The Renin-Angiotensin System During Acute Myocardial Ischemia in Dogs

Robson A.S. Santos, Jose M. Brum, K. Bridget Brosnihan, and Carlos M. Ferrario

We used the technique of high-performance liquid chromatography combined with radioimmunoassay to establish the profile of angiotensin peptides in the periphery and across the circulation of the dog's heart. Data were obtained before and after blockade of angiotensin converting enzyme, and after acute myocardial ischemia produced by occlusion of the left anterior descending coronary artery. Baseline values of plasma renin activity and immunoreactive angiotensin II were higher in the aortic root than in the coronary sinus but concentrations of angiotensin I and angiotensin-(1-7) were similar. In untreated animals, coronary occlusion produced significant increases in renin activity and arterial and venous levels of angiotensin I and angiotensin II. Inhibition of converting enzyme with benazeprilat (CGS-14,831) increased baseline circulating levels of angiotensin I, whereas angiotensin II and its carboxyl terminal fragments were reduced markedly. Baseline plasma levels of angiotensin-(1-7) and its fragments did not change. Myocardial ischemia in benazeprilat-treated dogs increased plasma renin activity and circulating levels of angiotensin I. Concentrations of angiotensin II and angiotensin-(1-7) did not change either in peripheral blood or across the coronary circulation. These results indicate that angiotensin peptides can be formed endogenously by enzymatic pathways alternate to converting enzyme. Furthermore, these data provide the basis for a further understanding of the role of the renin-angiotensin system after myocardial ischemia. (Hypertension 1990;15[suppl I]:I-121-I-127)

Newer studies of the renin-angiotensin system (RAS) emphasize that tissue production of angiotensins serves a local regulatory role.1-2 The existence of angiotensin-forming enzymes in the vascular wall, the heart, and the brain suggest that they contribute to the maintenance of vascular resistance and arterial pressure. Although angiotensin II (Ang II) is the key bioactive product of the RAS, recent studies have uncovered biological actions of amino (N-) and carboxyl (C-) terminal angiotensin peptide fragments. In this regard, we showed that the N-terminal heptapeptide angiotensin-(1-7)[Ang-(1-7)] is as potent as Ang II in stimulating the release of vasopressin3 although it has no myotropic or dipsogenic activity.4 Additionally, Ang-(1-7) mimics the actions of Ang II when injected into the vagal-solitarii complex of the rat6 or after superfusion of the peptide onto isolated slices of the canine medulla oblongata.6 Others have shown that the C-terminal heptapeptide Ang-(2-8) has potent actions on aldosterone secretion and neuronal activity.7,8

In this study, we used chromatographic procedures developed in the laboratory9-10 to determine the profile of angiotensin peptides across the peripheral and cardiac circulations. Data were obtained before and after pharmacological blockade of angiotensin converting enzyme (ACE) and also after acute myocardial ischemia produced by occlusion of the left anterior descending (LAD) coronary artery. This strategy gave us the opportunity to characterize for the first time the effect of ACE inhibition on circulating levels of endogenous angiotensins after acute cardiac ischemia.

Methods

Anesthesia was induced in 21 male mongrel dogs (20–22 kg) with sodium thiamylal (25 mg/kg i.v.). After endotracheal intubation, anesthesia was maintained with 1% halothane (Halocarbon Laboratories, Hackensack, New Jersey) in a gas mixture of 70% O2 and 30% room air. Catheters were placed into the left carotid artery and a jugular vein. Under x-ray fluoroscopy, either a 4- or 5-F Pruitt angiographic balloon-catheter (Bionomy Co., Tampa, Florida) was inserted into the LAD coronary artery.
above the level of the first diagonal branch. Another 5-F Goodale-Lubin catheter (USCI-BARD, Billerica, Massachusetts) was placed into the coronary sinus. A third catheter was advanced into the thoracic aorta through a femoral artery. Aortic blood pressure was measured with a solid-state strain-gauge transducer (Gould DTX, Oxnard, California). Both the arterial pressure and the lead II of the electrocardiogram were recorded continuously on a multichannel direct-writing oscillograph (Gould Inc, Cleveland, Ohio). Additional catheters were placed in the right atrium and aortic root through a femoral vein and artery for infusions of drugs and sampling of blood.

**Protocol 1.** In these experiments (13 dogs), we determined the effects of an acute occlusion of the LAD coronary artery on the profile of angiotensin peptides present in blood collected from the aorta, the right atrium, and the coronary sinus. Blood collections (12 ml) were done 30 minutes after an intravenous infusion of 20 ml of 0.9% saline (untreated group), and within 3 minutes at p values less than 0.05.

**Biochemical Procedures**

Blood was drawn in sterile ice-cold plastic syringes rinsed with a 15% NH₂EDTA/9.1 mM O-phenanthroline. Blood for plasma renin activity (PRA) was placed in ice-cold silicone-coated glass vials containing 50 µl/ml of the previous enzyme inhibitors mixture. For measurement of angiotensin peptides, a 2.5 mM pepstatin solution (50 µl/ml blood) was also used. Plasma was stored at −80° C after centrifugation (20,000g for 15 minutes at 4° C). PRA and plasma angiotensin measurements were done as described previously. Plasma angiotensins were processed for both direct radioimmunoassay (RIA) and high-performance liquid chromatography (HPLC) combined with RIA. Identification of angiotensin peptides by HPLC–RIA was performed by comparison of their elution times with those of synthetic angiotensin standards, as described elsewhere. The specificity of the three separate polyclonal antibodies used for the measure of immunoreactive angiotensin I (Ang I), Ang II, and Ang-(1–7) is also documented. The analysis includes studies of cross-reactivity with other peptides and the description of interassay and intraassay levels of variability and minimal detection values. For clarity, estimates of total immunoreactivity by the direct radioimmunological procedure are denoted throughout the text by the “ir” prefix. Direct measurements of the concentration of angiotensin peptides by HPLC combined with RIA are not denoted by this qualifier.

**Statistics**

Results are expressed as mean ± SEM. Statistical analyses were performed using either the Student’s t test for paired observations or a one- and two-way analysis of variance. Statistical differences are noted at p values less than 0.05.

**Results**

Table 1 shows the baseline values of PRA and ir angiotensins in normal anesthetized dogs. PRA was slightly higher in blood drawn from the aorta than from the coronary sinus. The PRA arteriovenous difference across the heart averaged 0.75±0.28 ng Ang l/ml/hr (p<0.05). Additionally, irAng II was also significantly higher in the aortic root than in the coronary sinus. In contrast, there were no arteriovenous differences for irAng I and irAng-(1–7). As expected, irAng II was significantly lower in the right atrium than in the aorta (Table 1). But no significant differences existed in the concentrations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aortic root</th>
<th>Coronary sinus</th>
<th>Right atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>3.8±0.8</td>
<td>3.1±0.8*</td>
<td>3.3±1.1</td>
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<td>Immunoreactive Ang I (fmol/ml)</td>
<td>42.1±11.9</td>
<td>44.9±12.1</td>
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<td>Immunoreactive Ang II (fmol/ml)</td>
<td>27.1±6.8</td>
<td>15.4±3.7*</td>
<td>8.5±2.6*</td>
</tr>
<tr>
<td>Immunoreactive Ang-(1-7) (fmol/ml)</td>
<td>5.8±3.4</td>
<td>3.7±1.9</td>
<td>4.2±1.9</td>
</tr>
</tbody>
</table>

Data are mean±1 SEM of values obtained in 12 of 13 dogs from protocol 1. Values from right atrium are from five dogs.

*p<0.05 as compared with concentrations in blood obtained from aorta.

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**TABLE 1. Baseline Values of Renin-Angiotensin Components in Anesthetized Dogs**

- **Immunoreactive Ang I** refers to the concentration of angiotensin I (Ang I) in blood collected from the aorta, the right atrium, and the coronary sinus. The values are expressed in femtomoles per milliliter (fmol/ml). The table shows the baseline values obtained in 12 of 13 dogs from protocol 1. The values from the right atrium were from five dogs.

- **Immunoreactive Ang II** and **Immunoreactive Ang-(1-7)** are similarly denoted for angiotensin II and Ang-(1-7), respectively. The values are also expressed in femtomoles per milliliter (fmol/ml). The table indicates the baseline concentrations in blood collected from the aorta, the right atrium, and the coronary sinus.

- **Plasma renin activity (PRA)** is measured in nanograms of renin per milliliter per hour (ng/ml/hr). The table shows the baseline values obtained in 12 of 13 dogs from protocol 1. The values from the right atrium were from five dogs.

- The statistical significance of differences between groups is noted with asterisks (*p<0.05).
FIGURE 1. Bar graph showing effect of occlusion of left anterior descending (LAD) coronary artery on components of renin-angiotensin system. Data are mean±SEM obtained in untreated (n=6) and CGS-14,831-treated (n=6) dogs. Blood samples were collected from coronary sinus (CS), Aortic root (Ao), and right atrium (RA) before and 10 minutes after LAD coronary artery occlusion. *Indicate significant difference (p<0.05) when compared with values determined before occlusion. PRA, plasma renin activity; Ang, angiotensin; ir, immunoreactivity; ND, not detectable (1 ml plasma).

of PRA and plasma irAng-(1–7) between the right atrium and the aorta. In this group of untreated dogs, baseline mean arterial pressure (MAP) averaged 90±8 mm Hg and heart rate (HR) was 89±8 beat/min.

Figure 1 shows the effect of temporary occlusion of a LAD coronary artery on PRA and levels of irAng I, irAng II, and irAng-(1–7) in dogs with and without previous treatment with benazeprilat. Figure 2 shows the specific levels of the angiotensin
peptides as determined by the combination of HPLC and RIA. The data showed that plasma concentrations of irAng I, irAng II, and irAng-(1-7) were in part explained by other cross-reacting circulating fragments. These included 1) the Ang I fragments, Ang-(2-10) and Ang-(3-10); 2) the C-terminal fragments of Ang II, Ang-(2-8), Ang-(3-8), and Ang-(4-8); and 3) the C-terminal fragments of Ang-(1-7), the peptides Ang-(2-7), Ang-(3-7), and Ang-(4-7) (Figure 2). Ang I and Ang II represented most of the total irAng I and irAng II (70-85%) measured in either arterial or venous blood of untreated dogs (Figures 1 and 2). In contrast, irAng-(1-7) in blood obtained from both the aorta and the right atrium but not from the coronary sinus was more evenly distributed among three other C-terminal fragments of Ang-(1-7). The large average value of Ang-(1-7) in the coronary
sinus of the untreated group (Figure 2) was because of a high level of the heptapeptide in one of three dogs. HPLC analysis of baseline plasma samples of untreated dogs revealed that the concentration of the C-terminal fragments Ang-(2-8), Ang-(3-8), and Ang-(4-8) in the blood drawn from the aorta was greater than that obtained from the right atrium. This finding suggests that these peptides are generated across the pulmonary circulation.

Nine of 13 untreated (protocol 1) and six of eight dogs given benazeprilat (protocol 2) survived the procedure of temporary occlusion of the LAD coronary artery. Ventricular fibrillation developed in the other dogs and they died within minutes after coronary artery occlusion. In dogs not given benazeprilat, MAP decreased from 101±4 to 81±8 mm Hg, whereas HR (107±5 vs. 106±9 beats/min) remained unchanged after LAD coronary artery occlusion. Occlusion of the LAD coronary artery for 10 minutes was associated with hyperreninemia and significant increases in the concentrations of irAng I and irAng II in the aorta, the coronary sinus, and the right atrium (Figure 1). In contrast, irAng-(1-7) did not change. The increases in irAng I and irAng II after occlusion of a coronary artery were explained by a predominant rise in the concentrations of authentic Ang I and Ang II (Figure 2). Additionally, acute cardiac ischemia had no discernible effect on the arteriovenous differences of angiotensin peptides across either the systemic or the cardiac circulation (Figures 1 and 2). On the other hand, induction of myocardial ischemia abolished the arteriovenous difference in PRA. Similar results were found in blood samples taken 3 minutes after occlusion (data not shown).

Compared with untreated animals, an intravenous injection of benazeprilat produced no significant changes in baseline MAP (92±4 mm Hg) and HR (94±10 beats/min) but marked changes in the levels of angiotensin peptides. Within 30 minutes of ACE blockade, PRA and irAng I increased two and threefold, respectively, in both arterial and venous blood (Figure 1). In contrast, irAng II decreased to values below the detection limit of the RIA (<1 pg/ml) in five of the six dogs, whereas in one dog, the concentration of irAng II was similar to that measured in untreated dogs. irAng-(1-7) was not detected in either arterial or venous blood after benazeprilat administration (Figure 1). HPLC analysis performed in three of the six treated dogs showed that most of the increase in irAng I was because of rises in the blood levels of Ang I, Ang-(3-10), and Ang-(4-10) (Figure 2). In untreated dogs, Ang-(3-10) and Ang-(4-10) contributed 10–14% and 0.1–0.5%, respectively, to the levels of irAng I. In dogs given benazeprilat, these fragments were responsible for 22–42% and 10–20% of the amount of irAng I (Figure 2). ACE inhibition produced an increase in the plasma concentration of the C-terminal fragments of Ang-(1-7), Ang-(3-7), and Ang-(4-7). In contrast, Ang II fragments decreased to values below the detection level of the RIA after HPLC separation (Figure 2).

Occlusion of the LAD coronary artery in benazeprilat-treated dogs caused a decrease in MAP from 92±4 mm Hg to 75±5 mm Hg, no change in HR (95±10 beats/min vs. 99±9 beats/min), and further increments in PRA (Figure 1). Acute cardiac ischemia increased circulating levels of Ang I peptides assayed in blood obtained from either the coronary sinus, the aortic root, or the right atrium, whereas there was no change in the concentrations of Ang II and Ang-(1-7) (Figure 2).

Discussion

The combination of HPLC and RIA enabled the specific measurement of angiotensin peptides circulating in the blood obtained from the right atrium, the coronary sinus, and the aorta of anesthetized dogs subjected to temporary occlusion of a coronary artery both in the absence and presence of ACE blockade. The profile of the content of angiotensin peptides across the circulation of the heart, as measured from arteriovenous differences, showed no evidence of a spillover of these components from a source that is endogenous to the heart. Neither inhibition of ACE nor temporary occlusion of the LAD coronary artery, alone or in combination, demonstrated that the heart contributes to the content of angiotensin peptides circulating in peripheral blood. Although a local cardiac angiotensin system might play a role in the regulation of myocardial function and perfusion, its activity is not revealed in the blood draining from the coronary sinus.

We9,13 and others14 have resorted to specific measures of angiotensin peptides to uncover the mode of action of drugs that inhibit production of Ang II. Measures of total immunoreactivity prevent accurate determinations of low concentrations of Ang II and do not exclude the contribution of other cross-reactive products and potential immunoreactive artifacts. The method perfected by us circumvents these problems and yields a true picture of the relative concentrations of the various fragments that contribute to the radioimmunological determinations of Ang I and Ang II.10 Additionally, we now report the presence of Ang-(1-7) and related fragments in arterial and venous plasma of anesthetized dogs. Linkage between the HPLC procedure and RIA showed that, in anesthetized dogs, the concentrations of irAng I, irAng II, and irAng-(1-7) are in part determined by the presence of corresponding fragments that cross-react with epitopes from each of the corresponding antisera. In these experiments, Ang I, Ang II, and Ang-(1-7) were the major peptides, whereas Ang-(2-10), Ang-(3-10), Ang-(3-8), Ang-(4-8), and Ang-(2-7) were minor components of the total irAng I, irAng II, and irAng-(1-7), respectively. We also found that Ang-(1-7) was present at low concentrations. Furthermore, the content of the heptapeptide in blood from either
the aorta, the coronary sinus, or the right atrium shows significant variability. Nevertheless, the finding of Ang-(1-7) in the peripheral circulation of the dog is important because recent studies suggest a physiological action of this fragment in vasopressin secretion and in the control of arterial pressure by the brain.\(^5,6\) Ang-(2-8) is another biologically active angiotensin that was also found to circulate at very low concentrations.

Acute blockade of ACE with benazeprilat depleted the content of Ang II and its C-terminal fragments from both the arterial and venous circulations. The finding that ACE blockade did not alter the relatively low concentrations of Ang-(1-7) and its related C-terminal fragments emphasizes the existence of alternate enzymatic pathways for the generation of angiotensin peptides not only in the brain\(^4,13\) but also in the peripheral circulation. We also found higher concentrations of Ang-(4-8) and Ang-(3-8) in blood drawn from the aorta and coronary sinus of untreated dogs both before and during temporary occlusion of a coronary artery, as compared with their concentration in the right atrium. We interpret these findings as suggesting the pulmonary circulation as a processing site. Moreover, these peptides can be produced through an ACE-dependent pathway because benazeprilat administration inhibited their productions.

Studies of the RAS suggest that tissue angiotensins have a local function.\(^1,2\) The detection in the heart of both renin and angiotensinogen genes supports the existence of an endogenous RAS.\(^1\) In the isolated rat heart, pretreatment with an ACE inhibitor reduced the conversion of Ang I to Ang II.\(^2\) Studies suggest that ACE inhibitors might reduce myocardial infarct size and prevent ventricular fibrillation during reperfusion.\(^15\) These data were interpreted to suggest that ACE inhibitors have a cardioprotective function by preventing local formation of Ang II.\(^1,2,15\) Liang et al.\(^16\) however, found no evidence that ACE inhibitors have a direct effect on myocardial performance or infarct size in conscious dogs. Pfeffer et al.\(^17\) showed that captopril treatment for 3 months reduced left ventricular dilation and enhanced the pumping ability of rats with chronic myocardial infarction. In our experiments, we noted that pretreatment with benazeprilat was associated with a slight reduction of the incidence of ventricular fibrillation after acute coronary artery occlusion. The small size of the sample, however, precludes any conclusions. Thus, in agreement with previous studies,\(^16,17\) any beneficial effects of ACE inhibitors might be related to blockade of the peripheral hemodynamic and neural actions of circulating Ang II. Furthermore, we find no direct evidence that the heart is a source for the release of angiotensin peptides even after the imposition of cardiac ischemia. The disappearance of the arteriovenous difference for PRA after coronary occlusion, however, suggests local changes in renin release or activation.

The potent effect of benazeprilat in eliminating the production of Ang II and related C-terminal fragments suggests that the cardioprotective action of the ACE inhibitors is because of inhibition of the effects of circulating Ang II on the sympathetic nervous system and the baroreceptors.\(^2\) It is conceivable, however, that the cardioprotective effect of ACE inhibitors might be in part related to the activity of other angiotensin peptides for which their production is not inhibited. This possibility requires investigation of the novel biological activities of fragments of Ang II\(^4\) and Ang-(1-7).\(^4\)

Using HPLC procedures developed in this laboratory, we gained knowledge of the profile of angiotensin peptides in the peripheral and cardiac circulation of the anesthetized dog and a description of their fate after occlusion of the LAD coronary artery both in the absence and presence of ACE blockade. Analyses of the molecular forms of angiotensin peptides in these conditions affirm the importance of the endocrine actions of Ang II. The research also showed that valid assessment of the role of angiotensin peptides requires the use of methods that are more sensitive than the direct RIA. The data constitute a base for further study of the dynamic pathways by which the family of angiotensin peptides convey actions at target tissues.\(^4\)

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References


**KEY WORDS** • angiotensin II • angiotensin-(1–7) • ischemia • converting enzyme • renin-angiotensin system • benazeprilat
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