ACE inhibitors in HF restore canine pulmonary endothelial function and ANG II vasoconstriction

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Straeter-Knowlen, Ingrid M., Louis J. Dell’Italia, J un Dai, Gerald H. Hankes, A. Raymond Dillon, R. Earl Cartee, Gerald M. Pohost and David D. Ku. ACE inhibitors in HF restore canine pulmonary endothelial function and ANG II vasoconstriction. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1924–H1930, 1999.—Chronic mitral regurgitation (MR) in dogs results in pulmonary congestion and increased cardiac angiotensin-converting enzyme (ACE) activity and angiotensin (ANG) II levels. ACE could contribute to altered pulmonary vasomotion in heart failure, and ACE inhibitor (ACEI) therapy may normalize pulmonary vasomotion. We evaluated pulmonary artery (PA) responses to ANG II and bradykinin (BK) in control dogs, in dogs with 4 mo of MR, in MR dogs treated with the ACEI ramipril (MR + R), and in control dogs treated with ramipril (C + R). Mean PA systolic pressure increased in MR dogs (21 ± 4 mmHg) but was normal in MR + R dogs (13 ± 1 mmHg). Constriction of PA rings to ANG II was depressed in MR dogs. ACEI treatment (MR + R) restored ANG II responsiveness, but peak ANG II response (3.6 ± 0.2 g) in MR + R dogs remained lower than in C + R dogs (4.7 ± 0.2 g). Endothelium-dependent relaxation to BK was decreased (−87 ± 4% C, −65 ± 4% MR; P < 0.05). Ramipril (MR + R) restored relaxation to BK. This demonstrates that pulmonary congestion results in impaired pulmonary vasomotion to ANG II and BK, which ACEIs could normalize, supporting the use of ACEIs in clinical management of chronic congestive heart failure.

ramipril; bradykinin; acetylcholine; pulmonary artery; mitral regurgitation; heart failure; angiotensin-converting enzyme

CHRONIC HEART FAILURE LEADS to abnormalities in vaso-
motor tone at rest, in response to vasodilatory stimuli, and during exercis 
(11, 34, 35). It has been postulated that these abnormalities may result from changes in neural input, circulating and local hormonal factors, and vessel wall structure (15). The renin-angiotensin system (RAS), catecholamines, endothelin, vasopres 
sin, and atrial natriuretic factor have all been implicated in the altered regional and overall cardiovascular function in heart failure. The influence of these neuro-
hormones has been described in large part in the systemic peripheral vasculature. A direct effect of these traditional neurohormonal pathways on the develop-
ment of pulmonary hypertension and pulmonary vaso-
constriction of congestive heart failure has not been extensively studied. This could have important implications because increased pulmonary vascular resistance can lead to right ventricular functional impairment, which is a strong predictor of mortality in heart failure (10, 24, 25, 27).

The Studies of Left Ventricular Dysfunction (SOLVD) and Survival and Left Ventricular Enlargement (SAVE) demonstrated that chronic angiotensin-converting enzyme (ACEI) therapy prevented further deterioration in left ventricular function and decreased coronary artery disease morbidity and mortality in patients with left ventricular dysfunction without overt heart failure (26, 30). These beneficial effects of ACEIs in asymptomatic patients suggest that factors other than blood pressure reduction alone may be operating under certain conditions (33). One such hypothesis is that ACEIs may exert an important local effect on the RAS in both vascular and cardiac tissues, whereas the circulating RAS remains normal in patients with left ventricular dysfunction without overt heart failure (8, 14, 18). However, there are few or no data regarding the beneficial effect of chronic ACEI therapy in pulmonary vascular function.

Normal pulmonary vasomotor function, as in the other vascular beds, depends on the delicate balance between the vasodilatory and vasoconstrictory mecha-
nisms. Increased ACE activity resulting in increased degradation of bradykinin (BK) and increased ANG II could contribute further to altered vasomotor function in heart failure. Thus chronic ACEI therapy could provide two beneficial effects, decreased ANG II formation and an increased preservation of BK. Pulmonary vascular response to chronic ACEI therapy in an ani-
mal model of chronic heart failure, however, has not been extensively evaluated (4).

We (7) have previously demonstrated that chronic volume overload hypertrophy caused by mitral regur-
gitation (MR) in the dog results in twofold increases in intracardiac ACE and chymase activity and threefold increase in intracardiac ANG II peptide levels in the left ventricle. This model is also characterized by increased pulmonary pressures and increased circulating RAS components. We hypothesized that heightened activity of the RAS in this model of heart failure would result in abnormal vasomotor function in the pulmo-
nary vasculature and that these abnormalities could be
isolated intralobar PA ring preparations. At the time of death, the dogs were anesthetized as described previously, a left lateral thoracotomy was performed, and the heart was KCl arrested. The left caudal lung lobe was harvested as quickly as possible and immersed in ice-cold Krebs-Henseleit (KH) solution. The intralobar PA was then identified in the left caudal lung lobe, isolated, and cleaned of surrounding tissues in prewarmed (37°C) and oxygenated (95% O2-5% CO2) KH solution containing (in mM) 118 NaCl, 4.6 KCl, 27.2 NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, 1.75 CaCl2, 0.03 Na2EDTA, and 11.1 glucose as previously described (16). Each vessel was cut into rings 5-mm long and mounted by means of two L-shaped 27-gauge stainless steel needles and placed in 10- or 5-ml jacketed chambers containing prewarmed and oxygenated KH solution. The upper needle was attached to a force-displacement transducer (Grass FT.03C) by a silk suture. The vessels were passively stretched to 2 g for isometric force recording on a Grass polygraph (model 7C). After 40 min of equilibration, the vessel rings were exposed to two successive dosages of maximum depolarizing KCl (80 mM). When contractile responses plateaued, the vessel rings were rinsed with KH and allowed to equilibrate for 1 h with 5 μM indomethacin before start of the experiment. It has previously been shown (17) that this concentration of indomethacin results in complete inhibition of cyclooxygenase and production of prostanoids. Pretreatment with indomethacin will block the EC-dependent cyclooxygenase-derived contribution to vasodilation (i.e., prostacyclin). All drug concentrations given are the final concentrations as they appear in the tissue baths.

For endothelium-dependent relaxation, data are expressed as percentages of relaxation or percentages of decrease in phenylephrine (PE)-induced constriction. BK or ACh was added in cumulative fashion to the bath to establish dose-response curves as soon as tension had stabilized. To document the role of endothelium and nitric oxide on the observed relaxation, studies were repeated in separate series of pulmonary arteries with disrupted endothelium by mechanical abrading of the lumen of each vessel with a wooden applicator and pretreatment of the vessels with a specific nitric oxide synthase inhibitor, Nω-monomethyl-L-arginine (L-NMMA, 0.25 mM) before BK and ACh testing. For the contraction studies, cumulative dose responses to ANG II and phenylephrine were performed in all endothelium-disrupted pulmonary arteries. The vasconstrictor effects of each drug tested were normalized by expressing the data as a percentage of the maximum constriction induced by KCl (80 mM) in the same vessel ring.

Drugs and chemicals. ACh, ANG, BK, indomethacin, phenylephrine, and sodium nitroprusside were purchased from Sigma Chemical (St. Louis, MO). L-NMMA was purchased from Calbiochem, (La Jolla, CA). Losartan was kindly provided by Merck Pharmaceutical. All drug solutions were prepared just before use. Laboratory reagents and chemicals used for the preparation of KH solution were purchased from Fisher Chemical (Pittsburgh, PA).

Plasma ANG II peptide levels. Cardiac ANG II peptide concentrations were determined by a method recently described from our laboratory using solid-phase extraction (SPE), HPLC, and RIA (20). AG50WX4 (200-400 mesh) cation exchange resin was used in an SPE procedure for sample purification. The recovery from the SPE procedure has been previously determined in our laboratory using both labeled and unlabeled ANG peptides (21). With the use of 125I-labeled ANG I (1.4 × 107 counts/min) and 125I-labeled ANG II (9 × 106 counts/min), recoveries were 93 ± 2% (n = 6) and 91 ± 2% (n = 6), respectively. With the use of 0.5, 1.0, or 1.5 mmol of unlabeled ANG I and II, recoveries were 91 ± 9%.
(n = 6) and 90 ± 1% (n = 6), respectively (20). 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 5.4). Aliquots (100 ml) of each relevant fraction of column effluent were subjected to RIA immediately on 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 heart tissues examined. Antibodies to ANG I and II were raised in our laboratory in New Zealand White rabbits immunized against peptides conjugated to poly-L-lysine, as previously described (20). The 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 and for ANG II was 2 pg/ml.

**RESULTS**

**Hemodynamics.** Four months after induction of MR, pulmonary arterial (21 ± 3.5 mmHg) and pulmonary capillary wedge (15 ± 2.2 mmHg) pressures increased significantly in the MR dogs compared with the control values (13.3 ± 1.4 and 9.2 ± 1.4 mmHg, respectively). However, in the ramipril-treated MR dogs, at the end of the 4-mo period, no significant change in the pulmonary arterial (13 ± 1.0 mmHg) and pulmonary capillary wedge (9 ± 0.6 mmHg) pressures were noted. These data demonstrate the presence of pulmonary congestion and related heart failure in our experimentally induced MR dogs. Chronic treatment with the ACEI ramipril was effective in reversing these hemodynamic changes. Clinically, there were no significant differences between the groups, including necessity of treatment with furosemide.

**Vasoconstrictor responses of PA rings.** Addition of ANG II (0.1–10 nM) produced a potent dose-dependent contraction in isolated intralobar pulmonary arteries of control dogs, reaching a maximum of 4.7 ± 0.2 g and ED50 of −log 9 (Table 1). Figure 1A shows the actual contractile tension developed in response to increasing concentrations of ANG II in control, MR, and MR + R dogs. Figure 1B shows the same results after they were normalized as a percentage of maximum KCl contraction in each vessel ring. Chronic pulmonary congestion and heart failure in MR dogs resulted in a significant shift of the dose-response curve to the right and decreased both sensitivity (ED50 of −log 8.5) and extent of maximum contractile response (3.5 ± 0.4 g) to ANG II. Treatment of control dogs with ramipril did not alter the ANG II constrictor response (data not shown), followed by the Student’s unpaired t-test. P < 0.05 was considered statistically significant.

Table 1. Effects of MR and ACEI treatment on ANG II-induced vasoconstriction on isolated, endothelium-denuded canine pulmonary arteries

<table>
<thead>
<tr>
<th>n</th>
<th>Peak ANG II Response, g</th>
<th>ED50, −log</th>
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<tbody>
<tr>
<td>Control</td>
<td>35</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>MR</td>
<td>15</td>
<td>3.5 ± 0.4*</td>
</tr>
<tr>
<td>MR + ACEI</td>
<td>27</td>
<td>3.6 ± 0.2*</td>
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Values are means ± SE; n is no. of arteries. MR, mitral regurgitation; ACEI, angiotensin-converting enzyme inhibitor. *Statistically significant difference from control.

**Fig. 1.** Concentration-response relationships of ANG II-induced contraction in isolated, endothelium-denuded intralobar pulmonary arteries of control (○), mitral regurgitation (MR; ■), and MR and ramipril-treated (MR + R; ▲) dogs. A: actual contractile tension developed following cumulative addition of ANG II (0.1–10 nM). B: same data when contractile responses were expressed as percentage of maximum contraction observed in 80 mM KCl in same vessel rings. Data points represent means (control, 18 rings; MR, 18 rings; MR + R, 22 rings); vertical lines indicate ±SE. *Statistical difference from control at P < 0.05.
whereas similar ramipril treatment in the MR dogs completely restored the sensitivity of the pulmonary arteries to ANG II constrictor response. The maximum contractile response of the MR + R dogs, however, remained significantly lower (3.6 ± 0.2 g) than in the control dogs (Table 1, Fig. 1). The dissociation constants (K_B) of ANG II-induced contraction, determined in a subset of control and MR dogs in the presence of the specific ANG II receptor (AT_1) blocker losartan (10 nM), were not significantly different between the control (K_B = 14 nM) and the MR (K_B = 9.5 nM) dogs (Fig. 2). These results indicate that the decreased response to ANG II in MR dogs was probably related to a downregulation of the AT_1 receptors and not caused by AT_1 receptor dysfunction in the MR dogs with chronic congestive heart failure.

To further investigate the mechanism(s) of altered ANG II response in the MR dogs, dose-dependent contractile responses to α-adrenergic receptor activation by phenylephrine were also evaluated. Figure 3 shows that the dose-response curves to phenylephrine-induced contraction were not significantly different among the control, MR, and MR + R pulmonary arteries, suggesting that the changes in ANG II responses in MR dogs were probably related to a downregulation of the AT_1 receptors and not caused by AT_1 receptor dysfunction in the MR dogs with chronic congestive heart failure.

Relaxation response of PA rings. In all phenylephrine-precontracted and indomethacin-pretreated control pulmonary arteries, cumulative addition of 0.1 nM to 0.1 µM of BK resulted in a dose- and endothelium-dependent relaxation reaching a maximum of −87 ± 4%. Mechanical disruption of intimal endothelium and pretreatment with the specific inhibitor of nitric oxide synthase, 0.25 mM L-NMMA, completely abolished the observed BK relaxation (data not shown). Induction of chronic congestive heart failure with MR in MR dogs resulted in a significant depression of the BK-induced endothelium-dependent relaxation. As shown in Fig. 4, IC_50 of BK-induced relaxation was increased from 9 to 90 nM, respectively, in the control and the MR dogs. Similarly, the maximum relaxation to 0.3 µM BK was significantly reduced to −66 ± 4% in the MR dogs. However, chronic treatment of the MR dogs with the ACEI ramipril completely restored the BK relaxation response (Fig. 4).

As shown in Fig. 5, similar alterations in ACh-induced, endothelium-dependent relaxation in the MR dogs and their restoration when treated with ramipril were also observed. Maximum relaxation response to 3 µM ACh was decreased from −88 ± 3% (control dogs) to

Fig. 2. Effects of losartan on ANG II-induced contraction in isolated, endothelium-denuded pulmonary arteries in control (○; A) and MR (■; B) dogs. Losartan (0.01 and 0.1 µM; ○ and ■, respectively) was added to tissue bath 20 min before ANG II testing. Each point represents mean (control, 2 dogs, 4 rings; MR, 2 dogs, 4 rings); vertical lines indicate ± SE.

Fig. 3. Concentration-response relationships of cumulative addition of phenylephrine (0.01–0.3 µM) in isolated, endothelium-denuded pulmonary arteries in control (○), MR (■), and MR + R (▲) dogs. Each point represents mean (control, 15 rings; MR, 16 rings; MR + R, 18 rings); vertical lines indicate ± SE.
The maximum ACh relaxation in the ramipril-treated MR dogs was $283\pm4\%$, which was not significantly different from the control.

Circulating ANG II peptide levels. Plasma ANG II peptide levels were significantly elevated in a subset of MR dogs compared with control dogs (286 ± 27 pg/nl in control, n = 2, vs. 435 ± 299 pg/nl in MR dogs, n = 3) (Fig. 6). Treatment of MR dogs with the ACEI ramipril prevented the increases in circulating ANG II peptide levels (78 ± 34 pg/nl; n = 3).

DISCUSSION

We previously reported that percutaneous chordal rupture of the mitral valve in dogs resulted in pulmonary venous congestion and volume overload-induced left ventricular heart failure (7). More importantly, we reported that myocardial hypertrophy associated with this pathological state was accompanied by marked increases in cardiac ACE activity and tissue ANG II levels (7). Results of the present study confirmed these findings and further demonstrated that initiation of ACEI therapy, either early (24 h after the surgical mitral valve chordal rupture) or late (3 wk after induction of MR), prevented the associated pulmonary congestion and hypertension in these dogs with mitral valve insufficiency. A significant decrease in plasma ANG II levels was also observed in the animals treated with the ACEI ramipril compared with MR dogs not treated with the ACEI. These findings suggest a close relationship between the elevation of ANG II levels and the volume overload induced congestive heart failure. Thus the present animal model of congestive heart failure caused by chronic MR provides a useful tool for the investigation of the role of ANG II in the regulation of cardiovascular function.

ANG II is one of the most potent vasoconstrictors known and has also been implicated in the development of various pathophysiological hypertensive diseases (9, 13). In addition to the direct action on vasomotion, ANG II has also been shown to induce marked structural changes in the vasculature, and the resulting vascular hypertrophy and remodeling may enhance vasoconstriction and sustain hypertension (9). Indeed, a similar role of ANG II in the regulation of pulmonary vascular function and its associated increases during the development of pulmonary hypertension have been reported (4, 24). Accordingly, it is presumed that ANG II mediates a potent vasoconstrictor response in the systemic and pulmonary vasculature in vivo.

Our in vitro studies demonstrate a significant decrease in the contractile response and sensitivity to ANG II in the PA of MR dogs, whereas the $\alpha$-adrenergic receptor-mediated contraction was not altered in the same pulmonary vessels. This suggests a specific and selective perturbation of ANG II receptor function in the lungs of dogs with MR-induced heart failure. The marked elevated plasma ANG II levels in our MR dogs could have mediated a compensatory decrease in ANG II receptor function, possibly via an alteration in the...
specific signal transduction pathways, thereby accounting for the decreased ANG II constrictor response. Indeed, Cheng and co-workers (5) demonstrated a decreased contractility response to ANG II in isolated cardiocytes from dogs with chronic heart failure caused by rapid pacing. Alternatively, it is possible that a specific downregulation of AT1 receptor numbers could occur during chronic MR. This is substantiated by receptor binding studies in numerous other animal models of hypertension and heart failure, which demonstrated that AT1-receptor density in the heart and kidney was significantly reduced after ANG II infusion in the rat in vivo (29). Recently, heart failure in human patients resulted in a selective downregulation of the AT1 receptor (6, 31). Because ANG II is known to be one of the most potent factors in the regulation of gene expression of RAS components, it could account for the altered ANG II receptor function observed in the pulmonary vasculature. However, to our best knowledge, this present study is the first study to describe decreased functional response to ANG II in PA vessels in heart failure. However, one study (28) in the literature found that arterial vasoconstrictor response to ANG II is enhanced in pacing-induced heart failure in the dog. Another study demonstrated that canine lung lobes after pacing-induced heart failure and pulmonary hypertension show enhanced arterial vasoconstriction induced by norepinephrine after β-blockade (32). The reason for the difference between their data and ours may be that in the lung lobes, changes in vascular tone are dominated by those in resistance microvessels rather than the larger conduit vessels studied in our dogs. Also, results from the present paper are obtained under isometric conditions, whereas those in lung lobes are under isotonic conditions, which can lead to differences in sensitivity (3). Furthermore, there could be model-specific changes in pulmonary vascular function, i.e., pacing versus MR.

Our findings that chronic treatment with an ACEI such as ramipril is able to reverse and normalize the sensitivity of the PA to ANG II further supports our contention that adaptation of ANG II receptor function and/or density may have occurred. However, it is interesting to note that although the sensitivity of ANG II response was recovered, the peak ANG II response in the MR + R dogs remained decreased compared with the control dogs. The potential role of this complex interaction in mediating the response of pulmonary pressures in vivo requires further investigation.

Another important finding of this study was the significantly decreased EC-dependent relaxation of the pulmonary arteries of MR dogs in response to both BK and ACh. Bradykinin exerts its effects on the endothelium through BK2 receptors by activating endothelial nitric oxide synthase and also by activating arachidonic acid conversion to prostacyclin. ACh is a choline ester that exerts a vasodilatory response of most vascular beds through muscarinic receptors located on the endothelial cells with subsequent release of endothelium-derived relaxing factor (EDRF) (1, 2, 12, 22). Thus the decrease in the vasodilatory response to BK and ACh in the pulmonary arteries of MR dogs suggests a generalized endothelial dysfunction in this vascular bed rather than a BK-specific alteration in the endothelium-mediated relaxation. Such a generalized endothelial dysfunction could be caused by a diminished EDRF (nitric oxide) production and/or release in the MR pulmonary arteries. Similar findings have been reported with endothelial cell dysfunction during heart failure leading to enhanced vasoconstriction at rest and decreased vasodilatation in response to exercise and ischemia, further contributing to heart failure (23).

Our findings of endothelial cell dysfunction in the lungs of our dogs with chronic congestive heart failure concur with other reports in the literature. Ontkeane et al. (23) reported decreased EDRF-mediated relaxation to ACh in isolated PA rings taken from rats with congestive heart failure. However, Mathew et al. (19) found no significant difference in response to ACh and BK of canine pulmonary arteries between pacing-induced congestive heart failure and control groups but the vasodilatation to isoprotrenol and prostacyclin was significantly diminished. The differences between these findings and our study may be related to different neurohormonal activation of the heart failure models. However, to our knowledge, this is the first report of complete prevention or reversal of the pulmonary endothelial dysfunction with treatment of an ACEI in MR dogs.

In summary, in dogs with chronic MR, pulmonary congestion results in a significant decrease in the vasoconstrictor response to ANG II. Furthermore, relaxation to BK in these pulmonary arteries is also significantly decreased. These pulmonary vasomotion alterations are normalized in dogs with MR treated with an ACEI. Dogs with MR chronically treated with ramipril exhibit normal PA pressures in the presence of normal ANG II sensitivity but increased peak ANG II response. These in vitro and in vivo findings support the beneficial use of ACEIs. Additionally, these results could suggest that BK-NO signaling mechanisms may be important in pulmonary vasomotor tone in heart failure and may supersede the vasoconstrictor effects of ANG II. Whether these findings are applicable to the in vivo pulmonary circulation requires further investigation.

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