Cardiac Renin and Angiotensins

Uptake From Plasma Versus In Situ Synthesis

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Abstract The existence of a cardiac renin-angiotensin system, independent of the circulating renin-angiotensin system, is still controversial. We compared the tissue levels of renin-angiotensin system components in the heart with the levels in blood plasma in healthy pigs and 30 hours after nephrectomy. Angiotensin I (Ang I)–generating activity of cardiac tissue was identified as renin by its inhibition with a specific active site–directed renin inhibitor. We took precautions to prevent the ex vivo generation and breakdown of cardiac angiotensins and made appropriate corrections for any losses of intact Ang I and II during extraction and assay. Tissue levels of renin (n=11) and Ang I (n=7) and II (n=7) in the left and right atria were higher than in the corresponding ventricles ($P<.05$). Cardiac renin and Ang I levels (expressed per gram wet weight) were similar to the plasma levels, and Ang II in cardiac tissue was higher than in plasma ($P<.05$). The presence of these renin-angiotensin system components in cardiac tissue therefore cannot be accounted for by trapped plasma or simple diffusion from plasma into the interstitial fluid. Angiotensinogen levels (n=11) in cardiac tissue were 10% to 25% of the levels in plasma, which is compatible with its diffusion from plasma into the interstitium. Like angiotensin-converting enzyme, renin was enriched in a purified cardiac membrane fraction prepared from left ventricular tissue, as compared with crude homogenate, and 12±3% (mean±SD, n=6) of renin in crude homogenate was found in the cardiac membrane fraction and could be solubilized with 1% Triton X-100. Tissue levels of renin and Ang I and II in the atria and ventricles were directly correlated with plasma levels ($P<.05$), and in both tissue and plasma the levels were undetectably low after nephrectomy. We conclude that most if not all renin in cardiac tissue originates from the kidney. Results support the contentions that in the healthy heart, angiotensin production depends on plasma-derived renin and that plasma-derived angiotensinogen in the interstitial fluid is a potential source of cardiac angiotensins. Binding of renin to cardiac membranes may be part of a mechanism by which renin is taken up from plasma. (**Hypertension**. 1994;24:37-48.)

Key Words • renin • angiotensinogen • angiotensins • renin-angiotensin system • kininase II

Angiotensin I (Ang I) is produced in the circulating blood by the action of renin from the kidney on angiotensinogen produced by the liver. Ang I is converted to Ang II, a potent vasoconstrictor, by angiotensin-converting enzyme (ACE) located on the luminal surface of the vascular endothelium. It is now well established that Ang I and II are not only produced in the blood compartment but also locally in tissues. Recent evidence suggests that complete local renin-angiotensin systems (RAS) are present in a number of organs, for instance, kidney, adrenal gland, and ovary.[1-3] In such local RAS, the production of Ang I and II is thought to depend on in situ synthesized renin rather than plasma-derived renin.

A local cardiac RAS has also been postulated.[4,5] However, direct evidence for Ang I and II production in the heart by in situ synthesized renin is still lacking. Renin mRNA levels in the heart are usually low and can be detected only by polymerase chain reaction.[6-8] Early studies showed Ang I–generating activity in left ventricular tissue of the canine heart,[9] but it is not known whether this activity was caused by renin or reninlike enzymes, such as cathepsin D.[10] Recently, both renin mRNA and renin itself were demonstrated in cultured myocytes and fibroblasts from neonatal rat hearts.[5]

Angiotensinogen mRNA and angiotensinogen have been detected in rat[11] and human[12] cardiac tissue as well as in cultured myocytes and fibroblasts from neonatal rat hearts.[5] It has also been reported that angiotensinogen is released by the isolated perfused rat Langendorff heart,[13] but it remains to be proved that this angiotensinogen was not derived from plasma.

ACE mRNA can be readily detected in the heart.[14] ACE has been demonstrated in the rat heart by autoradiography, using a radiolabeled ACE inhibitor,[15] as well as by measurement of ACE activity in cardiac tissue homogenates.[16] Infusion of Ang I into the coronary circulation resulted in the prompt appearance of Ang II in the coronary effluent, and this was blocked with an ACE inhibitor.[17,18] It is not known whether cardiac ACE is present on myocytes or is limited to the endothelium of the coronary circulation.

Attempts have been made to measure Ang I and II in cardiac tissue. Results showed a wide range of levels, from barely detectable to very high.[4,19-21] These variable results are probably caused by methodological difficulties related to the lack of specificity of some angiotensin assays and to angiotensin breakdown during the preparation of tissue extracts. Recently, with a very sensitive and specific technique, it was found in the rat that after

nephrectomy Ang II was still present in cardiac tissue.[21] However, Ang II was also still present in plasma, and uptake of Ang II from plasma therefore remained a possibility.

The favorable effects of ACE inhibitors in heart failure, which at least in part do not appear to be related to their effect on blood pressure,[22] have stimulated interest in the possible role of a cardiac RAS. Because of this possibility, and in view of the uncertainties about the evidence of such a local system, we undertook the present study to further assess the origin of the RAS components in the heart. We measured prorenin, renin, angiotensinogen, and Ang I and II in hearts from anesthetized pigs, addressing the difficulties in measuring tissue levels of these RAS components. We compared the levels in cardiac tissue with those in plasma. To assess to what extent RAS components in the heart depend on the kidney, we studied the effects of nephrectomy on both the cardiac and circulating levels.

Methods

Collection of Cardiac Tissue

Hearts were obtained from pigs (crossbred Yorkshire× Landrace, Hedelse Varkens Combinatie) with a body weight of 22 to 30 kg. All procedures followed were in accordance with institutional guidelines. The pigs (n=24) either had been used for a series of acute pharmacologic experiments, in which the cardiac effects of dobutamine after a 30-minute occlusion of the left anterior descending coronary artery were studied, or had served as a control (neither coronary occlusion nor drug administration). Two additional pigs were bilaterally nephrectomized to determine whether cardiac renin originates from the kidney. These animals were then allowed to recover and were kept alive for 30 hours. The animals were anesthetized with 160 mg/kg α-chloralose (Merck) injected into the superior caval vein, followed by continuous infusion of low-dose pentobarbital (5 mg/kg per hour IV).

The heart was quickly removed, and the right and left atria and parts of the right and left ventricles (pieces of 2 to 3 g) were excised on the spot. The tissues were immediately frozen in liquid nitrogen and stored at −70°C until assay. To examine whether angiotensin levels remained stable for some time after the heart had been removed from the body, we divided the various tissue pieces from two hearts into two portions; one was frozen immediately and the other after it had been kept for 30 minutes at room temperature.

Collection of Blood Samples

Two peripheral venous blood samples were obtained immediately before the heart was removed, one to measure angiotensins and one to measure renin and angiotensinogen. The blood sample for angiotensin measurements was collected in a prechilled polystyrene tube containing the following inhibitors (0.5 mL inhibitor solution in 10 mL blood): 0.01 mmol/L of the renin inhibitor Ro 42,5892 (Hoffmann–La Roche), 6.25 mmol/L disodium EDTA, and 1.25 mmol/L 1,10-phenanthroline (final concentrations in blood). With this inhibitor mixture, virtually complete inhibition of renin, ACE, and angiotensinases is achieved.[17] The blood sample was immediately transferred into a polystyrene tube and centrifuged at 3000g for 10 minutes at 4°C. Plasma was stored at −70°C and assayed within 2 days. The second blood sample, which was taken for renin and angiotensinogen measurements, was collected into a polystyrene tube containing trisodium citrate (0.2 mL citrate solution in 10 mL blood; final citrate concentration in blood, 0.013 mol/L). The sample was centrifuged at 3000g for 10 minutes at room temperature. Plasma was stored at −20°C and assayed within 1 week.

Extraction, High-Performance Liquid Chromatographic Separation, and Measurement of Angiotensins

Liquid nitrogen–frozen cardiac tissue (2 to 3 g) was rapidly minced with scissors into small pieces and homogenized (1:10, wt/vol) with a Polytron PT10/35 (Kinematica) in an iced solution of 0.1 mol/L HCl/80% ethanol, as described by Chappell et al.[23] Homogenates were centrifuged at 20 000g for 20 minutes at 4°C, and the supernatant was stored for 12 hours at −20°C. The pellet was discarded. The supernatant obtained after a second centrifugation step (20 000g, 20 minutes, 4°C) was diluted 1:1 (vol/vol) with 0.1% orthophosphoric acid and stored for 4 to 6 hours at 4°C. The diluted supernatant was again centrifuged (20 000g, 20 minutes, 4°C). The final supernatant was further diluted 1:1 (vol/vol) with 0.02% orthophosphoric acid and then concentrated by reversible adsorption to octadecylsilyl silica (Sep-Pak C18, Waters Chromatography Division, Millipore Corp). Plasma was directly applied to the Sep-Pak cartridges.

The Sep-Pak cartridges were conditioned with 4 mL methanol and equilibrated with 2 times 4 mL cold water. Samples were passed through the cartridge at 4°C, followed by a wash with 2 times 4 mL cold water. Adsorbed angiotensins were eluted with 3 mL methanol into polypropylene tubes, and the methanol was evaporated under vacuum rotation at 4°C using a Speed Vac concentrator (Savant Instruments). Separations were performed by reversed-phase high-performance liquid chromatography (HPLC), according to the method of Nussberger et al,[24] using a Nucleosil C18 steel column (250×4.6 mm and 10-μm particle size). Mobile phase A was 0.085% orthophosphoric 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 methanol extracts were dissolved in 100 μL HPLC solvent (mobile phase A) and injected. 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 20-second fractions into polypropylene tubes coated with bovine serum albumin. The fractions containing Ang I and II were neutralized with 0.5 mol/L sodium hydroxide and vacuum dried at 4°C. The Ang I and II concentrations were measured by radioimmunoassay as described previously.[25] The lower limit of detection was 0.4 fmol per fraction for the Ang II assay and 1.0 fmol per fraction for the Ang I assay.

Recovery of angiotensins after homogenization, extraction, and HPLC separation was determined by addition of ^{125}I–Ang I or ^{125}I–Ang II (specific activity, 3.6×10^6 cpm/pmol) to the tissue (approximately 10 000 cpm/g) before homogenization. The concentrations of intact ^{125}I–Ang I and its radiolabeled metabolites in the HPLC fractions were measured in a gamma counter. Similar experiments were performed in which Ang I and II were added to the tissue (approximately 100 fmol/g) before homogenization.

Extraction of Renin and Prorenin

Liquid nitrogen–frozen cardiac tissue (2 to 3 g) was rapidly minced into small pieces and homogenized (1:2, wt/vol) with a Polytron PT10/35 in 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. The crude homogenate was centrifuged at 20 000g for 30 minutes at 4°C. The supernatant was stored at −20°C (supernatant 1). The pellet was washed with phosphate buffer (1:2, wt/vol) and centrifuged again at 20 000g for 30 minutes at 4°C. The second supernatant was also stored at −20°C (supernatant 2). The pellet was resuspended in phosphate buffer (1:2, wt/vol) using a Potter homogenizer (Heidolph Elektro KG) and was stored at −20°C (pellet).

Kidney renin was prepared from freshly obtained porcine kidneys. Kidney tissue was homogenized (1:1, wt/vol) in isotonic phosphate buffer, pH 7.4, and dialyzed at 4°C for 48 hours in 0.05 mol/L glycine buffer, pH 3.3, containing 0.001

mol/L disodium EDTA and 0.095 mol/L NaCl. This was followed by dialysis for 24 hours against a 0.1 mol/L phosphate buffer, pH 7.4, containing 0.001 mol/L disodium EDTA and 0.075 mol/L NaCl. The content of the dialysis bag was then collected, and denatured protein was removed by centrifugation at 20 000g for 20 minutes at 4°C. The resulting supernatant (semipurified porcine kidney renin) contained renin at a concentration of 650 pmol Ang I/min per milliliter, as assessed by incubation at pH 7.4 with sheep renin substrate.[26]

Recombinant human prorenin, used in recovery experiments (see below), was a kind gift of Dr W. Fischli (Hoffmann–La Roche, Basel, Switzerland). Traces of renin present in this preparation were removed by Cibacron Blue affinity chromatography.[27]

The pellet fraction always contained some renin activity, despite the fact that the pellet was washed by resuspension in isotonic phosphate buffer and recentrifugation. The pellet fraction, prepared as described above, contained most of the intracellular structures and organelles, such as myofibrils, cell nuclei, parts of the mitochondria, and lysosomes. To study whether any renin is bound to cardiac membranes, we modified the centrifugation procedure in such a way that the pellet fraction was almost devoid of myofibrils, nuclei, and mitochondria and was mainly composed of plasma membranes and sarcoplasmic reticulum. It should be noted that intact cardiac tissue contains not only myocytes but endothelial and vascular smooth muscle cells as well. Freshly obtained cardiac left ventricular tissue (20 g) was minced into small pieces and homogenized (1:10, wt/vol) with a Polytron PT10/35 in 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. The homogenate was centrifuged at 2000g for 15 minutes at 4°C. The supernatant was recentrifuged at 50 000g for 45 minutes at 4°C. The supernatant obtained after the second centrifugation was also discarded, whereas the remaining pellet was washed two times by resuspension in phosphate buffer and subsequent centrifugation. Renin measurements were performed in the washed pellet fraction (purified cardiac membrane fraction) and the crude homogenate. ACE, as a marker of membrane-bound enzymes, was measured in the purified membrane fraction with a commercial kit (ACEcolor, Fujirebio), containing p-hydroxybenzoyl-glycyl-L-histidyl-L-leucine as synthetic substrate. Total protein was measured according to the method of Lowry.[28]

To assess whether any loss of renin had occurred during homogenization and extraction of cardiac tissue, we added known amounts of semipurified porcine kidney renin or trypsin-activated recombinant human prorenin to frozen cardiac tissue before homogenization. Experiments in which known amounts of recombinant human prorenin were added to frozen tissue were also carried out to check for inadvertent activation of prorenin.

Measurement of Renin
Routine Enzyme-Kinetic Assay

Samples were assessed in duplicate by measurement of the rate of Ang I generation at pH 7.4 during incubation at 37°C with an excess of sheep renin substrate in the presence of inhibitors of angiotensinases, ACE, and serine proteases.[26] Ang I was measured by radioimmunoassay. Incubation time was maximally 6 hours. The incubation mixture consisted of 100 μL sample, 200 μL renin substrate, 14 μL inhibitor solution, and 100 μL 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. Renin substrate was prepared from plasma of nephrectomized sheep.[26] The renin activity of the renin substrate preparation was less than 1.5 fmol Ang I/min per milliliter. Two different inhibitor solutions were used, one with the specific renin inhibitor Ro 42,5892 and one without renin inhibitor. Both solutions contained phenylmethylsulfonyl fluoride (0.0024 mol/L), disodium EDTA (0.005 mol/L), 8-hydroxyquinoline sulfate (0.0034 mol/L), and aprotinin (100 kallikrein inhibitory units per milliliter) (final con-

centrations in the incubation mixture). The renin inhibitor Ro 42,5892 was used in a final concentration of 10^{-5} mol/L. Inhibition of porcine kidney renin is virtually complete at this concentration.[17] Any remaining Ang I–generating activity may be assumed to be caused by enzymes other than renin.

With plasma samples, the Ang I generation during incubation was linear over time. However, the crude cardiac homogenates, the supernatants 1 and 2, and the pellet showed no detectable increase of Ang I over time. ^{125}I–Ang I added to a crude homogenate of left ventricular tissue (1:10 diluted in phosphate buffer, see "Extraction of Renin and Prorenin") was rapidly broken down at 37°C ($t_{1/2}$=0.9 minute, mean of two experiments). A small quantity of ^{125}I–Ang II was formed (less than 5% of added ^{125}I–Ang I in 30 minutes). The formation of ^{125}I–Ang II could be completely prevented by captopril (0.04 mmol/L), and the half-life of added ^{125}I–Ang I with captopril was similar to that without captopril ($t_{1/2}$=1.0 minute, mean of two experiments). This indicates that Ang I breakdown in the homogenates was mainly caused by degradation into fragments other than Ang II. Addition of the inhibitor mix that was used in the routine renin measurements prolonged the $t_{1/2}$ of ^{125}I–Ang I (and unlabeled Ang I) in the crude homogenates, but it was still less than 10 minutes. This was also true for the supernatants 1 and 2 and the pellet fraction. Thus, the lack of an increase of Ang I during incubation with angiotensinogen in the routine enzyme-kinetic assay was due to angiotensinase activity, which could not be blocked by the inhibitor mix. To solve these problems we used an acidification step before incubation.[27,29] One milliliter of sample was dialyzed at 4°C for 48 hours in 0.05 mol/L glycine buffer, pH 3.3, containing 0.001 mol/L disodium EDTA and 0.095 mol/L NaCl. This was followed by dialysis at 4°C for 24 hours against a 0.1 mol/L phosphate buffer, pH 7.4, containing 0.001 mol/L disodium EDTA and 0.075 mol/L NaCl. The content of the dialysis bags was then collected, and the volumes were adjusted to 1 mL with phosphate buffer. With the acid-pretreated extracts, the recovery of Ang I that was added to the extracts before incubation with renin substrate was better than 98% (n=4) after 6 hours of incubation at 37°C. Acid pretreatment therefore led to effective removal of angiotensinase activity.

Antibody-Trapping Enzyme-Kinetic Assay

In this assay Ang I degradation is prevented by the addition of an excess of Ang I antibody before incubation at 37°C with renin substrate.[30] We used the same Ang I antiserum as in the routine assay described above. The incubation mixture consisted of 100 μL sample, 200 μL renin substrate, 10 μL Ang I antiserum (final dilution, 1:4800), 14 μL inhibitor solution, and 90 μL 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. Incubation time was maximally 60 minutes. For quantification of the trapped Ang I, the incubate was diluted 20-fold with ice-cold 0.15 mol/L Tris buffer, pH 7.4, containing ^{125}I–Ang I.[31] The antiserum dilution (1:96 000) in the diluted incubate was similar to the dilution used to measure Ang I in the routine renin assay. The diluted incubate was kept at 4°C for 18 hours. By dilution and cooling, Ang I generation is stopped, Ang I dissociates from the antibody and can be displaced by ^{125}I–Ang I, and bound and free radioactivity can be measured. Recovery of Ang I that was added to acid-pretreated cardiac tissue extracts (n=4) before the incubation at 37°C was better than 98% after 30 and 60 minutes of incubation. Recovery of Ang I added to non-pretreated cardiac tissue extracts was less complete; it was 75% to 83% after 30 minutes of incubation and 63% to 69% after 60 minutes of incubation (n=4). Recovery of Ang I added to plasma was better than 98% after both 30 and 60 minutes of incubation (n=4).

Measurement of Prorenin

Prorenin was measured with the routine enzyme-kinetic assay as described above, after it had been activated to renin.

Different activation procedures were investigated. As described above, renin in the cardiac tissue fractions could not be measured with the routine enzyme-kinetic assay because of the high angiotensinase activity of these extracts. Acid pretreatment of the extracts led to destruction of angiotensinases and enabled us to make accurate measurements of Ang I–generating activity. However, acid pretreatment also causes (partial) activation of prorenin.[27] To activate prorenin by acid pretreatment, the samples were acidified at pH 3.3 followed by neutralization to pH 7.4, as described under "Measurement of Renin."

A second method was acidification to pH 3.3 followed by incubation with plasmin at pH 7.4. At low pH, plasmin inhibitors are destroyed,[27] and the added plasmin will cause activation of prorenin at neutral pH.[29] After dialysis for 48 hours at 4°C in 0.05 mol/L glycine buffer, pH 3.3, containing 0.001 mol/L disodium EDTA and 0.095 mol/L NaCl, the samples were quickly adjusted to pH 7.4 with 1 mol/L NaOH. Then, 0.2 vol plasmin solution (final concentration, 1 casein unit per milliliter) in 0.15 mol/L NaCl was added, and the mixture was incubated for 48 hours at 4°C.

A third activation method consisted of incubation of 1000 μL of sample for 48 hours at 4°C with 200 μL trypsin coupled to Sepharose (final trypsin concentration, 8.8×10^3 Na-benzoyl-L-arginine ethyl ester units per milliliter of sample).[26] After incubation the trypsin was removed by centrifugation.

To investigate whether the activation of prorenin with the various procedures we attempted was complete, we added purified recombinant human prorenin to the tissue extracts before the activation step.

Identification of Cardiac Ang I–Generating Activity as Renin

Renin inhibitors with high specificity can be applied to distinguish between "true" renin and "pseudorenin" (for instance, cathepsin D). We used the competitive specific renin inhibitor Ro 42,5892 (molecular weight, 631), which has IC_{50} values of 7×10^{-10} mol/L for human renin and 3.5×10^{-5} mol/L for bovine cathepsin D.[32]

We measured percent renin inhibition by Ro 42,5892 in the routine enzyme-kinetic assay at inhibitor concentrations ranging from 10^{-11} to 10^{-5} mol/L. The inhibition curves for the various cardiac tissue extracts were compared with those for plasma and kidney renin. Porcine kidney renin was prepared as described under "Extraction of Renin and Prorenin" and was used in a 1:1000 dilution.

Measurement of Angiotensinogen

Angiotensinogen was measured in the same samples as were used for the renin measurement. The angiotensinogen concentration was determined as the maximal quantity of Ang I generated during incubation at 37°C and pH 7.4 with an excess of porcine kidney renin in the presence of inhibitors of ACE and angiotensinases.[33] The incubation volume consisted of 25 μL cardiac tissue extract or plasma (the latter diluted 1:10 in phosphate buffer), 150 μL semipurified porcine kidney renin (see "Extraction of Renin and Prorenin"), which was diluted 1:50 in phosphate buffer, and 10 μL inhibitor solution (see "Measurement of Renin"). The assay conditions were chosen in such a way that Ang I formation was complete within 30 minutes.

Results

Recovery Experiments

Angiotensins

We measured Ang I and II losses during the homogenization, extraction, and HPLC separation of cardiac tissue by adding known amounts of both radiolabeled and unlabeled Ang I and II to the tissue before homogenization (n=5 for ^{125}I–Ang I, ^{125}I–Ang II, Ang I, and Ang II). Recovery from tissue was 40% to 70% and from plasma 80% to 100%. There was no significant difference in recovery between Ang I and II or between radiolabeled and unlabeled peptides, in accordance with previous studies.[34,35] Routinely, therefore, all angiotensin measurements were carried out with ^{125}I–Ang I as internal standard, and results were corrected for incomplete recovery.

Renin and Prorenin

As assessed with the routine enzyme-kinetic assay in acid-pretreated tissue extracts, practically all (>90%) porcine kidney renin added to frozen cardiac tissue before homogenization was recovered from the acid-pretreated extracts (left ventricular tissue, sum of supernatants 1 and 2 and pellet, n=4), and the same was true for trypsin-activated recombinant human prorenin (n=6). The antibody-trapping assay also showed complete recovery of added porcine kidney renin and trypsin-activated recombinant human prorenin in the acid-pretreated extracts (n=4) as well as in the non-pretreated extracts (n=4). Porcine kidney renin (n=4) and trypsin-activated recombinant human prorenin (n=6) added to plasma were also fully recovered.

Less than 5% of nonactivated recombinant human prorenin added to frozen left ventricular tissue was found to be activated after homogenization and extraction, as assessed by the antibody-trapping assay in the nonacidified extracts obtained from these tissues (n=4). The same was true for recombinant human prorenin added to plasma.

Ang I and II Content of Cardiac Tissue

Cardiac tissue Ang I levels (expressed per gram wet weight) were similar to the Ang I levels in blood plasma, whereas cardiac Ang II was higher than plasma Ang II (Table 1). The ratio of cardiac Ang II to Ang I was two to three times that in plasma. The plasma content of cardiac tissue amounts to 5% to 10% of wet weight.[36] The cardiac Ang I and II levels we measured are therefore too high to be explained by trapped blood plasma. The high Ang II–Ang I ratio, relative to plasma, in cardiac tissue was not caused by conversion of Ang I to Ang II after the tissue had been taken out nor by degradation of Ang I into other peptides than Ang II. This was demonstrated by addition of ^{125}I–Ang I immediately before homogenization of the tissue in 0.1 mol/L HCl/80% ethanol. Addition of ^{125}I–Ang I did not result in the appearance of any ^{125}I-labeled metabolite of ^{125}I–Ang I in the tissue extracts (Fig 1).

Keeping cardiac tissue for 30 minutes at room temperature before freezing did not significantly alter the results of the Ang I and II measurements, nor did it change the measured Ang II–Ang I ratios (Table 2). This indicates either that no metabolic breakdown of angiotensins occurred as soon as the heart had been removed from the body or that angiotensins were still produced at rates matching their breakdown.

Tissue Ang I and II were higher in the atria than in the ventricles (Table 1).

Cardiac tissue levels of Ang I and II were directly correlated with their levels in plasma (Fig 2). Thirty hours after bilateral nephrectomy, Ang I and II were below the detection limit of the assay (approximately 1

TABLE 1. Angiotensin I and II Content of Cardiac Tissue and Plasma

Tissue	Ang I, fmol/g	Ang II, fmol/g	Ang II–Ang I Ratio
Plasma	16.6 (1.8-237)	9.8 (1.4-191)*	0.51 (0.26-1.33)*
Left atrium	15.1 (2.9-105)†	25.7 (11.6-244)†	1.96 (0.56-3.69)
Left ventricle	11.1 (2.0-61.0)	14.7 (2.4-217)	1.33 (0.62-12.3)
Right atrium	21.6 (8.1-56.9)†	21.1 (9.2-182)†	0.98 (0.34-3.21)
Right ventricle	13.2 (6.4-31.8)	13.4 (2.5-128.8)	1.02 (0.39-9.75)

Ang indicates angiotensin. Data are geometric means and ranges (n=7).
*$P<.05$, cardiac tissue vs plasma (Mann-Whitney U test for paired observations).
†$P<.05$, atrium vs corresponding ventricle.

fmol/g) in both cardiac tissue and plasma (Fig 3). The Ang I and II levels were directly correlated in both cardiac tissue (left ventricle: $r=.70$, $P<.05$, n=7) and plasma ($r=.93$, $P<.05$, n=7).

Renin Content of Cardiac Tissue

Table 3 summarizes cardiac renin levels measured at neutral pH by the routine enzyme-kinetic assay, with acid pretreatment of the samples. Renin could not be measured with this assay without acid pretreatment because the tissue samples contained a high concentration of angiotensinase activity, which could not be inhibited by the enzyme inhibitors we used. We therefore tried the antibody-trapping enzyme-kinetic assay (see "Measurement of Renin") in the hope that the rapid breakdown of Ang I which occurs in the non-pretreated samples might be prevented by binding Ang I to the Ang I antibody. With this assay, we found Ang I generation at 37°C in the non-pretreated samples to be linear for 30 minutes. After 30 minutes the rate of Ang I generation began to decline. In Fig 4 the results obtained with the antibody-trapping assay in non-pretreated and acid-pretreated cardiac tissue extracts are compared with those obtained with the routine assay in acid-pretreated samples. Results of the two assays were not different.

All measurements of cardiac renin were made in both the supernatants 1 and 2 and the pellet fraction (see "Extraction of Renin and Prorenin"). The renin data presented in Table 3 were calculated by summation of the results obtained in these tissue fractions. In four crude homogenates of left ventricular tissue, renin was measured with the routine enzyme-kinetic assay, with acid pretreatment. Similar measurements were made in the supernatants 1 and 2 and in the pellet fraction prepared from these crude homogenates. The results showed that the sum of the amounts of renin in the latter three fractions was equal to the amount of renin in the crude homogenate (Table 4).

As shown in Table 3, the renin levels in cardiac tissue (expressed per gram wet weight) were similar to those in blood plasma, and like the angiotensin levels, the renin levels are too high to be explained by trapped blood plasma. The tissue renin levels were higher in the atria than in the ventricles, which was also true for the angiotensins. The renin level in cardiac tissue was directly correlated with its level in plasma (Fig 2). Thirty hours after bilateral nephrectomy, renin was below or close to the detection limit of the assay (approximately 2 fmol Ang I/min per gram) in both cardiac tissue and plasma (Fig 3).
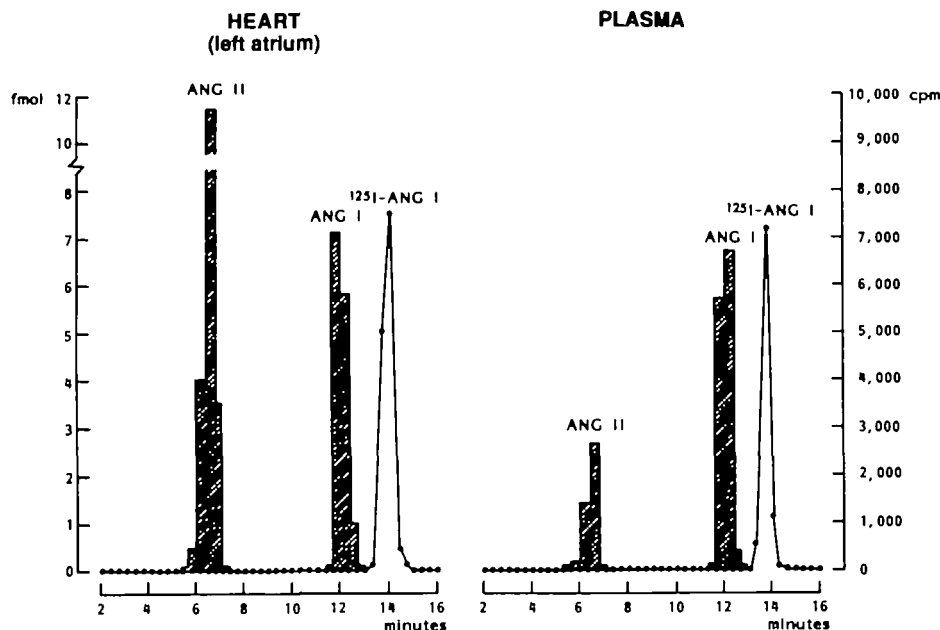

Fig 1. Chromatograms show high-performance liquid chromatographic elution profile of endogenous (hatched area) and radiolabeled (closed circles) angiotensins in porcine left atrial tissue extract (left) and plasma sample (right). ^{125}I-Angiotensin I was added as an internal standard during the purification procedure (see "Methods" for details). ANG indicates angiotensin.

TABLE 2. Effect of Delay of Freezing and Homogenization on Angiotensin I and II Content of Left Ventricular Tissue

Delay Period	Ang I, fmol/g	Ang II, fmol/g	Ang II–Ang I Ratio
No delay	21.3±13.4	14.3±6.5	0.83±0.31
30-Minute delay	28.5±29.0	17.3±9.6	0.86±0.34

Ang indicates angiotensin. Data are mean and SD (n=7).

The data presented in Tables 3 and 4 and Figs 2, 3, and 4 are corrected for Ang I–generating activity that was not caused by renin. The correction factor was calculated from measurements of the percentage of Ang I–generating activity that could be inhibited by the specific renin inhibitor Ro 42,5892 (10^{-5} mol/L). The inhibition curves (percent inhibition plotted against inhibitor concentration) of acid-pretreated porcine cardiac tissue extracts were similar to those of porcine kidney renin or plasma renin (Fig 5). The IC_{50} of Ro 42,5892 for porcine renin was approximately 5×10^{-8} mol/L, which is approximately two orders of magnitude higher than for human renin.[32] With the routine enzyme-kinetic assay it appeared that 91±12% (mean±SD, n=16) of Ang I–generating activity was inhibited by the renin inhibitor in acid-pretreated cardiac tissue fractions. With the antibody-trapping enzyme-kinetic assay, 88±2% (n=12) of Ang I–generating activity was found to be inhibited by the renin inhibitor in acid-pretreated cardiac tissue fractions and 74±19% (n=32) in non-pretreated fractions. Thus, most of the Ang I–generating activity we measured in the cardiac tissue fractions, both with and without acid pretreatment, was due to renin.

In non-pretreated plasma samples the Ang I generation was linear in both the routine and the antibody-trapping enzyme-kinetic assays and could be inhibited by more than 95% with the specific renin inhibitor Ro 42,5892. Ang I recovery was virtually complete, and the results were not different with the two assays. Thus, plasma renin could be reliably measured without acid pretreatment.

When the renin levels of the supernatants 1 and 2 and the pellet fraction were compared, it became apparent that the pellet fraction contained a substantial amount of renin even though the pellet had been extensively
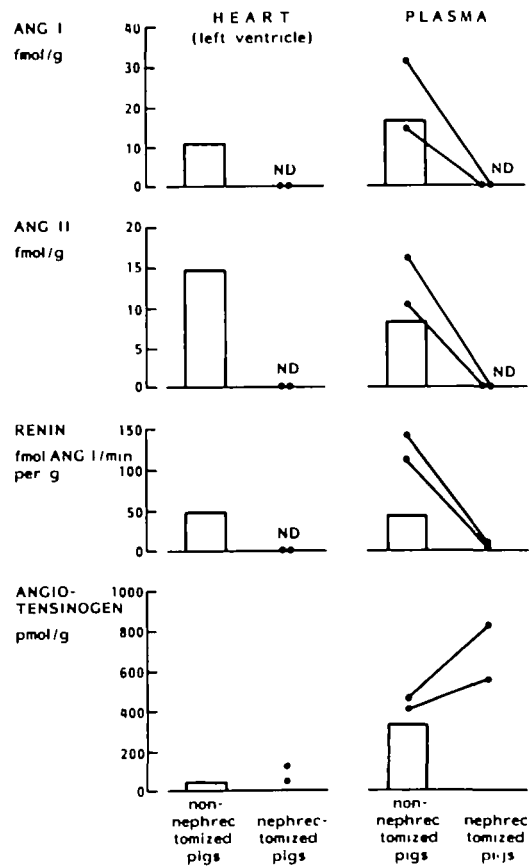

Fig 3. Graphs show left ventricular and plasma levels of angiotensin I (Ang I), Ang II, renin, and angiotensinogen in nonnephrectomized pigs (bars; n=7 for Ang I and II, geometric means; n=11 for renin and angiotensinogen means) and nephrectomized pigs (individual points). Plasma levels of Ang I, Ang II, renin, and angiotensinogen in nephrectomized animals before nephrectomy are also given. ANG indicates angiotensin; ND, not detectable.

washed. Table 4 gives the data for left ventricular tissue. It can be seen that the renin content of the pellet fraction was approximately 20% of the renin content in the supernatants 1 and 2. Similar results were obtained for right ventricular tissue and for right and left atria.

These results prompted us to investigate the pellet fraction in more detail and to prepare a more purified
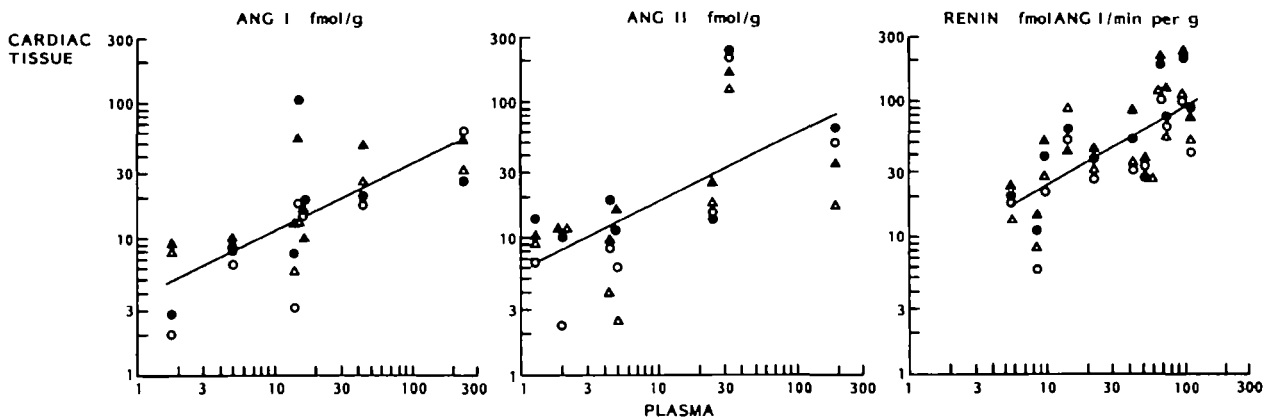

Fig 2. Plots show relation between cardiac tissue levels and plasma levels of angiotensin I (ANG I) (left; r=.70, P<.05), angiotensin II (ANG II) (middle; r=.64, P<.05), and renin (right; r=.72, P<.05). Closed circles indicate left atrium; open circles, left ventricle; closed triangles, right atrium; and open triangles, right ventricle.

TABLE 3. Renin and Angiotensinogen Content of Cardiac Tissue and Plasma

Tissue	Renin, fmol Ang I/min per gram	Prorenin, fmol Ang I/min per gram	Angiotensinogen, pmol/g
Plasma	46.0 (5.7-109)	27.7 (5.9-67.5)	340 (195-623)
Left atrium	74.5 (11.9-212)*	ND	71.8 (24.7-169)*
Left ventricle	46.6 (6.4-104)	ND	36.0 (4.1-64.8)
Right atrium	84.2 (15.1-215)*	ND	91.2 (28.8-176)*
Right ventricle	52.3 (8.4-118)	ND	57.8 (29.1-107)

Ang indicates angiotensin; ND, not detectable. Data are means and ranges (n=11).
*$P<.05$, atrium vs corresponding ventricle (Student's t test for paired observations).

cardiac membrane fraction. Despite repeated washings, the purified membrane fraction contained a measurable amount of renin (Table 5); it contained 12.2±2.9% (n=6) of the total amount of renin extracted from cardiac tissue (Fig 6). Expressed per gram protein, renin was enriched twofold to threefold in the purified membrane fraction (Table 5 and Fig 6). Treatment with 1% Triton X-100 dissolved virtually all renin present in this

fraction, thereby indicating that renin was bound to cell plasma, sarcoplasmic, or lysosomal membranes. It is possible that some dissolution of membrane-bound renin occurred during the washing and centrifugation procedures before treatment with Triton X-100. We therefore may have underestimated the quantity of membrane-bound renin.

ACE was measured as a marker of membrane-bound enzymes. The purified membrane fraction contained 20.3±5.0% (mean±SD, n=5) of the total amount of ACE extracted from left ventricular tissue. ACE was enriched fivefold to sixfold in the purified membrane fraction (Table 5 and Fig 6), and virtually all ACE activity in this fraction was dissolved by the addition of 1% Triton X-100, thereby confirming that ACE is a membrane-bound enzyme.

Prorenin Content of Cardiac Tissue

Prorenin was measured with the routine enzyme-kinetic assay at neutral pH after it had been activated to renin. As described under "Measurement of Prorenin," three activation procedures were examined: acid treatment, acid treatment followed by treatment with plasmin at pH 7.4, and treatment with trypsin-Sepharose at pH 7.4. Table 6 shows the results.

After trypsin-Sepharose treatment of the cardiac tissue extracts, Ang I generation was not detectable during incubation with renin substrate. We therefore were unable to measure prorenin reliably in trypsin-treated tissue extracts.

After acid treatment the Ang I generation during incubation with renin substrate was linear, and acid
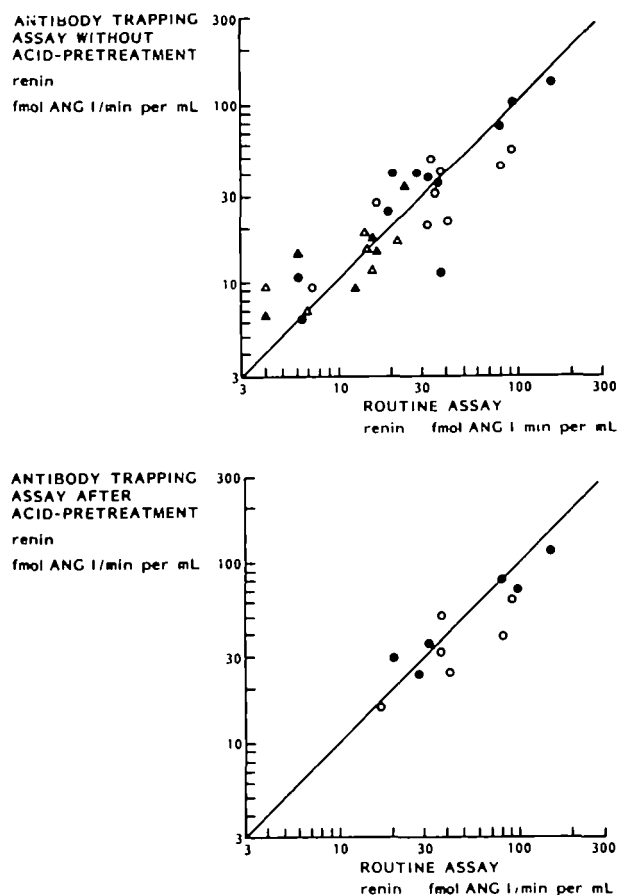

FIG 4. Plots show comparison between angiotensin I–generating activities measured in cardiac tissue by antibody-trapping enzyme-kinetic assay and routine enzyme-kinetic assay. Top, Antibody-trapping assay in non-pretreated extracts. Bottom, Antibody-trapping assay in acid-pretreated extracts. The routine assay was performed in acid-pretreated extracts. The relation between the two types of measurements is not different from the line of identity. ANG indicates angiotensin; closed circles, atria (supernatant 1); open circles, ventricles (supernatant 1); closed triangles, atria (supernatant 2); and open triangles, ventricles (supernatant 2). See "Methods" for details.

TABLE 4. Renin and Angiotensinogen Content of Various Left Ventricular Tissue Fractions

Tissue Fraction	Renin, fmol Ang I/min per fraction	Angiotensinogen, pmol per fraction
Crude homogenate	65.3	157.4
Supernatant 1	35.1	96.1
Supernatant 2	26.9	33.6
Pellet	12.0	9.8
Supernatants 1 and 2+pellet	75.0	139.6

Ang indicates angiotensin. Data are means (n=4). Supernatants 1 and 2 and the pellet were prepared from the crude homogenate. The sum of the contents of supernatants 1 and 2 and pellet is not significantly different from the content of the crude homogenate for both renin and angiotensinogen.
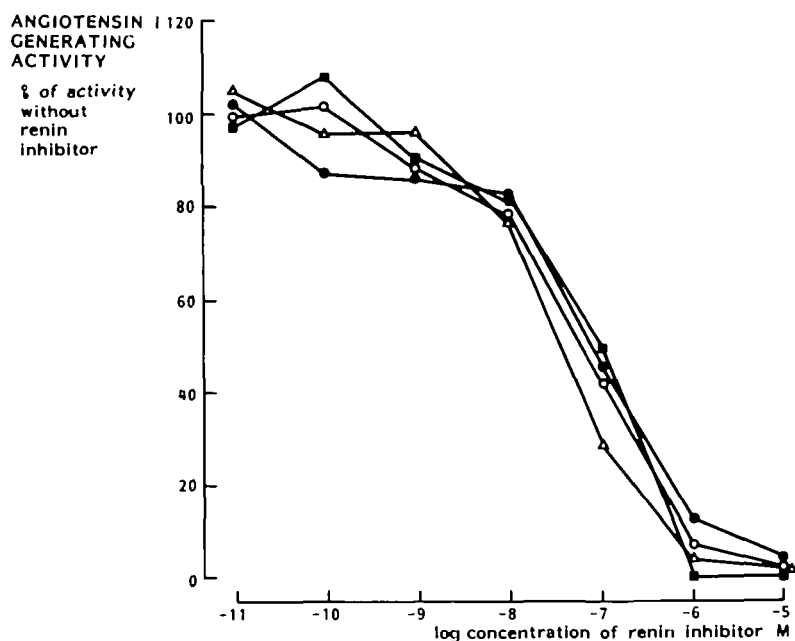
ANGIOTENSIN I
GENERATING
ACTIVITY

% of activity
without
renin
inhibitor

FIG 5. Line graph shows inhibition of angiotensin I–generating activity by increasing concentrations of the renin inhibitor Ro 42,5892. Closed circles indicate acid-pretreated crude cardiac tissue homogenate (n=3); closed squares, acid-pretreated purified cardiac membrane fraction (n=1); open circles, kidney renin (n=2); and open triangles, plasma renin (n=3).

treatment caused complete activation of added recombinant human prorenin without loss of prorenin (Table 6).

Acid treatment followed by treatment with plasmin at pH 7.4 yielded Ang I–generating activities that were 19±7% (mean±SD, n=12) lower than with acid treatment alone ($P<.05$, Table 6). Results for added recombinant human prorenin were 14±6% (n=4) lower with the combined acid and plasmin treatment than with acid treatment alone. It therefore appears that plasmin had caused some destruction of prorenin or renin.

With both the combined acid and plasmin treatment and the treatment with acid alone, more than 80% of the Ang I–generating activity was inhibited by the renin inhibitor Ro 42,5892 (Table 6).

As described above, renin in the acid-pretreated tissue extracts could be measured with both the antibody-trapping assay and the routine enzyme-kinetic assay. Both methods gave similar results. In non-pretreated extracts reliable measurements of renin were possible only with the antibody-trapping assay and not with the routine assay. Results obtained with the antibody-trapping assay in non-pretreated extracts were not different from those in acid-pretreated extracts. It therefore appears that the cardiac tissue extracts contained predominantly renin and little or no prorenin (Table 3).

In porcine plasma, treatment with trypsin-Sepharose was the most effective way to activate prorenin (Table

6). This confirms earlier findings on prorenin activation in bovine and human plasma.[26,29] Acid treatment and acid treatment followed by treatment with plasmin at neutral pH did not lead to an increase in Ang I–generating activity of porcine plasma (Table 6), whereas these procedures are known to activate prorenin in bovine and human plasma.[27,29] Ang I–generating activity was linear over time in both the routine and antibody-trapping enzyme-kinetic assays, and it was completely (>98%) inhibited with the renin inhibitor Ro 42,5892 (10^{-5} mol/L). The concentration of prorenin in plasma was 0.6±0.4 times (n=11) the concentration of renin (Table 3).

Angiotensinogen Content of Cardiac Tissue

The angiotensinogen levels in cardiac tissue (expressed per gram wet weight) were 10% to 26% of those in blood plasma (Table 3). Like the renin and angiotensin levels, the angiotensinogen levels are too high to be explained by trapped blood plasma (5% to 10% of wet weight).

All measurements of cardiac angiotensinogen were made in both the supernatants 1 and 2 and the pellet fraction. The angiotensinogen data presented in Table 3 were calculated by summation of the results obtained in these tissue fractions. As with renin, the sum of the amounts of angiotensinogen in the three fractions was equal to the amount of angiotensinogen in the crude

TABLE 5. Enrichment of Renin and ACE in Purified Membrane Fraction From Left Ventricular Tissue

Tissue Fraction	Renin, fmol Ang I/min per gram protein	Angiotensinogen, pmol/g protein	ACE, U/g protein
Plasma	591 (312-960)	11.6 (5.4-18.9)	1.4 (0.20-2.3)
Crude homogenate (H)	327 (60-523)	0.80 (0.55-1.5)	1.6 (1.1-3.0)
Purified membrane fraction (M)	798 (192-1044)	0.23 (0.04-0.53)	8.4 (3.8-15.0)
M-H ratio (relative specific activity)	2.7 (1.8-3.6)*	0.19 (0.06-0.65)*	5.4 (2.8-9.0)*

ACE indicates angiotensin-converting enzyme; Ang, angiotensin. Data are means and ranges (n=6-8).
*$P<.05$, difference from 1.0 (Student's t test for paired observations).
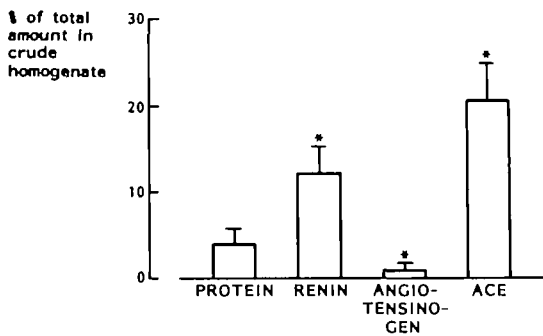
Fig 6. Bar graph shows total protein, renin, angiotensinogen, and angiotensin-converting enzyme (ACE) in purified cardiac membrane fraction. Values are percentage of the total amount in crude homogenate. *$P<.05$ compared with protein (n=6-8).

homogenate from which these fractions were prepared (Table 4).

Like renin and the angiotensins, angiotensinogen was higher in the atria than in the ventricles. Cardiac and plasma angiotensinogen levels did not correlate. Thirty hours after bilateral nephrectomy, plasma angiotensinogen had increased (Fig 3), and the left ventricular angiotensinogen levels in the nephrectomized animals were above the levels in nonnephrectomized animals.

The purified cardiac membrane fraction contained little or no angiotensinogen (Table 5 and Fig 6), suggesting that angiotensinogen is not bound by cardiac membranes.

Discussion

In the present study renin, angiotensinogen, Ang I, and Ang II were found to be present in cardiac tissue obtained from anesthetized, healthy pigs. Although the Ang I and II levels we measured in cardiac tissue were too high to be explained by trapped blood plasma, they were linearly correlated with the levels in the circulation. In fact, after bilateral nephrectomy, when Ang I and II in plasma were below the detection limit of the assay, their cardiac levels were also undetectable. It is not known whether the angiotensins in the heart are locally synthesized or derived from plasma. If one assumes that the angiotensins enter the tissue by simple

diffusion from plasma and that their localization in the tissue is restricted to the interstitial fluid compartment, their concentration in this compartment should not exceed that in blood plasma. In heart tissue the extracellular fluid compartment accounts for 20% to 25% of tissue weight. On the basis of the assumption that the angiotensins in the heart are restricted to this compartment, it can be calculated from our results that the Ang I and II levels would be 5 to 10 times higher in the extracellular fluid than in plasma. When the same assumption is applied to our results on angiotensinogen, the level of angiotensinogen in the extracellular fluid would be similar to that in plasma. Thus, it appears that the presence of angiotensins in the heart is not due to simple diffusion from plasma into the interstitium or, if it is due to diffusion, that their localization is not restricted to the extracellular fluid compartment.

With respect to angiotensinogen, however, our results are compatible with the assumption that this component of the RAS enters the tissue by diffusion from plasma and is mainly present in extracellular fluid. The lack of a significant correlation between the angiotensinogen levels in cardiac tissue and in plasma, a finding that contrasts with the results on Ang I, Ang II, and renin, may be attributed to the fact that the angiotensinogen results were within a narrow range compared with the levels of the angiotensins and renin. Another explanation might be that cardiac angiotensinogen is at least partly locally produced in the heart. Angiotensinogen mRNA has been detected in cardiac tissue homogenates.[11,12]

Are the cardiac Ang I and II levels we measured the true in vivo levels? We took precautions to prevent the ex vivo generation and breakdown of angiotensins. Cardiac tissue samples were frozen in liquid nitrogen immediately after the heart had been taken out, and the frozen tissue pieces were homogenized in 0.1 mol/L HCl/80% ethanol. The possibility that some ex vivo generation and breakdown of angiotensins occurred in the short time that elapsed before the tissue was frozen cannot be excluded. However, our observation that the Ang I and II levels were not significantly altered when cardiac tissue pieces were deliberately left for some time (30 minutes) at room temperature before they were frozen and extracted indicates that, if there was ex

TABLE 6. Effect of Various Procedures Known to Activate Prorenin on Angiotensin I–Generating Activity of Left Ventricular Tissue Fractions

Tissue Fraction	n	No Pretreatment		Acid Pretreatment		Plasmin Pretreatment		Trypsin Pretreatment	
		Activity, (fmol Ang I/min)/mL	Maximal Inhibition by Renin Inhibitor, %	Activity, (fmol Ang I/min)/mL	Maximal Inhibition by Renin Inhibitor, %	Activity, (fmol Ang I/min)/mL	Maximal Inhibition by Renin Inhibitor, %	Activity, (fmol Ang I/min)/mL	Maximal Inhibition by Renin Inhibitor, %
Plasma	3	54.0	98	42.4	85	30.8	92	90.0	99
Human prorenin (hPR)	1	14.4	98	326	97	307	97	131	99
Crude homogenate	4	ND		98.8	82	65.5	84	ND	
Crude homogenate+hPR	4	ND		491	89	387	91	ND	
Supernatant 1	4	ND		43.7	94	41.1	93	ND	
Supernatant 2	4	ND		14.1	92	10.3	91	ND	
Pellet	4	ND		8.9	83	8.6	92	ND	

Ang indicates angiotensin; ND, not detectable because of high angioteninase activity. Data are means. Prorenin activation procedures are described in more detail under "Measurement of Prorenin."

vivo generation of angiotensins, it could keep up, at least for some time, with their breakdown. Similar observations have been made on the ex vivo generation of angiotensins in the rat kidney.[1] These observations lend support to the validity of our angiotensin measurements as a true measure of the in vivo content of cardiac tissue.

The Ang II–Ang I ratio in cardiac tissue was higher than in plasma. This finding is in agreement with a number of reports on the levels of Ang I and II in various tissues, including the heart.[1,2,4,21] We could not measure Ang II in cardiac tissue 30 hours after nephrectomy. This is at variance with observations in rats, in which cardiac Ang II decreased by 50% in the first 24 hours and reached the detection limit after 48 hours.[21] Disappearance of cardiac angiotensins after nephrectomy may occur more slowly in the rat.

Reports on renin measurements in cardiac tissue are scarce, and it is not known whether it is really renin that was measured.[9,10] We used an enzyme-kinetic assay, which was based on the linear generation of Ang I at 37°C during incubation at neutral pH with an excess of renin substrate added to the tissue extracts. The specificity of the assay was assessed by addition of a specific renin inhibitor in a high concentration to the incubates, and a correction was made for any Ang I–generating activity that could not be inhibited. Ang I breakdown during incubation was prevented by acid pretreatment of the extracts or by rapid binding of the formed Ang I to Ang I antibody (antibody trapping).[30,31]

Acid pretreatment causes activation of prorenin, and this may have led to overestimation of the renin content of cardiac tissue and to underestimation of the prorenin content. However, measurements in non-pretreated tissue extracts with the antibody-trapping assay yielded results that were not different from those obtained in the acid-pretreated extracts with both the antibody-trapping and routine assays. Thus, the cardiac tissue extracts contained little or no prorenin. The porcine tissue homogenates contained trapped plasma, in which prorenin is known to be present. However, trapped plasma makes up for only 5% to 10% of cardiac tissue weight,[36] and the prorenin concentration of plasma is too low to contribute significantly to our renin measurements in cardiac tissue. Activation of cardiac prorenin during the homogenization and extraction procedures should be considered. However, the results of the antibody-trapping assay in the nonacidified cardiac tissue extracts showed practically no activation of human prorenin that was added to the tissue before homogenization and extraction. Also, in rat adrenal tissue and porcine reproductive organs, renin and not prorenin could be detected.[2,37]

As with Ang I and II, the renin content of the heart was much too high to be explained by trapped blood plasma. Along the same lines of reasoning that were followed to explain the angiotensin results, it can be concluded from our renin measurements that the presence of renin in cardiac tissue is not due to simple diffusion from plasma or, if it is due to simple diffusion, that the localization of renin is not restricted to the extracellular fluid compartment. That renin in cardiac tissue is not restricted to extracellular fluid is indicated by our finding that at least 12% of the total amount of renin in left ventricular tissue could be recovered from a purified membrane fraction. ACE, which is known to be a membrane-bound enzyme, was enriched fivefold to sixfold in the membrane fraction, compared with a twofold to threefold enrichment of renin. The difference in enrichment between ACE and renin may be due at least in part to dissolution of renin during the centrifugation procedure. ACE has a carboxy-terminal hydrophobic segment anchored to the cell membrane[38] and may be more tightly bound to the membrane than renin.

The direct and linear correlations of the renin levels in cardiac tissue with those in circulating plasma, together with the finding that the cardiac renin levels were practically zero 30 hours after bilateral nephrectomy when renin in plasma is also practically zero, are strong evidence that by far the most renin in cardiac tissue is derived from the circulation. As most, if not all, renin in plasma is of renal origin, this interpretation of our results implies that cardiac renin is also of renal origin. This conclusion is supported by studies showing low or undetectable renin mRNA levels in cardiac tissue from adult animals.[6-8] Uptake of renin in vascular tissue has been demonstrated in the early experiments by Loudon et al.[39] Studies in which bolus injections of radiolabeled renin were given to monkeys or rats showed that radioactivity accumulated not only in liver and kidney but also, albeit in lower amounts, in heart and blood vessels.[40,41] Recently, it was found that renin in human arteries was predominantly present in the endothelial layer, where it may have been taken up from the blood.[42] With the use of immunohistochemical techniques, it was shown that renin in the human left ventricle was present only in the endothelium of coronary blood vessels.[43] A renin-binding protein has been described in various organs, including the heart.[44] Recently, it was proposed that prorenin becomes catalytically active after binding to a cell receptor.[45] Receptor binding of prorenin would unfold its prosegment, and this renders the active site accessible to angiotensinogen. One may therefore speculate that membrane-bound renin is in fact "activated" prorenin. However, there are no supporting data for this interesting proposal yet.

We are aware of the fact that the present study is no proof of the cardiac production of Ang I at local tissue sites, although our results are certainly compatible with such local production. If local production is occurring, then it is clear from our data that most of it depends on kidney-derived renin that is taken up from the circulation. The direct correlations we observed between the levels of renin and the angiotensins in the heart and their levels in plasma are in agreement with this conclusion.

The dependency of cardiac Ang I production on renin from plasma is also supported by previous work from our group, which showed that the overflow of intracardially produced Ang I into the coronary circulation is directly proportional to the level of circulating renin.[46] It is also in agreement with experiments in the isolated perfused rat Langendorff heart, in which Ang I and II were detected in the coronary effluent only after renin had been added to the perfusion fluid.[13] In agreement with our observations in the pig, in a recent study in the rat the cardiac tissue levels of Ang I and II were also found to be reduced after nephrectomy.[21]

Taken together, the following sequence of events appears to emerge from our results. Renin, taken up by the heart from the circulation, acts in the tissue on angiotensinogen to form Ang I. Ang I is then locally converted to Ang II by ACE. Plasma-derived angiotensinogen in the interstitial fluid is a potential source of cardiac angiotensins.

Enzymes other than ACE may be involved in the conversion of Ang I to Ang II. In the human heart, for instance, a highly specific serine proteinase ("human heart chymase"), which converts Ang I to Ang II, has been demonstrated.[47] A similar enzyme may exist in porcine cardiac tissue.

The animals in our study were healthy 3- to 4-month-old pigs. It is possible that during fetal development or under pathological conditions some of the genes of the RAS are switched on in the heart. Cultured myocytes and fibroblasts from neonatal rat hearts contain mRNA for renin and angiotensinogen and release both peptides into the culture medium.[5] Cardiac angiotensinogen mRNA is increased in postinfarction left ventricular remodeling,[11] and ACE gene expression is stimulated during pressure overload–induced ventricular hypertrophy.[14]

The Ang II levels (expressed per gram tissue wet weight) that we measured in cardiac tissue extracts were somewhat higher than the levels in plasma. In the intact tissue the levels might have been even higher in some localized compartment. High Ang II concentrations are known to have chronotropic and inotropic effects[48] and may lead to arrhythmias[49] and myocyte necrosis.[50] A role of Ang II as a myocardial growth factor has also been suggested.[51] Finally, the therapeutic effect of ACE inhibitors in patients with congestive heart failure, an effect that may be at least partly independent of the effect of these compounds on blood pressure,[22] suggests that cardiac angiotensin production is involved in the development of heart failure.

Acknowledgment

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