Compartmentalization of Angiotensin II Generation in the Dog Heart

Evidence for Independent Mechanisms in Intravascular and Interstitial Spaces

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Abstract

Angiotensin-converting enzyme inhibitors have beneficial effects that are presumably mediated by decreased angiotensin II (ANG II) production. In this study, we measure for the first time ANG I and ANG II levels in the interstitial fluid (ISF) space of the heart. ISF and aortic plasma ANG I and II levels were obtained at baseline, during intravenous infusion of ANG I (5 μM, 0.1 ml/min, 60 min), and during ANG I + the angiotensin-converting enzyme inhibitor captopril (cap) (2.5 mM, 0.1 ml/min, 60 min) in six anesthetized open-chested dogs. ISF samples were obtained using microdialysis probes inserted into the left ventricular myocardium (3–4 probes/dog). ANG I increased mean arterial pressure from 102±3 (SEM) to 124±3 mmHg ($P < 0.01$); addition of cap decreased MAP to 95±3 mmHg ($P < 0.01$). ANG I infusion increased aortic plasma ANG I and ANG II (pg/ml) (ANG I = 101±129 to 370±158 pg/ml, $P < 0.01$; and ANG II = 22±40 to 466±49, $P < 0.01$); addition of cap further increased ANG I (1,790±158, $P < 0.01$) and decreased ANG II (33±49, $P < 0.01$). ISF ANG I and ANG II levels (pg/ml) were > 100-fold higher than plasma levels, and did not change from baseline (8,122±528 and 6,333±677), during ANG I (8,269±502 and 6,139±695) or ANG I + cap (8,753±502 and 5,884±695). The finding of very high ANG I and ANG II levels in the ISF vs. intravascular space that are not affected by IV ANG I or cap suggests that ANG II production and/or degradation in the heart is compartmentalized and mediated by different enzymatic mechanisms in the interstitial and intravascular spaces. (*J. Clin. Invest.* 1997. 100:253–258.) Key words: angiotensin-converting enzyme inhibitors • left ventricular function • angiotensins • heart • heart hypertrophy

Introduction

Angiotensin II (ANG II)[1] has a wide range of cardiovascular effects that have been implicated in the pathophysiology of cardiac hypertrophy and heart failure. In addition to its well-known vasopressor actions, ANG II can promote deleterious hypertrophy and fibrosis because it is a growth factor for cardiocytes and fibroblasts (1, 2). ANG II may also have arrhythmogenic and cytotoxic effects on the heart because it stimulates the release of norepinephrine from cardiac sympathetic nerves (3). Components of the renin–angiotensin system (RAS) have been demonstrated in the heart by biochemical, immunohistochemical, and molecular biological techniques (4). Increased gene transcript levels of the components of the RAS, including renin (5), angiotensinogen (6), angiotensin receptors (7), and angiotensin-converting enzyme (ACE) (8–10) have been identified in experimental models of pressure (6–8) and volume overload (5, 9, 10) in the rat heart. Thus, there is increasing evidence that ANG II formation in the heart is mediated by a local RAS, acts independently of the circulating RAS components, and is upregulated by hemodynamic stress.

The pathophysiologic significance of ACE in the formation of ANG II was emphasized by clinical trials demonstrating that chronic ACE inhibitor therapy prevented further deterioration in left ventricular function, and decreased coronary artery disease morbidity and mortality in patients with left ventricular dysfunction (11, 12). These beneficial effects could not be attributed solely to a decrease in blood pressure, and were presumably mediated in part by decreased ANG II formation in the heart. The recent Evaluation of Losartan in the Elderly (ELITE) trial has shown superior survival benefit of the ANG II receptor blocker Losartan over the ACE inhibitor captopril in elderly patients with congestive heart failure, thus adding further strength to this hypothesis (13).

Interpretation of the origins of ANG II in the heart is complicated by the finding of multiple ANG II–forming pathways in cardiac tissue (14). In particular, a serine protease with extremely high affinity for ANG I, chymostatin-sensitive angiotensin generating enzyme (heart chymase), has been found in high concentrations in the human heart (15). Chymase has a higher specificity and catalytic activity for conversion of ANG I to ANG II than does ACE, accounting for > 80% of ANG II formation in nonsoluble human cardiac membrane preparations (15). We have reported previously that > 80% of ANG II–forming enzyme activity was blocked by the chymase inhibitor chymostatin, while only 6% was blocked by captopril in

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1. *Abbreviations used in this paper:* ACE, angiotensin-converting enzyme; ANG II, angiotensin II; HA, hippuric acid; HHL, hippuryl histidyl leucine; ISF, interstitial fluid; IVC, inferior vena cava; LAD, left anterior descending; LV, left ventricular; MAP, mean arterial pressure; RAS, renin–angiotensin system; SPE, solid-phase extraction.

tissue extracts of normal dog heart and of heart from dogs subjected to chronic volume overload hypertrophy, suggesting that chymase rather than ACE is the main source of ANG II in the dog heart in vitro (16). Subsequent studies, however, demonstrated that > 60% of ANG II formation across the myocardial circulation in vivo was inhibited by intracoronary infusion of captopril, while only 15% was inhibitable by infusion of chymostatin (17). This difference may be related to the distribution and compartmentalization of chymase and ACE in the heart.

In situ hybridization and electron microscopy immunocytochemical studies in the human heart have demonstrated that chymase is synthesized and stored in the intracellular compartment of mast cells and other types of interstitial cells (18). A high level of chymase-like immunoreactivity is localized to the cardiac interstitium, and is likely associated with the interstitial extracellular matrix (18). In contrast, ACE is bound to the cell membranes of endothelial cells, with its catalytic site exposed to the luminal surface (19). Thus, intravascular ANG II levels may reflect the activity of ACE, while interstitial ANG II levels may reflect the activity of chymase in conversion of ANG I to ANG II. In vitro assay conditions for measurement of cardiac ANG II disrupt cardiac tissue, and thus cannot distinguish this compartmentalization of ANG II formation in the heart. Previous work has demonstrated the feasibility of measuring neurohormone levels in the interstitial fluid (ISF) compartment of the brain (20, 21), kidney (22), and heart (23–25) in animal models in vivo using a microdialysis technique. ISF immunoreactive ANG levels in the kidney have been reported to be substantially higher than plasma levels (22).

We hypothesized that ANG II generation in the heart is compartmentalized, and that different enzymatic mechanisms mediate the generation of ANG II in the interstitial and intravascular compartments. Accordingly, we used the microdialysis technique to test the hypotheses that ANG II levels are higher in the ISF compartment of the dog heart than in plasma, that ISF ANG II levels are not sensitive to alterations in ANG II concentrations in the coronary circulation, and that ISF ANG II levels are not reduced by acute intravenous ACE inhibitor infusion.

Methods

Animal preparation

Six adult mongrel dogs (25–30 kg) were screened to rule out *Ehrlichia canis et platys* and dirofilaria immitis, and underwent general anesthesia using pentobarbital and mechanical ventilation (ventilator; Harvard Apparatus, Inc., South Natick, MA). A median sternotomy was performed, and the heart was exposed and suspended in a pericardial cradle. A No. 8 French sheath (Cordis, Miami. FL) was inserted into the right carotid artery, and was positioned in the ascending aorta. Descending thoracic aortic pressure was monitored continuously using a 4 French microtip Millar catheter (Millar Instruments, Houston, TX) inserted through a femoral artery cut-down. A 7 Fr. pediatric feeding tube with multiple side holes was inserted into the coronary sinus through the right atrial appendage, and was stabilized with a purse string suture. A Doppler coronary flow probe (Transonic Systems Inc., Ithaca, NY) was inserted around the left anterior descending artery distal to the takeoff of the diagonal branch for coronary flow measurement during all drug infusions. Three to four microdialysis probes were inserted into the left ventricular (LV) myocardium in the region perfused by the left anterior descending (LAD) coronary artery at the base, mid, and apical regions of the anterior wall of the LV by inserting a curved 25-gauge needle through the myocardium and inserting one end of the probe inlet tubing through the needle. The needle was then withdrawn, and the probe was pulled through the tissue, placing the dialysis membrane fully inside the muscle as previously described (23).

Protocol

Blood samples for ANG I and ANG II assay were collected from the aorta and coronary sinus during baseline, after a 60-min infusion of ANG I (5 μM ANG I at 0.1 ml/min) into the inferior vena cava (IVC), and after a 60-min infusion of ANG I plus captopril (5 μM ANG I plus 2.5 mM captopril at 0.1 ml/min) into the IVC. ISF for ANG I and ANG II assay was collected from the microdialysis probes over a 60-min collection period during baseline, during ANG I infusion, and during ANG I plus captopril infusion. Heart rate and systemic arterial pressure were recorded continuously, and the LAD coronary flow was recorded at 1-min intervals throughout the protocol.

Cardiac microdialysis

The cardiac microdialysis technique is similar to the technique used by Van Wylen et al. (23). Each microdialysis probe (Clirans; Terumo Corp. Tokyo, Japan) is a semipermeable membrane probe with a molecular cutoff of 35 Kd and an inner diameter of 200 μm which is connected to methyl deactivated silica capillary tubing (OD 0.17 mm). Thus, each microdialysis probe consists of a single 200-μm dialysis fiber and two hollow tubes inserted, adjusted, and sealed within the dialysis fiber such that the distance between the ends of the silica tubes is 4 cm. The probe is perfused by a precision infusion syringe pump (BAS, West Lafayette, IN) at a flow rate of 2.5 μl/min. In each animal, three to four microdialysis probes were implanted into the LV midmyocardium, in the region perfused by the LAD coronary artery at the base, mid, and apex of the LV anterior wall. After insertion of the microdialysis probes, the inflow capillary tube of each probe was connected via the larger deactivated silica tube to a gas-tight glass syringe filled with lactated Ringer's solution and perfused at 2.5 μl/min. The effluent, or dialysate, was collected from the outflow silica tube in small plastic tubes with 50 μl of acetic acid (5 M) and was frozen (−80°C) until biochemical analysis.

Cardiac microdialysis is based on the principle that, as the dialysate solution passes through the microdialysis fiber, diffusion occurs between the fluid within the fiber and the ISF surrounding the fiber (23). The dialysate concentration is therefore an estimate of intramyocardial ISF concentration. At the flow rates that are used in the microdialysis experiments in vivo, however, it is unlikely that complete equilibration occurs between the lactated Ringer's solution within the fiber and the cardiac ISF in the vicinity of the fiber. Therefore, we performed in vitro experiments to estimate recovery from our microdialysis probes using the method described by Van Wylen et al. (23). Assuming that all probes have the same area available for diffusion, the recovery (determined by comparing the concentration in the dialysis probe effluent with that of the medium, i.e., the percent recovery) depends primarily on the perfusion rate through the dialysis fiber. We perfused microdialysis probes ($n = 5$) at 0.5, 1.0, and 2.5 μl/min with isotonic saline in a beaker containing a bathing medium of isotonic saline (maintained at 37.5°C) and ^{3}H ANG II (49.2 Ci/mmol; DuPont NEN, Boston, MA) at a concentration of 1 mCi/ml. The recoveries for all flow rates are depicted in Fig. 1. At 2.5 μl/min, the rate that was used for the in vivo experiments, the percent recovery was 17±2%. Our recovery of 17% was used in the final calculation of ISF values, and thus represents an estimate of ISF levels since diffusional exchange may differ between a beaker and the beating heart.

In vivo stability of our microdialysis probes was assessed by determining the concentration of the stable compound acetaminophen in the effluent from the probes in three dogs (three probes/dog) for three 1-h collections in each dog. Concentrations of acetaminophen were calculated using HPLC analysis of the dialysis probe infusate and the dialysis probe effluent. The initial concentration in the infusate was considered to be 100%. We found that 83% of acetaminophen was detected in the effluent, indicating that 17% diffused into

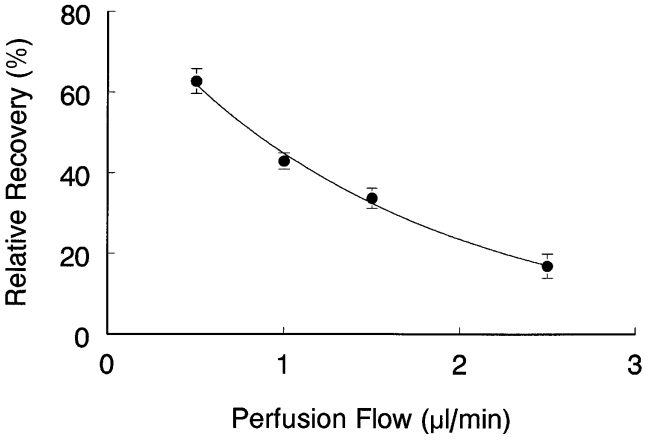

Figure 1. Graph of percent recovery data from all flow rates tested in vitro.

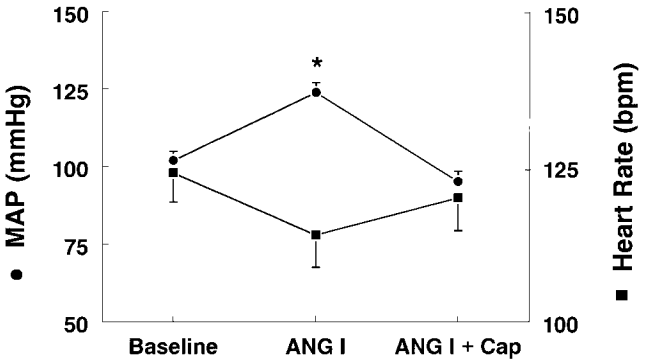

Figure 2. MAP and heart rate at baseline, during intravenous ANG I infusion, and during intravenous ANG I + captopril (*Cap*) infusion in the six dogs. $^{*}P < 0.01$ vs. baseline and ANG I + Cap.

the ISF, since the compound is not degraded under these conditions. This was a constant finding for the three 1-h perfusions for all nine probes. Thus, these experiments document that our dialysis probes did not become plugged or break down over the time course of the in vivo experiments.

Biochemical analyses

Angiotensin peptide levels. ISF and plasma ANG I and ANG II concentrations were determined by a method recently described from our laboratory that combines solid-phase extraction (SPE), HPLC, and RIA (26). AG50WX4 (200–400 mesh) cation exchange resin was used in an SPE procedure for sample purification. The recovery from the SPE procedure has been determined previously in our laboratory using both labeled and unlabeled ANG peptides (27). Using [^{125}I]-ANG I (1.4×10^7 cpm) and [^{125}I]-ANG II (9×10^6 cpm), recoveries were 93±2% ($n = 6$) and 91±2% ($n = 6$), respectively. Using 0.5, 1.0, or 1.5 μmol unlabeled ANG I and II, recoveries were 91±9% ($n = 6$) and 90±1% ($n = 6$), respectively. Separation was performed by reversed-phase HPLC on a phenyl silica gel column with an eluent consisting of 20% acetonitrile in 0.1 M ammonium phosphate buffer, pH = 4.9. Each HPLC fraction was 300 μl. Aliquots (100-μl) of each relevant fraction of column effluent were subjected to RIA immediately upon collection. Elution of standard ANG peptides under isocratic conditions revealed clear resolution of ANG I, II, and III and $ANG_{1–7}$ and $ANG_{3–8}$ peptides. RIA of relevant peaks revealed detectable levels of ANG I and II in all plasma and ISF samples examined. Antibodies to ANG I and II were raised in our laboratory in white New Zealand rabbits immunized against peptides conjugated to poly-L-lysine, as previously described (26). Cross-reactivity of anti–ANG I antiserum with ANG II, and of anti–ANG II antiserum with ANG I was < 0.5%. The sensitivity of the RIA for ANG I was 4 pg/ml; for ANG II, 2 pg/ml.

ACE activity of the ISF using hippuryl histidyl leucine (HHL) as substrate

ISF ACE activity was measured using an assay developed in our laboratory. According to this method, the reaction product hippuric acid (HA) is isolated from the reaction mixture by reverse-phase HPLC (16, 28). ISF (50-μl) samples obtained during baseline and during intravenous ANG I plus captopril infusion were incubated with 500 μl of a reaction mixture containing 0.3 M NaCl, 10^{-4} M $ZnCl_2$, and 15 mM HHL as substrate in 0.1 M phosphate buffer, (0.01 U purified ACE, rabbit lung; Sigma Chemical Co., St. Louis, MO), pH = 8.3 at 37°C for 30 min. The enzymatic reactions were terminated by addition of 500 μl of 1 N HCl. The HA formed by action of ACE on HHL was extracted from the acidified solution into 1.5 ml of ethyl acetate by vortex mixing for 15 s. After brief centrifugation, a 1-ml aliquot of each ethyl acetate layer was transferred to a clean tube and dried by heating at 120°C for 30 min. The dried HA samples were redissolved in 0.5 ml HPLC mobile phase containing 15% acetonitrile (CH_3CN) in 0.1 M ammonium phosphate buffer (vol/vol), pH 6.8, and applied to a reverse-phase Alltima 5 micron-phenyl HPLC column (Alltech Associates Inc., Deerfield, IL). The HPLC separation of HHL and HA was carried out at 35°C at a flow rate of 1.0 ml/min. The HA was quantitated by measuring its absorbance at 228 nm (tunable model 484 UV detector; Waters Associates, Milford, MA).

Statistical analysis

All data are presented as mean±standard error. ANOVA with Newman-Keul post-hoc comparison was used to compare hemodynamics and ANG I and ANG II levels at baseline, during ANG I infusion, and during ANG I plus captopril infusion. $P < 0.05$ was required for significance.

Results

Hemodynamics. ANG I increased mean arterial pressure (MAP) from 102±3 to 124±3 mmHg ($P < 0.01$), while addition of captopril decreased MAP to 95±3 mmHg ($P < 0.01$). Heart rate did not differ among baseline (124±5 bpm), ANG I infusion (114±5 bpm), and ANG I + captopril (120±5 bpm) periods (Fig. 2). In addition, LAD coronary blood flow did not

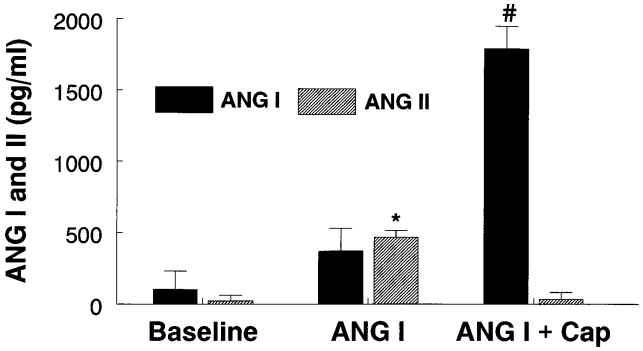

Figure 3. Mean ANG I and ANG II levels in aortic plasma during baseline and during intravenous infusion of ANG I and ANG I + captopril (*Cap*) in the six dogs. $^{*}P < 0.01$ vs. baseline and ANG I + Cap; $^{\#}P < 0.01$ vs. baseline and ANG I.

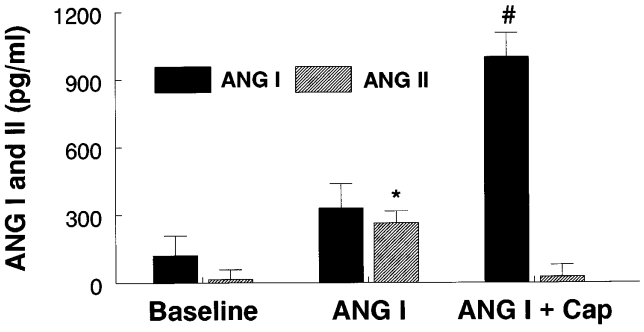

Figure 4. ANG I and ANG II levels in the coronary sinus plasma during baseline and during intravenous infusion of ANG I and ANG I + captopril (*Cap*) in the six dogs. $^{*}P < 0.01$ vs. baseline and ANG I + Cap, $^{\#}P < 0.01$ vs. baseline and ANG I.

change from baseline (20±2 ml/min) to ANG I infusion (18±2 ml/min) to ANG I + captopril (20±2 ml/min) periods.

Plasma and ISF ANG peptide levels. ANG I infusion increased aortic plasma ANG I and ANG II (pg/ml) (ANG I = 101±129 to 370±158 pg/ml, $P < 0.01$; and ANG II = 22±40 to 466±49 pg/ml, $P < 0.01$); while addition of captopril increased plasma ANG I (1,790±158 pg/ml, $P < 0.01$) and decreased plasma ANG II (33±49 pg/ml, $P < 0.01$) (Fig. 3). Coronary sinus plasma ANG I and ANG II levels demonstrated a similar response (Fig. 4). ANG I infusion increased coronary sinus ANG I and ANG II levels (ANG I = 121±87 to 330±107 pg/ml, $P < 0.01$; and ANG II = 16±43 to 264±53 pg/ml, $P < 0.01$); while addition of captopril increased ANG I (999±107 pg/ml, $P < 0.01$) and decreased ANG II (27±53 pg/ml, $P < 0.01$). ISF ANG I and ANG II levels were > 100-fold higher than plasma levels, and did not change from baseline (8,122±528 and 6,333±677 pg/ml), during ANG I (8,269±502 and 6,139±695 pg/ml), or during ANG I + cap (8,753±502 and 5,884±695 pg/ml) (Fig. 5).

ISF ACE activity assay using HHL as substrate. One additional adult mongrel dog was studied under the experimental conditions described above to address whether captopril infused into the IVC entered the ISF in concentrations sufficient to inhibit ACE-mediated ANG II production. ANG I + captopril was infused into the IVC, and ISF was collected from four microdialysis probes. First, HPLC analysis of ISF collected during ANG I + captopril infusion into the IVC demonstrated a clear captopril peak. Second, we assessed the ability of the ISF to inhibit conversion of the ACE specific substrate HHL to HA by added purified ACE. Generation of HA was almost completely inhibited in ISF collected during IVC infusion of ANG I + captopril, while there was no inhibition of HA production in ISF collected during baseline (Fig. 6). These experiments demonstrate clearly that intravenously administered captopril crosses the endothelial barrier and enters the ISF in concentrations sufficient to inhibit ACE-mediated ANG II production.

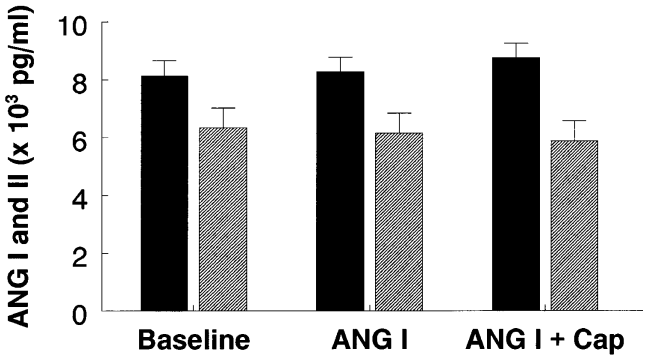

Figure 5. Mean ANG I and ANG II levels in the ISF compartment during baseline and during intravenous infusion of ANG I and ANG I + captopril (*Cap*) in the six dogs. *Black bar*, ANG I; *striped bar*, ANG II.

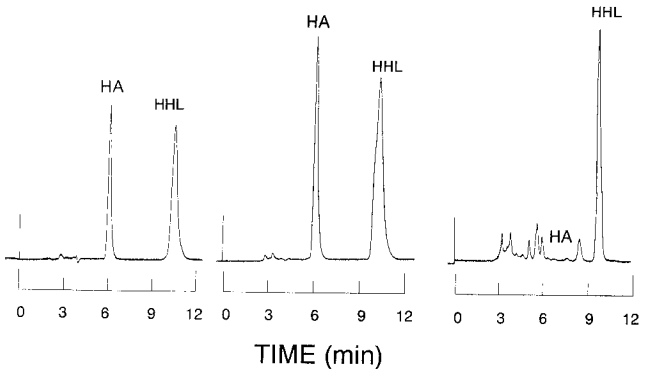

Figure 6. HPLC chromatograms. *Left*: 0.01 μg HA and HHL standards. *Middle*: Chromatogram from 50 μl ISF obtained during baseline to which had been added 0.01 U of purified ACE. ISF + ACE was incubated for 30 min with HHL, and was extracted and subjected to HPLC analysis as previously described for the ACE assay. Generation of HA at levels observed for the reaction carried out in lactated Ringer's solution above (not shown) was observed, indicating that ISF obtained under baseline conditions did not inhibit the catalytic activity of exogenous purified ACE. *Right*: Chromatogram from 50 μl ISF obtained during IVC infusion of ANG I + captopril, to which had been added 0.01 U of purified ACE. ISF + ACE was incubated for 30 min with HHL, extracted, and subjected to HPLC analysis as previously described for the ACE activity assay. Generation of HA was almost completely inhibited, indicating that intravenously administered captopril entered the ISF in concentrations sufficient to inhibit the catalytic activity of exogenous purified ACE.

Discussion

The results of the current investigation demonstrate very high ANG I and ANG II levels in the ISF versus the intravascular space of the dog heart in vivo. The concentration of ANG II in the ISF was 100-fold greater than in plasma at baseline. ISF ANG I and ANG II levels were unaffected by intravenous infusion of ANG I and infusion of ANG I plus captopril, in spite of entrance of captopril into the ISF. In contrast, aortic and coronary sinus plasma ANG II increased 10-fold during ANG I infusion and decreased to baseline after addition of captopril, while plasma ANG I increased 17-fold during captopril plus ANG I infusion. These data suggest that there are separate compartments for ANG I and ANG II production and/or degradation in the intravascular and ISF spaces of the heart, and that ANG II generation in these compartments is mediated by different enzymatic mechanisms.

We used microdialysis to demonstrate for the first time that

ANG I and ANG II concentrations in the ISF of the dog heart are far greater than in the coronary circulation, and are not sensitive to major alterations in intracoronary ANG levels. The ability to measure neurohormones and metabolites in the ISF of the dog heart in vivo can provide insight into physiologic functions at the cellular level within the myocardium that are separate from the intravascular space. For example, the adenosine hypothesis has suggested that reduced myocardial oxygen supply relative to demand results in increased adenosine release into the ISF, causing coronary vasodilation via interaction with adenosine receptors on vascular smooth muscle, leading to an improvement in the supply/demand imbalance. Sampling of coronary sinus blood during ischemic episodes to test this hypothesis, however, has produced inconclusive data because of intracellular binding of adenosine and metabolism of adenosine by endothelial cells (29). Using the microdialysis technique to sample intramyocardial adenosine, Van Wylen et al. demonstrated that dobutamine infusion resulted in a 2.5-fold increase in ISF adenosine, and that regional ischemia resulted in a significant 13-fold increase in ISF adenosine concentrations (23). These results supported the adenosine hypothesis, and suggested that cardiac microdialysis provided a reliable technique for sampling intramyocardial ISF. The current study is the first to examine ANG concentrations in the ISF of the normal dog heart, and to test their relationship to both static and changing (via infusion of exogenous ANG I and ACE inhibitor) intracoronary ANG levels.

The finding of extremely high concentrations of ANG II in the ISF compartment is consistent with recent work in neonatal rat cardiac myocytes. Sadoshima et al. detected high ANG II levels (400–500 pM) in the culture medium of neonatal rat cardiocytes after a 10-min stretch (30). The ANG II content of stretched myocytes was less than half that of control myocytes (24.0 ± 4.2 vs. 55.2 ± 7.2 pg per 10^7 cells, $P = 0.006$), suggesting that stretched myocytes released their contents of ANG II into the culture medium. In contrast, ANG I content did not differ between stretched and control myocytes (73.0 ± 10.8 vs. 74.8 ± 10.0 pg per 10^7 cells). The increase in ANG II in response to stretch was not prevented by pretreatment with captopril, and was accompanied by a 4.8-fold increase in angiotensinogen mRNA in 24 h. This demonstration of the capability of cardiac myocytes to release large quantities of ANG II into the extracellular space is consistent with our finding of high ANG II levels in the ISF of the dog.

Another mechanism to explain the high ISF ANG II levels in our dogs is the action of chymase in the cardiac interstitium. Chymase has higher specificity and catalytic activity for conversion of ANG I to ANG II than does ACE; the K_{cat} for conversion of ANG I to ANG II is 20-fold higher for chymase than for ACE (160/s vs. 8.3/s) (31, 32). Electron microscopy immunocytochemical and in situ hybridization studies have suggested that human heart chymase is synthesized and stored in secretory granules in mast cells, endothelial cells, and mesenchymal cells. Upon release from these cells, chymase is localized mainly in the interstitial compartment of the myocardium (18). Thus, chymase is a likely source for ANG II in the ISF because of its high affinity for ANG I and its location in the cardiac interstitium. Noda et al. demonstrated an increase in ANG II release into the anterior interventricular vein during acute ischemia in bilaterally nephrectomized dogs (33). This increase in ANG II was suppressed by the serine protease inhibitors nafamostat and chymostatin, and was unaffected by captopril. In another study, systemic infusion of [Pro^{11}D-Ala^{12}] ANG I, a substrate selective for human chymase, but not for ACE, produced systemic arterial vasoconstriction in the conscious baboon that was reversed by an ANG II receptor blocker, but not by captopril (34). These studies support a physiologic effect of chymase in the cardiovascular system of the dog and baboon. In the current investigation, the lack of change in ISF ANG II levels as intravascular ANG II decreased during intravenous captopril infusion supports the hypothesis of intravascular and interstitial compartmentalization of ANG II formation from ACE and chymase, respectively, in the heart.

The finding of very high ANG I levels in the ISF vs. intravascular space that are not affected by IV ANG I or captopril, also suggests compartmentalization for ANG I in the heart. Our additional experiments clearly demonstrate that ACE inhibitor (captopril) is present in the ISF dialysate in concentrations sufficient to inhibit extensively ACE activity after acute intravenous administration of captopril. Danser et al. demonstrated rapid ANG I degradation within the cardiac and systemic vasculature of the pig in vivo, and an increased ANG I degradation rate during captopril administration, most likely due to aminopeptidase and endopeptidase action on increased concentrations of ANG I substrate (35). Previous studies by Bailie et al. demonstrated that, after injection into the renal artery of the dog, significant amounts of ^{14}C ANG II did not appear in the renal lymph or the urine, suggesting that metabolism of circulating ANG peptide occurs primarily in the vascular compartment (36). In a related fashion, the endothelial cell has been shown to metabolize very actively adenosine, and thus function as a physiological barrier for passage of adenosine into the ISF (37). Taken together, these results support the possible role of the endothelial cell as a functional metabolic barrier for access of ANG I and ANG II into the ISF space. Whether the ISF space is the site of local ANG II production cannot be answered directly by the current study.

Using the microdialysis technique in the dog kidney, Siragy et al. demonstrated 1,000-fold higher ISF immunoreactive ANG peptide levels compared with circulating ANG II in response to acute stress (22). This finding suggests that ANG II formation in the ISF is a regulatable process that responds to stresses/stimuli known to affect the activity of the RAS in other tissue compartments, e.g., the intravascular space and the intracellular compartment. These findings support the hypothesis that ANG II formed locally in the ISF of the heart can exert an important effect on myocytes and fibroblasts independent of the circulating RAS.

One potential limitation of the current investigation is that the ANG II levels in the ISF may result from tissue damage during implantation of the probes. We began ISF collections 20 min after microdialysis probe insertion. Our experiments were designed in this way because Van Wylen and co-workers showed that adenosine levels were increased in the first 20 min after probe insertion, but decreased to baseline after this time (23). Therefore, they (and we) began collections after a 20-min stabilization period. Furthermore, we found no change in ISF ANG I or ANG II levels over the three 1-h collection periods, suggesting that our ANG II levels were not a result of acute tissue damage.

We describe, to our knowledge, the first experiments using the microdialysis technique for studying the RAS in the heart in vivo. Our results suggest that local production of ANG II

may be substantial within the myocardium, supporting the hypothesis of local physiologic effects on cardiac myocytes and/or interstitial cells. Future studies will investigate the compartmentalization theory for ANG II production in the intravascular and ISF spaces of the heart by performing experiments (*a*) during acute hemodynamic stresses, and (*b*) during intravenous and ISF infusion of selective inhibitors of ANG II formation and of selective substrates of ACE and chymase. This approach will provide a direct assessment of the milieu to which cardiomyocytes and interstitial cells are exposed in vivo, as well as further insight into the enzymatic mechanisms of ANG II formation in the interstitial and intravascular spaces.

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References

1. Yamazaki, T., I. Komuro, S. Kudoh, Y. Zou, I. Shiojima, T. Mizuno, H. Takano, Y. Hiroi, K. Ueki, K. Tobe, et al. 1995. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. *Circ. Res.* 77:258–265.

2. Sadoshima, J., and S. Izumo. 1993. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts: critical role of the AT_1 receptor subtype. *Circ. Res.* 73:413–423.

3. Brasch, H., L. Sieroslawski, and P. Dominiak. 1993. Angiotensin II increases norepinephrine release from atria by acting on angiotensin subtype 1 receptors. *Hypertension (Dallas).* 22:699–704.

4. Lindpaintner, K., M. Jin, M.J. Wilhelm, F. Suzuki, W. Linz, B.A. Schoelkens, and D. Ganten. 1988. Intracardiac generation of angiotensin and its physiologic role. *Circulation.* 77(Suppl.):1–18.

5. Boer, P.H., M. Ruzicka, W. Lear, E. Harmsen, J. Rosenthal, and F.H.H. Leenen. 1994. Stretch-mediated activation of the cardiac renin gene. *Am. J. Physiol. (Heart Circ. Physiol. 36).* 267:H1630–H1636.

6. Baker, K.M., M. Chernin, S.K. Wixon, and J.F. Aceto. 1990. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. *Am. J. Physiol. (Heart. Circ. Physiol. 28).* 259:H324–H332.

7. Everett, A.D., A. Tufro-McReddie, A. Fisher, and R.A. Gomez. 1994. Angiotensin receptor regulates cardiac hypertrophy and transforming growth factor-B_1 expression. *Hypertension (Dallas).* 23:587–592.

8. Schunkert, H., V.J. Dzau, S.S. Tang, A.T. Hirsch, C.S. Apstein, and B.H. Lorell. 1990. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy: effects on coronary resistance, contractility and relaxation. *J. Clin. Invest.* 86:1913–1920.

9. Iwai, N., H. Shimoike, and M. Kinoshita. 1995. Cardiac renin-angiotensin system in the hypertrophied heart. *Circulation.* 92:2690–2696.

10. Pieruzzi, F., Z.A. Abassi, and H.R. Keiser. 1995. Expression of renin-angiotensin system components in the heart, kidneys, and lungs of rats with experimental heart failure. *Circulation.* 92:3105–3112.

11. Pfeffer, M.A., E. Braunwald, L.A. Moye, L. Basta, E.J. Brown, Jr., T.E. Cuddy, B.R. Davis, E.M. Geltman, S. Goldman, G.C. Flaker, et al. 1992. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. *N. Engl. J. Med.* 327:669–677.

12. The SOLVD Investigators. 1991. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N. Engl. J. Med.* 327:685–691.

13. Pitt, B., R. Segal, F.A. Martinez, G. Meurers, A.J. Cowley, I. Thomas, P.C. Deedwania, D.E. Ney, D.B. Snavely, and P.I. Chang, on behalf of the ELITE STUDY Investigators. 1997. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study, ELITE). *Lancet.* 349:747–752.

14. Dzau, V.J. 1989. Multiple pathways of angiotensin production in the blood vessel wall: evidence, possibilities and hypotheses. *J. Hypertens.* 7:933–936.

15. Urata, H., B. Healy, R.W. Stewart, F.M. Bumpus, and A. Husain. 1990. Angiotensin II- forming pathways in normal and failing human hearts. *Circ. Res.* 66:883–890.

16. Dell'Italia, L.J., Q.C. Meng, E. Balcells, I.M. Straeter-Knowlen, G.H. Hankes, R. Dillon, R.E. Cartee, R. Orr, S.P. Bishop, S. Oparil, and T. Elton. 1995. Increased ACE and chymase-like activity in cardiac tissue of dogs with chronic mitral regurgitation. *Am. J. Physiol. (Heart Circ. Physiol. 38).* 269: H2065–H2073.

17. Balcells, E., Q.C. Meng, G. Hageman, R.W. Palmer, J. Durand, and L.J. Dell'Italia. 1996. Angiotensin II formation in dog heart is mediated by different pathways in vivo and in vitro. *Am. J. Physiol. (Heart Circ. Physiol. 38).* 271: H417–H471.

18. Urata, H., K.D. Boehm, A. Philip, A. Kinoshita, J. Gabrovsek, F.M. Bumpus, and A. Hussain. 1993. Cellular localization and regional distribution of an angiotensin II-forming chymase in the heart. *J. Clin. Invest.* 91:1269–1281.

19. Johnston, C.I. 1994. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. *Hypertension (Dallas).* 23: 258–268.

20. Van Wylen, D.G.L., T.S. Park, R. Rubio, and R.M. Berne. 1986. Increase in cerebral interstitial fluid adenosine concentration during hypoxia, local potassium infusion, and ischemia. *J. Cereb. Blood Flow Metab.* 6:522–528.

21. Robinson, T.E., and J.B. Justice. 1991. Microdialysis in the neurosciences. Elsevier, Amsterdam, Holland. 450 pp.

22. Siragy, H.M., N.L. Howell, N.V. Ragsdale, and R.M. Carey. 1995. Renal interstitial fluid angiotensin. Modulation by anesthesia, epinephrine, sodium depletion, and renin inhibition. *Hypertension.* 25:1021–1024.

23. Van Wylen, D.G.L., J. Willis, J. Sodhi, R.J. Weiss, R.D. Lasley, and R.M. Mentzer. 1990. Cardiac microdialysis to estimate interstitial adenosine and coronary blood flow. *Am. J. Physiol.(Heart Circ. Physiol. 27).* 258:H1642–H1649.

24. Wang, T., R.M. Mentzer, and D.G.L. Van Wylen. 1992. Interstitial adenosine with dipyridamole: effect of adenosine receptor blockade and adenosine deaminase. *Am. J. Physiol.* (*Heart Circ. Physiol.* 32). 263:H552–H558.

25. Wang, T., J. Sodhi, R.M. Mentzer, and D.G.L. Van Wylen. 1994. Changes in interstitial adenosine during hypoxia: relationship to oxygen supply, demand imbalance, and effects of adenosine deaminase. *Cardiovasc. Res.* 28: 1320–1325.

26. Meng, Q.C., J. Durand, Y. Chen, and S. Oparil. 1993. Simplified method for quantification of angiotensin peptides in tissue. *J. Chromatogr.* 614:19–25.

27. Meng, Q.C., J. Durand, Y. Chen, and S. Oparil. 1995. Effects of dietary salt on angiotensin peptides in kidney. *J. Am. Soc. Nephrol.* 6:1209–1215.

28. Meng, Q.C., E. Balcells, L. Dell'Italia, and S. Oparil. 1995. Sensitive method for quantitation of angiotensin-converting enzyme (ACE) activity in tissue. *Biochem. Pharmacol.* 50:1445–1450.

29. Olson, R.A., and R. Bunger. 1987. Metabolic control of coronary blood flow. *Prog. Cardiovasc. Dis.* 29:369–287.

30. Sadoshima, J., Y. Xu, H.S. Slayter, and S. Izumo. 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell.* 75:977–984.

31. Kinoshita, A., H. Urata, F.M. Bumpus, and A. Husain. 1991. Multiple determinants for the high substrate specificity of an angiotensin-forming chymase from the human heart. *J. Biol. Chem.* 266:19192–19197.

32. Husain, A., A. Kinoshita, S.S. Sung, H. Urata, and F.M. Bumpus. 1994. Human heart chymase. *In* The Cardiac Renin-Angiotensin System. K. Lindpaintner and D. Ganten, editors. Futura Publishing Co., Inc., Armonk, NY. 309–332.

33. Noda, K., M. Sasaguri, M. Ideishi, M. Ikeda, and K. Arakawa. 1993. Role of locally formed angiotensin II and bradykinin in the reduction of myocardial infarct size in dogs. *Circ. Res.* 27:334–340.

34. Hoit, B., Y. Shao, A. Kinoshita, M. Gabel, A. Husain, and R.A. Walsh. 1995. Effects of angiotensin II generated by an angiotensin converting enzyme-independent pathway on left ventricular performance in the conscious baboon. *J. Clin. Invest.* 95:1519–1527.

35. Danser, A.H.J., M.M.G. Koning, P.J.J. Admiraal, F.H.M. Derkx, P.D. Verduow, and M.A.D.H. Schalekamp. 1992. Metabolism of angiotensin I by different tissues in the intact animal. *Am. J. Physiol. (Heart Circ. Physiol. 32).* 263:H418–H428.

36. Bailie, M.D., F.C. Rector, and D.W. Seldin. 1971. Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. *J. Clin. Invest.* 50:119–126.

37. Nees, S., V. Herzog, B.F. Becker, M. Brock, C. Des Rosiers, and E. Gerlach. 1985. The coronary endothelium: a highly active metabolic barrier for adenosine. *Basic Res. Cardiol.* 80:515–529.