The Renin-Angiotensin System During Acute Myocardial Ischemia in Dogs

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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 end 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 rat5 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 (n = 3) or 10 minutes (n = 6) after occlusion of the LAD coronary artery. The position of the balloon catheter and the degree of inflation that achieved complete obstruction of flow into the distal portion of the LAD coronary artery were verified by injection of contrast material (Isovue-370, Squibb, New Brunswick, New Jersey) into the aortic root.

**Protocol 2.** The ACE inhibitor, CGS-14,831 (benazeprilat, CIBA-Geigy Co., Summit, New Jersey), was injected into another eight dogs 30 minutes before occlusion of the LAD coronary artery (treated group). The drug was given into a vein for 2.5 minutes at a concentration of 0.2 mg/kg and at a rate of 8 ml/min. Blood samples were collected before and within 10 minutes after LAD coronary artery occlusion. All other maneuvers were done as described for protocol 1.

**Biochemical Procedures**

Blood was drawn in sterile ice-cold plastic syringes rinsed with a 15% NH₄EDTA/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 angiotensins 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 measurement 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 1/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...
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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...
FIGURE 2. Bar graphs showing high-performance liquid chromatography (HPLC) profiles of plasma angiotensin peptides in coronary sinus (CS), aortic root (Ao), and right atrium (RA). Blood samples were taken before (B) and after (A) coronary artery occlusion in untreated (n=3) and CGS-14,831-treated (n=3) dogs. Samples corresponding to 3–5 ml plasma were processed by HPLC using LKB 2150 gradient system equipped with Nova Pak C8 column (3.9x150 mm, Millipore, Milford, Massachusetts). Column was equilibrated with 20% mobile phase A (0.13% heptafluorobutyric acid [HFBA]) and 30% mobile phase B (80% acetonitrile/0.13% HFBA) at flow rate of 1 ml/min. Plasma angiotensins were separated with 30-minute concave gradient 30–50% mobile phase B. Fractions of 12 seconds were collected and processed by three different radioimmunoassays: 3–9 minutes, Ang-(1-7); 9–15 minutes, Ang II; 15–22 minutes, Ang I. ir, immunoreactivity; Ang, 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 C-terminal fragments of Ang-(1-7). The large average value of Ang-(1-7) in the coronary
were responsible for 22-42% and 10-20% of the Ang-(3-10), and Ang-(4-10) (Figure 2). In untreated because of rises in the blood levels of Ang I, ANG-(1-7), and Ang-(2-10), Ang-(3-10), Ang-(4-8), and Ang-(2-7) contributed 10-14% and 0.1-0.5%, respectively, to the levels of irAng I was measured in untreated dogs. irAng-(l-7) was not detected in either arterial or venous blood after benazeprilat administration. 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.
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. 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 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. The detection in the heart of both renin and angiotensinogen genes supports the existence of an endogenous RAS. In the isolated rat heart, pretreatment with an ACE inhibitor reduced the conversion of Ang I to Ang II. Studies suggest that ACE inhibitors might reduce myocardial infarct size and prevent ventricular fibrillation during reperfusion. These data were interpreted to suggest that ACE inhibitors have a cardioprotective function by preventing local formation of Ang II. Liang et al., however, found no evidence that ACE inhibitors have a direct effect on myocardial performance or infarct size in conscious dogs. Pfeffer et al. 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, 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. 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 I and Ang-(1-7).

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.

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