Cardiac Angiotensinogen and Its Local Activation in the Isolated Perfused Beating Heart

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Increasing evidence suggests that the renin-angiotensin system modulates cardiovascular homeostasis both via its circulating, plasma-borne components and through locally present, tissue-resident systems with site-specific activity. The existence of such a system in the heart has been proposed, based on biochemical studies as well as on the demonstration of renin and angiotensinogen messenger RNA in cardiac tissue. We conducted the present study to determine whether biologically active angiotensin peptides may be cleaved within the heart from locally present angiotensinogen. Isolated, perfused rat hearts were exposed to infusions of purified hog renin; the coronary sinus effluent was collected and subsequently assayed for angiotensin I (Ang I) and angiotensin II (Ang II) by high-pressure liquid chromatography and specific radioimmunoassay. Both Ang I and II were undetectable under control conditions but appeared promptly after the addition of renin. Dose-dependent peak values for Ang I release ranged from 2.42±0.65 fmol/min to 1.38±0.18 pmol/min during renin infusions at concentrations between 10 microunits/ml and 5 milliunits/ml. Ang II levels measured in the perfusate reflected a mean fractional intracardiac conversion of Ang I to Ang II of 7.18±1.09%. Generation of Ang I and Ang II was inhibited in the presence of specific inhibitors of renin and converting enzyme, respectively. To investigate the source of angiotensinogen, we measured spontaneous angiotensinogen release from isolated perfused hearts. In the absence of renin in the perfusate, angiotensinogen was initially released in high, but rapidly declining, concentrations and subsequently at a low, but stable, rate. Prior perfusion with angiotensinogen-rich plasma resulted in enhanced early angiotensinogen release but did not alter the second, delayed phase, suggesting that, in addition to plasma-derived substrate, locally produced angiotensinogen may also participate in the intracardiac formation of angiotensin. Supporting this interpretation, hearts from animals pretreated with dexamethasone showed increased angiotensinogen messenger RNA concentrations as well as increased rates of angiotensinogen release not only during the early but also during the late phase. Our study newly demonstrates that Ang I and II may be formed within the isolated heart from locally present substrate, which appears to be derived in part from the circulating pool and in part from endogenous synthesis. These findings add support to the concept of a functionally active and locally integrated cardiac renin-angiotensin system and emphasize its potential physiological and pathological relevance. (Circulation Research 1990;67:564–573)

There is increasing recognition that the renin-angiotensin system exerts its modulatory influences on circulatory homeostasis not only through its circulating, plasma-borne components but also through generation and activation of these same components at a local level in various tissues.1-3 The complete catalytic cascade for the formation of angiotensin has been identified in a number of organs4-7 as well as in individual cell lines,8-11 suggesting the need for a redefinition of the renin-angiotensin system as representing a circulating endocrine as well as an almost ubiquitous paracrine or autocrine mediator

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system. Among tissue renin-angiotensin systems, the brain renin-angiotensin system has been investigated most extensively, providing evidence for both site-specific and systemic effects as well as for the potential physiopathologic role of such systems.12–14

The recent identification of components of the renin-angiotensin system in the heart7,15,16 is of particular interest 1) in view of the central role this system is known to play in circulatory homeostasis, 2) because of the unique therapeutic effectiveness of converting enzyme-inhibitors in cardiac failure,17–19 and 3) with respect to the long-recognized direct cardiomyotropic and neuromodulatory actions of angiotensins.20 Demonstration of gene expression of both renin and angiotensinogen in cardiac tissue16,21,22 was a pivotal step in documenting the potential for intracardiac synthesis of both the rate-limiting enzyme and its substrate. Based on these data, the existence of an intrinsic cardiac renin-angiotensin system has been proposed.

Demonstration of the components of the renin-angiotensin system in a given tissue does not, however, necessarily imply functional integration of its separate elements into a locally independent and biologically functional entity. Addressing one aspect of this question, we performed the present study to investigate whether locally present angiotensinogen can be activated within the heart to angiotensin I (Ang I) and angiotensin II (Ang II) via classic catalytic pathways.

Materials and Methods

Experimental Animals and Isolated Heart Preparations

Female Wistar-Kyoto rats (150–200 g) were used for all experiments. One hour before decapitation, they received an intraperitoneal injection of heparin sodium at a dose of 1,000 units/kg. The thoracic cavity was opened, and the heart was arrested with cold (4°C) normal saline and rapidly excised. The ascending aorta was tied into a perfusion cannula and mounted in a custom-built Langendorff apparatus.23 Perfusion was begun immediately using Krebs-Henseleit solution containing (mM) NaCl 113.8, NaHCO3 22, KCl 4.7, KH2PO4 1.2, CaCl2 2.5, MgSO4 1.1, and glucose 11 at a constant flow of 4 ml/min. This flow rate was chosen due to limitations in the amount of purified renin available and after pilot studies had shown no difference in the amount of Ang I generation at flow rates of 4 and 12 ml/min, the latter representing a more physiological value. The buffer was equilibrated with 95% O2-5% CO2, held at a constant temperature of 37°C, and titrated to a pH of 7.4. To maximize constancy of experimental conditions, we used a setup that allowed simultaneous perfusion of five hearts and collection of individual coronary sinus effluents. In each experiment, an additional cannula was perfused in parallel with the hearts according to the same protocol, serving as a blank channel from which background levels were determined. Background levels averaged 3.00±1.14% of signal. We used two side ports in the system to infused renin and inhibitors.

Experimental Protocols

After an equilibration period of 20 minutes, during which only Krebs-Henseleit solution was perfused, an infusion of hog renin at various concentrations was started. We exposed five hearts to renin at each of the following concentrations: 10, 50, and 200 microunits/ml, and 1 and 5 milliunits/ml. The duration of renin infusions ranged from 10 minutes at the highest concentrations to 3 hours at the lowest. Throughout the infusion period we collected fractions of the coronary sinus effluent. We chose a fraction volume of 8 ml (representing a collection period of 2 minutes) for the highest concentration, a volume of 20 ml (i.e., 5 minutes) for two intermediate concentrations, and a volume of 40 and 80 ml (i.e., 10 and 20 minutes, respectively) for the two lowest concentrations. In addition, a 10-minute fraction was collected at the beginning and at the end of each experiment while no renin was infused. In an additional experiment without infusion of renin, fractions of the effluent were collected over 1 hour and pooled. Fractions were immediately cooled to 2–4°C by passage through cooling coils immersed in an ice bath and maintained at that temperature until further processing, which took place within the next 2 hours. Samples from the blank channel were treated identically. The purified hog renin used in all experiments had been prepared in our laboratory according to a modification24 of previously published methods.25,26

In separate experiments, again employing five hearts each, we infused renin at a concentration of 200 microunits/ml simultaneously with 5×10−5 M HOE S850057, a renin inhibitor with an IC50 for hog renin of 3×10−7 M. After collection of two 5-minute fractions of the coronary sinus effluent, infusion of the inhibitor was terminated, and the renin infusion alone continued for an additional 20 minutes, during which further fractions were collected. In a similar fashion, we also conducted experiments using parallel perfusion of renin and the converting enzyme inhibitor captopril at a concentration of 10−6 M.

In an additional series of experiments, spontaneous release of angiotensinogen from the isolated heart was examined. From five hearts perfused with buffer only, 10-minute fractions of the coronary effluent were collected after a 30-minute equilibration period (to maintain constancy with other protocols) and subsequently assayed in vitro for angiotensinogen. Similar measurements were performed after the hearts had been exposed to a 10-minute infusion of angiotensinogen-rich plasma prepared from 24-hour–nephrectomized rats, followed again by a 30-minute equilibration period. In an additional experiment, rats were given a 7 mg/kg dose of dexamethasone or an equal volume of saline intraperitoneally 9 hours before decapitation. After subjecting the hearts to extracorporeal perfusion and collection of the effluent for angiotensinogen overflow measurements as above, these hearts were dis-
sected into right and left atria and ventricles and were snap-frozen for subsequent angiotensinogen messenger RNA (mRNA) determinations. Similarly, the livers of these rats were excised and stored at −80°C for analysis in parallel with the cardiac tissue samples.

Biochemical Analysis

Within 2 hours after completion of each experiment, fractions were applied to OSD silica C₁₈ cartridges (Sep-Pak, Waters Associates, Milford, Mass.) from which peptides were eluted using a previously described method. Eluates were subsequently taken to dryness in a vacuum centrifuge. The dry residues were dissolved in 0.1 M Tris acetate buffer containing 1 mM phenylmethylsulfonyl fluoride and 0.1% bovine serum albumin at pH 7.4. Aliquots of each sample were subjected to radioimmunoassay (RIA) for Ang I and II. The sensitivity for determination of Ang I and II was 1 pg. The Ang I antibody showed 0.05% cross-reactivity with Ang II, and the Ang II antibody had 1% cross-reactivity with Ang I and 100% cross-reactivity with des-Asp Ang II. For the Ang I RIA, we measured free radioactivity after binding to charcoal, whereas the low concentrations of Ang II made it necessary to modify the assay by adapting a double-antibody technique with determination of bound radioactivity.

To confirm the specificity of measurements made by RIA, aliquots of the perfusate were loaded onto a 7-μm Nucleosil C₁₈ column (Macherey, Nagel and Co., Düren, FRG) and subjected to high-pressure liquid chromatography (HPLC) (Waters). The elution was carried out using a linear methanol gradient (30% to 80% over a period of 35 minutes) in 10 mM ammonium acetate buffer, pH 5.4, at a flow rate of 1 ml/min. Fractions of 500 μl each were collected and assayed by RIA for Ang I and II. Synthetic Ang I and II were used for calibration of the HPLC column, as well as for determination of recovery, which was found to be 78% for Ang I and 91% for Ang II.

Angiotensinogen concentrations in the coronary sinus effluent were measured by in vitro incubation in the presence of 200 microunits/ml purified hog renin for 1 hour at 37°C. Samples were subsequently processed over OSD silica cartridges, lyophilized, and assayed for Ang I as described above.

mRNA Determinations

Angiotensinogen mRNA concentrations were measured by specific-solution hybridization assay as described previously. Briefly, total mRNA was extracted from tissues by using homogenization in a LiCl/urea buffer, followed by phenolization and ethanolic precipitation. Assays were performed using either a 716 base pair BamHI or a 289 base pair BamHI/Pvu II rat angiotensinogen complementary DNA fragment cloned into the polylinker site of pSPT18 and pGEM4, respectively. These fragments were used to prepare radiolabeled complementary RNA probe (using in vitro transcription from the SP6 promoter in the presence of [α-32P]UTP) and unlabeled synthetic mRNA (from the T7 promoter, used as standard). Aliquots of the RNA to be analyzed were ethanol-precipitated in an Eppendorf tube (Eppendorf Inc., Fremont, Calif.), washed, and dried. The RNA pellet was dissolved in 30 μl of 40% formamide, 400 mM NaCl, 1 mM EDTA, and 40 mM PIPES, containing 30,000 cpm freshly labeled angiotensinogen complementary RNA. The mixture was covered with two drops of paraffin oil and incubated in a 68°C water bath for 20 hours. Nonannealed RNA was subsequently digested during a 1-hour incubation at 30°C after the addition of 300 μl of a buffer containing 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl (pH 7.5), 40 μg/ml RNase A, and 2 μg/ml RNase T1 to each tube. This reaction was stopped by the addition of 1 ml ice-cold 10% trichloroacetic acid and 1.5% NaH₂PO₄. Subsequently, the contents of each tube were filtered over nitrocellulose membranes prewetted with the same solution. Filters were washed twice, dried, and immersed in 3-ml scintillation fluid; radioactivity was counted in a liquid scintillation counter. Tissue angiotensinogen mRNA concentrations were calculated using a calibration curve derived from parallel hybridization of known amounts of synthetic angiotensinogen mRNA, using a factor of 2.5 or 6.2 to correct for the difference between full-length angiotensinogen mRNA (1.8 kilobase) and the two probes (716 and 289 bp) used. The specificity of this solution hybridization assay has previously been validated against Northern blotting and RNase protection assay and was confirmed in our experiments by repeated RNase protection assay, using a modification of the protocol described previously.

Statistical Analysis

Raw data were corrected for cross-reactivity and nonspecific background by subtracting the respective blank values and for recovery by adjustment with the appropriate factor. Peptide release into the coronary effluent was then calculated as femtomoles of recovered material per minute for each individual collection period (fraction). For each heart, the fractional conversion of Ang I to Ang II was determined for individual collection periods by calculating the ratio of Ang II to Ang I. For each experiment, results are expressed as the mean±SEM of data from five hearts. We used analysis of variance to compare effects of different concentrations of renin on peak values for the release of Ang I and II. Post hoc analysis of individual group differences was carried out using Scheffe’s test. To test for dose-effect relations, we calculated Pearson correlations after log transformation of raw data. Effects of converting enzyme inhibitor and renin inhibitor were assessed by comparing preinhibition and postinhibition values in each group using paired Student’s t-test. A value of p<0.05 was considered statistically significant. We have not repeated p values in the text when they appear in a figure. Lack of a p value indicates that a statistically significant difference does not exist.
Results

Renin-Induced Release of Ang I

The pooled and extracted effluent from a 1-hour perfusion in the absence of renin contained no measurable Ang I (data not shown). Similarly, no detectable levels of Ang I were present in the coronary sinus effluent during the 10-minute collection period preceding the renin infusion in each experiment. Immediately after the start of the renin infusion, however, Ang I appeared in the perfusate (Figure 1). Peptide concentrations showed a consistent pattern of a rapid rise followed by an initially steep and subsequently more gradual decline (Figure 2). Maximum Ang I release occurred earlier at higher renin concentrations (Figure 2) and showed a positive, statistically significant correlation with the concentration of renin infused (p<0.005, Figure 2, insert). Cumulative amounts of released Ang I were not significantly different at the three highest concentrations of infused renin (3.19±1.21, 4.74±1.85, and 3.76±1.14 pmol at renin concentrations of 0.2, 1, and 5 milliunits/ml, respectively) but were lower at renin infusions of 10 and 50 microunits/ml (0.11±0.03 and 0.55±0.17 pmol/ml, respectively).

To test for the specificity of the observed release of Ang I in response to renin infusions, we simultaneously infused renin at a concentration of 200 microunits/ml and the investigational pentapeptide renin inhibitor HOE 8810057 at a concentration of 5×10^-6 M (Figure 3A). No Ang I was detectable in the perfusate collected during this first phase of the experiment. After 8 minutes, we stopped the renin inhibitor but continued the infusion of renin. The fractions of the coronary sinus effluent collected during this second phase of the experiment showed a steady increase in the amount of released Ang I, which reached levels not statistically different from those seen with the same dose of renin in the absence of inhibitor. The slope of the increase in peptide concentration in the perfusate, however, was not as steep as in control experiments without inhibitor.

Generation of Ang II

Ang II, like Ang I, was also undetectable in the cardiac perfusate during the 10-minute control period before exposure to renin. Similarly, it promptly appeared in the coronary sinus effluent after the renin infusion was started (Figure 1). Using

![Figure 1](http://circres.ahajournals.org/)

**FIGURE 1.** Representative example of the time course of angiotensin I (ANG I) and angiotensin II (ANG II) recovery in the coronary sinus effluent of isolated perfused hearts in response to renin infusion (data shown represent infusion of renin at 1 milli-unit/min; arrowhead marks the start of the infusion). Figure inset shows results of radioimmunoassay for ANG I and ANG II performed on high-performance liquid chromatography fractions of coronary sinus effluent sample.

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2.** Time course of angiotensin I (ANG I) release in response to renin infusion at different concentrations. For clarity, only three of five concentrations used are shown. Figure inset shows correlation of concentration of infused renin and peak rate of mean ANG I formation/release.
Figure 3. Panel A: Angiotensin I (Ang I) formation and release during renin infusion (200 microunits/ml) in the absence and presence of renin inhibitor HOE 5850057. HOE 5850057 infusion was begun 3 minutes before starting the renin infusion (indicated by arrowhead) and was discontinued 5 minutes thereafter while infusion of renin was continued. Panel B: Angiotensin II (ANG II) formation and release during renin infusion (1 milli-unit/ml) in the absence and presence of a concomitant infusion of $10^{-6}$ M captopril.

simultaneous determinations of Ang I and II in the perfusate, we calculated the mean fractional conversion rate of Ang I to Ang II as 7.18±1.09%.

To test if formation of Ang II was dependent on the classical pathway involving converting enzyme, we simultaneously infused renin at a concentration of 200 microunits/ml and the converting enzyme inhibitor captopril at a concentration of $10^{-6}$ M (Figure 3B). Ang I levels measured in the perfusate were similar to those found in previous experiments, whereas generation of Ang II was almost completely suppressed.

Specificity of Peptide Determinations

As an additional test for specificity of peptide determinations, samples of the coronary sinus effluent were processed over HPLC. We confirmed coelution of peptide peaks from our experimental samples with those of synthetic Ang I and II standards at 50 and 33 minutes, respectively (Figure 1, insert). A small additional peak at 27 minutes, demonstrating competition for the Ang II radioligand, coeluted with synthetic des-Asp Ang II.

Angiotensinogen Release

To investigate the spontaneous release of angiotensinogen from the heart we measured in vitro generation of Ang I by collecting 10-minute fractions of the coronary sinus effluent and incubating them at 37°C for 1 hour in the presence of renin. The data obtained show two distinct phases of release: during the first hour, initially high amounts of angiotensinogen release declined steeply; thereafter, release stabilized asymptotically at a low level (Figure 4).

To further examine the nature of this release and to delineate the contribution to overall release by angiotensinogen adsorbed from the circulating pool, we added angiotensinogen-rich plasma from 24-hour-nephrectomized rats to the perfusion buffer for 10 minutes. After termination of this infusion, early rate of release was markedly higher, and the slope of its decline was steeper than in control experiments (Figure 4, dotted curve); during the second phase, however, release was not different from that observed under control conditions.

Next, we measured angiotensinogen concentrations in the effluent from hearts of rats pretreated with dexamethasone, a potent stimulus for hepatic as well as extrahepatic angiotensinogen synthesis. We reasoned that this maneuver would increase not only the circulating (primarily liver-derived) pool but also local tissue angiotensinogen synthesis. As expected, release during the initial phase was markedly increased in hearts of dexamethasone-treated rats.
compared with hearts of sham-treated rats. However, in contrast to the above-mentioned experiments using perfusion with angiotensinogen-rich plasma, we also found a sustained, statistically significant increase of angiotensinogen overflow during the second, late phase of release in the dexamethasone-exposed hearts (Figure 4, bold curve).

**Tissue mRNA Determinations**

Angiotensinogen mRNA concentrations in the cardiac chambers and liver, as determined by solution hybridization assay, were comparable with previously measured values.28 Specificity of the solution-hybridization assay was confirmed by RNase protection assay (Figure 5). After dexamethasone treatment, angiotensinogen mRNA concentrations were significantly increased \((p > 0.05)\) in all four cardiac chambers, along with increased hepatic angiotensinogen mRNA concentrations (Figure 6). The increase was most pronounced in the right and left atrium, followed by the right and left ventricle.

**FIGURE 4.** Graph showing angiotensinogen release from isolated, perfused hearts under baseline conditions, after a 10-minute perfusion with angiotensinogen-rich plasma, and after pretreatment of experimental animals with 7 mg/kg i.p. dexamethasone. ANG I, angiotensin I. *Statistically significant differences between baseline and dexamethasone-stimulated angiotensinogen release during the late phase of angiotensinogen release \((p<0.05)\).

**FIGURE 5.** RNase protection assay validating specificity of hybridization between probe and target RNA in solution hybridization assays. Lane 1: Molecular weight marker (pUC19 digested with SauIII). Lane 2: [\(^{32}\)P]-labeled 289 bp complementary RNA probe, no RNase treatment. Lanes 3–6: RNase-treated hybridization products with transfer RNA (lane 3), 2.5 \(\mu\)g hepatic RNA (lane 4), and 300 \(\mu\)g (lane 5) and 500 \(\mu\)g (lane 6) left ventricular RNA. The apparent size difference between radiolabeled probe and specific hybridization products is due to the presence of a noncomplementary region encoded by the multiple cloning site of pGEM4 in native complementary RNA.
Discussion

Our understanding of the renin-angiotensin system has evolved over the past years to encompass not only its circulating elements as a classical hormonal system but also a growing number of organs in which local tissue renin-angiotensin systems have been demonstrated. Although the existence of a number of such systems has been confirmed by both biochemical and gene expression studies, there is little information available about their functional integration. Limited and indirect evidence, primarily from studies on the brain renin-angiotensin system, suggests that locally present components may in fact be linked to independently functioning and regulated units.12,13,32 In the present study we provide several lines of evidence indicating the potential for the functional local integration along classic catalytic pathways of a tissue renin-angiotensin system that has been identified in the mammalian heart, hitherto referred to as “cardiac renin-angiotensin system” (although the precise localization of its components within the heart remains obscure at present). Angiotensinogen was found to be locally present in the heart, spontaneously released into the coronary vasculature, and subject to enzymatic cleavage by a single-pass renin infusion. The resulting Ang I was demonstrated to be further converted to Ang II, the actual effector peptide. In addition, both enzymatic steps were found to be subject to pharmacological intervention, opening up the possibility that inhibitors of the renin-angiotensin system may have a local cardiac mechanism of action.

Our experiments demonstrate that perfusion of the isolated, beating heart with renin causes dose-dependent appearance of Ang I in the coronary sinus effluent. Undetectable levels of Ang I in the perfusate before exposure to renin as well as suppression of Ang I release in the presence of a renin-inhibitor strongly argue that the observed effects are mediated specifically by renin. In all experiments in which renin was infused, Ang I formation peaked early on and decreased steeply thereafter, suggesting the one-time processing of a nonreplenished pool of renin substrate, presumably sequestered previously from plasma. When renin infusions were maintained for longer time periods, however, an asymptotic plateau of angiotensin release at low rates became evident. (Due to limited availability of the purified renin, such prolonged infusions were only performed at low concentrations.) This second phase of rather stable peptide formation may represent the effects of infused renin on ongoing local synthesis and (constitutive) secretion of angiotensinogen.

To further test this hypothesis, we performed direct measurements of spontaneous release of angiotensinogen from the isolated perfused heart. We found a pattern of release similar to that observed in experiments employing renin infusions: during an early phase large, but rapidly declining, amounts of angiotensinogen appeared in the effluent, whereas a second, more prolonged period was characterized by the release of lower, but relatively constant, amounts. We interpret the initial phase as the washout of plasma-derived angiotensinogen still present in the heart. Calculated cumulative data suggest that this pool has a fairly constant size and is subject to earlier and progressively faster conversion to Ang I during single-pass exposure to increasing concentrations of renin within the coronary vasculature. In keeping with the hypothesis advanced above, the second, delayed phase of angiotensinogen release at a relatively low rate may represent overflow of locally synthesized protein. An alternative explanation, however, would be that this phase indicates the presence of a two-compartment system in which release of exogenously derived and sequestrated angiotensinogen occurs along two different equilibration kinetics. To differentiate between these two possibilities, we examined the effects of perfusion with angiotensinogen-rich plasma and treatment with dexamethasone on angiotensinogen release. Although both of these maneuvers raise circulating angiotensinogen levels, only the latter will result in stimulation of local angiotensinogen synthesis. If both early- and late-phase release were simply a function of sequestration and subsequent release of exogenous angiotensinogen, then both experimental conditions should similarly affect early and late phases of angiotensinogen release. If, however, the rate of angiotensinogen release during the delayed phase depended on local synthesis of angiotensinogen, then no changes would be expected in response to plasma perfusion, whereas dexamethasone exposure should result in sustained increases of angiotensinogen overflow during this phase. As expected, both perfusion with angiotensinogen-rich plasma and treatment with dexamethasone resulted in accentuated peak rates of early angiotensinogen release, which declined along steeper angiotensinogen release, which declined along steeper
nonreplenished pool, augmented by previous exposure to elevated levels of circulating angiotensinogen. While plasma perfusion had no effect on delayed-phase angiotensinogen release, stimulation of local angiotensinogen synthesis by dexamethasone treatment resulted in a significant and sustained increase of late-phase angiotensinogen release, despite the fact that initial rates of release were actually substantially higher after plasma perfusion. These data argue against the notion that late-phase release reflects liberation of previously sequestered, exogenous angiotensinogen from a second compartment. Instead, they indicate that the source of angiotensinogen released during the delayed phase is local synthesis and that the rate of this release is modulated by regulatory influences affecting the level of local angiotensinogen synthesis. This interpretation is corroborated and supported by the results of gene expression studies included in the present investigation, which document increased intracardiac levels of specific angiotensinogen mRNA after dexamethasone.

Peak Ang I concentrations measured when renin was infused at physiological concentrations (10 and 50 microunits/ml) were 0.52 and 4.25 pM, respectively, which is about one order of magnitude lower than normal plasma levels. This may reflect the limited amount of angiotensinogen available for conversion but may also be due in part to the use of a heterologous enzyme rather than a species-specific one. Also, by collecting 10-minute fractions of the coronary sinus effluent, we obtained average values that may not represent true peaks. By measuring only the amount of peptide present in the coronary sinus effluent, we may have further underestimated the amount generated within the heart. Ang I may be processed to Ang II intracellularly, limiting the amount that reaches the coronary sinus. In addition, Ang I secreted into the coronary circulation may be subject to reuptake into smooth muscle cells or cardiac myocytes, as suggested by Khairallah et al. Thus, local concentrations at the angiotensin receptor site might be considerably higher than apparent from our measurements and, thus, of potential physiological significance. Future studies using models in which functional parameters can be assessed will have to address this question. In addition to the effects of circulating renin examined in this investigation, local cardiac tissue renin, the presence of which is strongly suggested by the results of biochemical, immunologic, and molecular biological studies, may act on angiotensinogen taken up from the circulating pool or intrinsically produced within the heart.

The ratio between Ang I and Ang II observed in our study closely matches previous results obtained in a different system in which Ang I itself was infused. Inhibition of the conversion of Ang I to Ang II in the presence of a specific converting enzyme inhibitor indicates that the reaction was catalyzed by converting enzyme, most likely at the luminal surface of the coronary endothelium. However, both vascular smooth muscle cells and cardiac myocytes have been shown to contain the enzyme and must therefore also be considered potential sites of action. As discussed with respect to Ang I, total amounts of Ang II may have been underestimated in our study since we did not test for possible further enzymatic degradation of the peptide.

Several lines of evidence point to the potential importance of our findings. The recognized positive inotropic effects of Ang II, and perhaps also of Ang I indicate a possible influence of local cardiac angiotensin generation on contractile function. Its pronounced direct vasoconstrictor effects on the coronary vasculature may importantly influence myocardial perfusion. The facilitatory role of Ang II on sympathetic neurotransmission in the heart is well documented and may further enhance the aforementioned effects. Experimental evidence that converting enzyme inhibitors limit infarct size and suppress reperfusion arrhythmias emphasizes the possible functional relevance of an intrinsic cardiac renin-angiotensin system. Hence, the unique therapeutic effectiveness of converting enzyme inhibitors in congestive heart failure may in part be related to their modulatory influence on this system. Our demonstration that local activation of angiotensin within the heart may be modified by specific inhibitors raises the possibility of a cardiac site of action of these drugs.

In summary, our study confirms and extends previous evidence for the existence of a local cardiac renin-angiotensin system. Our data provide new evidence that the activity of this system is governed by classic catalytic pathways, thus suggesting the potential for its local integration as a functional paracrine or autocrine mediator system. In addition, our findings indicate that intracardiac angiotensinogen is derived from both the circulating pool and local synthesis and that local synthesis is capable of responding to regulatory stimulation. Our investigation was not intended to draw conclusions as to hemodynamic or other functional effects of the cardiac renin-angiotensin system, nor does it address the question concerning the precise cellular and subcellular localization of its components within the heart. The synthesis of results from a range of different, complementary approaches will be necessary to obtain a data base that will ultimately enable us to answer these questions and to assess the overall physiological relevance of this system. The knowledge gathered about the cardiac renin-angiotensin system so far, though, indicates that the continued study of this system promises to be an exciting and fruitful area of cardiovascular research.

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