiotensinogen perfusions and formed by the reaction of arterially delivered renin with arterially delivered angiotensinogen was converted to Ang II and ejected by the CE. Ang I to II conversion by the coronary vascular bed has been reported by others.[16,17] More interesting is our finding that during these combined renin/angiotensinogen perfusions, the concentration of Ang II in the IST was several times higher than in the CE, whereas during perfusions with Ang II, the Ang II concentration in the IST was lower than in the CE. These results indicate that a substantial part of Ang II in the ISF was not derived from the Ang II that was present in the perfusate.

At the time we finished this study, a report was published by Dell'Italia et al[18] on measurements of Ang II in the ISF space of the dog heart by using microdialysis probes. The Ang II level in the ISF was higher than in blood plasma and did not change after systemic infusion of Ang I. These results indicate compartmentalization of Ang II in the heart between the interstitial and intravascular fluid compartments. Our results are in agreement with this conclusion and suggest that Ang II in the ISF is formed outside the intravascular fluid compartment.

In the combined renin/angiotensinogen perfusion experiments, the Ang II level we measured in cardiac tissue (expressed per gram of tissue) was higher than in CE (expressed per milliliter of fluid). It was in fact as high as in the IST. This is an indication that the locally produced Ang II is not restricted to the extracellular fluid and that a large part

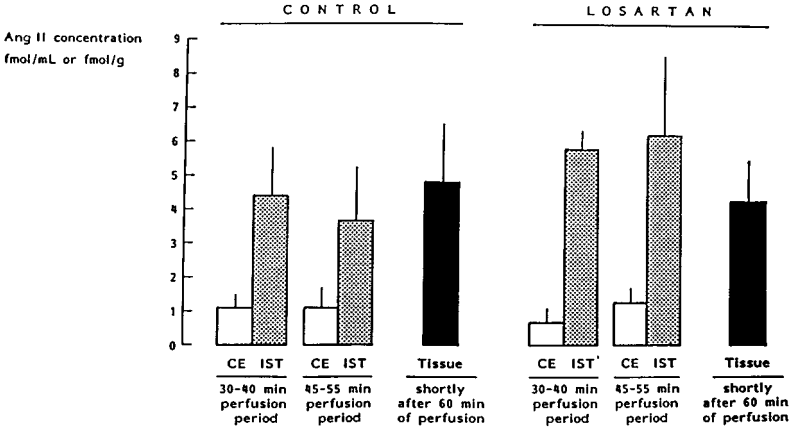


**Figure 6.** Steady-state Ang II concentration in CE and IST during combined renin/angiotensinogen perfusion for 60 minutes and the Ang II concentration in cardiac tissue shortly after the perfusion. Perfusions were carried out in the absence (n=3) or presence (n=3) of losartan ($10^{-6}$ mol/L). Data are presented as mean and half range.

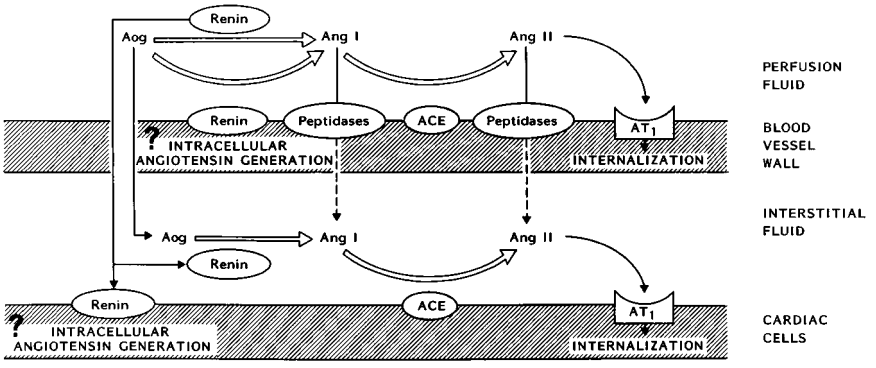


**Figure 7.** Proposed scheme of Ang I and II production in the heart: Intravascular and interstitial fluid compartments as well as blood vessel wall and cardiac cells (endothelial cells, myocytes, fibroblasts, macrophages) are depicted. Circulating renin and angiotensinogen (Aog) both enter the interstitial fluid compartment. Renin may also bind to the vascular wall and cardiac cells.[7,24] ACE is present on endothelial cells and possibly also on cardiac cells. Ang I and II are metabolized by peptidases while passing through the vascular wall. Ang I and II in the interstitial fluid are mainly generated outside the vascular fluid compartment. Tissue Ang I and II generation may occur not only in the interstitial fluid or on the cell surface but also within cells, for instance after renin uptake by the cells.[24] Binding of Ang II to the $AT_1$-receptor is followed by internalization of the $AT_1$-receptor–Ang II complex.

of this Ang II might be located in the cells, because the intravascular and interstitial fluid compartments comprise ≈38% and 23% of tissue weight, respectively.[7] Cellular binding and uptake through $AT_1$-type Ang II receptors is known to occur.[8–11] $^{125}$I-Ang II infused into the left cardiac ventricle of intact pigs is accumulated in cardiac tissue by an $AT_1$-receptor–dependent process, and the kinetics of Ang II accumulation and disappearance are compatible with receptor-mediated endocytosis.[12]

$AT_1$-receptor–mediated binding of Ang II to cardiac cells is also indicated by our results obtained during the Ang I or II perfusions, which showed that the cardiac tissue concentration of Ang II during these perfusions fell to an undetectably low level when the specific $AT_1$-receptor antagonist losartan was added to the perfusion fluid. Losartan, however, had no significant effect on the cardiac tissue concentration of locally formed Ang II during the combined renin/angiotensinogen perfusions. This is in accordance with observations in rats, which showed an increase and not a decrease in cardiac tissue Ang II after 8 days of treatment of the animals with losartan, an increase that is likely to be related to the stimulated renin release from the kidney after this drug.[19] The apparent discrepancy with respect to the observed effects of losartan, between the results of the Ang I and II perfusions on the one hand and the combined renin/angiotensinogen perfusion on the other hand, raises the possibility that during the combined renin/angiotensinogen perfusion, local Ang II production in the heart is taking place in a compartment that is not reached by losartan, that is, within the cells.

The finding that the measured levels of Ang II in cardiac tissue in the Ang I and II perfusion experiments were lower than calculated on the basis of their presence in the extracellular fluid may be explained as follows. Most of the extracellular fluid in the Langendorff heart preparation is localized in the intravascular compartment, that is, the coronary vascular bed and the right ventricle.[7] The half-life of Ang I and II is ≈1 minute in the ISF compartment.[7] In the intravascular compartment it is even shorter, ≈0.1 to 0.2 minute. It is therefore possible that the short period between the moment the perfusions had been stopped and the tissue had been transferred into liquid nitrogen was long enough for the endothelial peptidases to cause substantial breakdown of intravascular Ang I and II.

In the combined renin/angiotensinogen perfusion experiments these losses of extracellular Ang II might have been overcome, at least partly, by ongoing formation of this peptide during the time that elapsed until the tissue was frozen. The tissue levels of Ang II we measured in these combined renin/angiotensinogen perfusion experiments are therefore probably more close to the true levels at the time of perfusion than the tissue levels we measured in the Ang I and II perfusion experiments. Moreover, the Ang II concentration in the extracellular fluid was several orders of magnitude higher during the Ang I and II perfusions than during combined renin/angiotensinogen perfusion. Because of these higher levels of extracellular Ang II a smaller proportion of it will be bound to the cardiac cell $AT_1$-receptors and taken up in the cells, where it is protected against the rapid degradation to which extracellular Ang II is exposed.[11,12,20] Finally, if it is true that Ang II is produced within the cells during combined renin/angiotensinogen perfusion, this intracellular Ang II will similarly be protected against this rapid degradation.

The results of the renin/angiotensinogen perfusion experiments indicate that in contrast with Ang II, most of the locally produced Ang I in the heart is restricted to the cardiac extracellular fluid compartments. Likely sites of cardiac Ang I production are the ISF and a site closer to the blood compartment, possibly vascular surface-bound renin (Fig 7).[7] The question of where in the tissue the Ang II is formed is incompletely answered by the present study. Part of it is formed at the vascular endothelial and endocardial surfaces.[16,21–23] Our observations in the present study on the effects of blockade of $AT_1$-receptor–mediated uptake of Ang II, which did not show a change in the tissue level of locally produced Ang II as opposed to a decrease in the tissue level of arterially administered Ang II, raise the interesting possibility of intracellular Ang II formation (Fig 7). There is experimental evidence that neonatal rat cardiac myocytes are capable of binding and internalizing renin and prorenin through a mannose 6-phosphate receptor and that prorenin is activated in the cells.[24] Internalized Ang II has a long half-life, and there is growing evidence that intracellular Ang II can serve important functions.[25–27] $AT_1$-receptor–mediated uptake of Ang II and the evidence that the formation and degradation of Ang I and II in cardiac tissue are highly compartmentalized has implications for the effects of drugs

that interfere with Ang II receptor binding ($AT_1$-receptor antagonists) or reduce the production of Ang II (renin inhibitors, ACE inhibitors). These drugs may have different effects on the Ang II levels in different cardiac tissue compartments.

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