Right ventricular angiotensin converting enzyme activity and expression is increased during hypoxic pulmonary hypertension

Nicholas W. Morrell a,*, Sergei M. Danilov b, Krishna B. Satyan a, Kenneth G. Morris c, Kurt R. Stenmark a

a Lung Developmental Biology Laboratory, University of Colorado Health Sciences Center, Denver, CO 80262, USA
b Departments of Anesthesiology and Pharmacology, University of Illinois at Chicago, Chicago, IL 60612, USA
c Cardiovascular Pulmonary Laboratory, University of Colorado Health Sciences Center, Denver, CO 80262, USA

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Abstract

Objective: To determine whether local cardiac angiotensin converting enzyme (ACE) expression is upregulated during the development of hypoxia-induced right ventricular hypertrophy. Methods: ACE activity was measured in membrane preparations from the right ventricle and left ventricle plus septum in normoxic rats and animals exposed to chronic hypoxia for 8 and 14 days. Local cardiac ACE expression was studied by immunohistochemistry using a monoclonal antibody to ACE 9B9. Results: In the normal rat heart, ACE expression was confined to vascular endothelium, the valvular endocardium, and localized regions of parietal endocardium. We found that the development of pulmonary hypertension and right ventricular hypertrophy were associated with 2.6- and 3.4-fold increases in membrane-bound right ventricular ACE activity by 8 and 14 days of hypoxia, respectively. Right ventricular ACE activity was positively correlated with the degree of right ventricular hypertrophy ($r = 0.83$, $P < 0.001$). In contrast, left ventricular plus septal ACE activity was significantly reduced by approximately 40 and 60% by 8 and 14 days of hypoxia, respectively, compared to controls. In the right ventricle of chronically hypoxic rats, immunohistochemistry demonstrated increased ACE expression in areas of myocardial fibrosis. Interestingly, increased ACE expression was noted in the right ventricular epicardium in chronically hypoxic rats. In the free wall of the left ventricle there was a significant reduction in the number of myocardial capillaries which expressed ACE in chronically hypoxic rats. Conclusion: Chronic hypoxia has a differential effect on left and right ventricular ACE activity and that the sites of altered ACE expression are highly localized. We speculate that locally increased right ventricular ACE activity and expression may play a role in the pathogenesis of right ventricular hypertrophy secondary to hypoxic pulmonary hypertension.

Keywords: Hypoxia; Cor pulmonale; Endothelium; Fibrosis; Rat, ventricular myocardium; Pulmonary hypertension; ACE gene; ACE activity

1. Introduction

Although factors involved in hypertrophic growth of the left ventricle have been extensively investigated in systemic hypertension, few studies have examined the role of these factors in the setting of hypoxic pulmonary hypertension and right ventricular hypertrophy. During chronic hypoxia, hypertrophy of the right ventricle [1,2] may reflect an attempt to maintain right ventricular output in the face of a greatly increased afterload, consequent upon increased pulmonary vascular resistance [3]. However, continued hypertrophy and deposition of extracellular matrix may impair ventricular function and lead to decompensation of the right ventricle [4].

One system which appears to be important in the development of left ventricular hypertrophy is the renin–angiotensin system [5–8]. Angiotensin converting enzyme (ACE) activity and mRNA expression are increased in the hyper-
trophied left ventricle [8] and treatment of patients [9] and animals [7,10] with inhibitors of ACE reduces hypertrophy and improves survival. This effect on mortality has not been investigated in patients with pulmonary hypertension and right ventricular hypertrophy, though we have previously shown that ACE inhibition with captopril, or blockade of the angiotensin II type 1 receptor with losartan, reduces the pulmonary arterial pressure and right ventricular hypertrophy in rats with hypoxia-induced pulmonary hypertension [11]. This protective effect of ACE inhibition on right ventricular hypertrophy may be due to the decreased pulmonary vascular resistance and hence decreased afterload in rats treated with ACE inhibitors. Alternatively, some of the protective effect of ACE inhibition may also be due to an effect on local right ventricular ACE activity and a reduction in local cardiac angiotensin II formation.

Previous studies have not assessed changes in cardiac ACE during the specific situation of chronic alveolar hypoxia, in which right ventricular hypertrophy occurs with minimal effects on left ventricular function and mass. Therefore, we sought to determine whether local cardiac ACE may play a role in right ventricular hypertrophy associated with hypoxic pulmonary hypertension. Our results demonstrate that right ventricular hypertrophy is associated with an increase in membrane-bound right ventricular ACE activity and localized increases in ACE expression by immunohistochemistry. In contrast, ACE activity and expression were reduced in the left ventricle during chronic hypoxia. These results suggest a role for local overexpression of ACE in the pathogenesis of hypoxia-induced right ventricular hypertrophy.

2. Methods

2.1. Hemodynamic studies

Male Sprague-Dawley rats (weight approximately 250 g) were divided into 3 groups of 10 animals. One group served as a normoxic control and was maintained at Denver’s altitude of 5300 feet. Two experimental groups were maintained for 8 or 14 days at a simulated altitude of 17000 feet (equivalent to an inspired oxygen concentration of approximately 10%) in an hypobaric hypoxic chamber to induce pulmonary hypertension. After the specified period of hypoxia, subgroups of rats were anesthetized (intramuscular ketamine 75 mg/kg and xylazine 6 mg/kg); a polyethylene catheter was inserted into the right carotid artery for measurement of mean systemic pressure and a polyvinyl catheter was inserted into the right internal jugular vein and guided through the right ventricle into the main pulmonary artery to measure mean pulmonary arterial pressure, as described previously in detail [12]. Correct positioning of the pulmonary artery catheter was determined from the shape of the pressure tracing displayed on an oscilloscope. Cardiac output was measured by cardiogreen dye dilution [13]. These indices were calculated by an on-line personal computer. Cardiac output was calculated using an algorithm based on the standard Stewart-Hamilton method and normalized to body weight to obtain the cardiac index [14]. Blood samples were drawn from the carotid catheter into capillary tubes for measurement of hematocrit.

2.2. Assessment of right ventricular hypertrophy

Following hemodynamic studies the thorax was opened and the animal exsanguinated by cutting the inferior vena cava. To wash residual blood from the heart, a cannula was inserted through an incision at the apex of the right ventricle and flushed with 20 ml of phosphate-buffered saline. The heart and lungs were then removed en bloc. The heart was dissected free and kept on ice. The atria were removed and the right ventricle was carefully separated from the left ventricle and septum. The ventricles were blotted dry and weighed separately to determine indices of ventricular hypertrophy: the ratio of the weight of the right ventricle to that of the left ventricle plus septum (RV/LV + S) [15], the weight of the right ventricle to the animal’s body weight (RV/BW), and left ventricle plus septum-to-body weight ratio (LV + S/BW).

2.3. Processing of heart tissue

To study ventricular ACE activity in normoxic and chronically hypoxic rats, the separated ventricles were rapidly frozen in liquid nitrogen after weighing. Ventricles were then stored at −70°C for assay of ACE activity. For immunohistochemical studies of local ACE expression in subgroups of rats (n = 5 per group), the intact heart was embedded in medium for frozen tissue specimens (Tissue-Tek® OCT Compound, Miles Inc., Elkhart, IN), in a cryomold (Tissue-Tek®). Hearts were then immediately frozen in hexane cooled by dry ice in ethanol. Once frozen, blocks were stored at −70°C prior to sectioning.

2.4. Measurement of right and left ventricular ACE activity

The normally low level of cardiac ACE activity [16] was assayed in a crude ventricular homogenate and also in a tissue membrane preparation using a sensitive fluorometric assay [17]. The membrane preparation was used in an attempt to avoid contamination of the normally low level of cardiac ACE activity [16] by free ACE in serum. Ventricles were thawed and homogenized (Tekmar® Tissumizer, Cincinatti, OH) at 4°C in a 10-fold excess of Tris (0.05 M, pH 7.4) buffered saline (0.15 M NaCl). An
Table 1

Mean pulmonary artery pressure and resistance index, cardiac index, hematocrit, heart weights and mean systemic blood pressure in normoxic control, 8- and 14-day hypoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MPAP (mmHg)</th>
<th>CI (l/min/kg)</th>
<th>PVRI (mmHg/l/min/kg)</th>
<th>Hct (%)</th>
<th>RV/BW (g/kg)</th>
<th>LV+S/BW (g/kg)</th>
<th>RV/LV+S (g/kg)</th>
<th>MSAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic control</td>
<td>5</td>
<td>17.9±0.8</td>
<td>0.26±0.2</td>
<td>69.3±3.3</td>
<td>48±1</td>
<td>0.60±0.4</td>
<td>2.31±0.8</td>
<td>0.25±0.16</td>
<td>112±5</td>
</tr>
<tr>
<td>Hypoxic 8 days</td>
<td>5</td>
<td>26.4±1.2</td>
<td>0.25±0.2</td>
<td>105.6±5.6</td>
<td>61±25</td>
<td>0.92±0.4</td>
<td>2.22±1.2</td>
<td>0.40±0.15</td>
<td>98±3</td>
</tr>
<tr>
<td>Hypoxic 14 days</td>
<td>5</td>
<td>32.6±1.2</td>
<td>0.23±0.3</td>
<td>141.5±13.9</td>
<td>61±3</td>
<td>1.11±0.6</td>
<td>2.08±0.6</td>
<td>0.53±0.3</td>
<td>96±3</td>
</tr>
</tbody>
</table>

All values are mean± s.e. RV/LV+S = ratio of weight of right ventricle to that of left ventricle plus septum; BW = body weight; n = number of animals; MPAP = mean pulmonary artery pressure; PVRI = pulmonary vascular resistance index; MSAP = mean systemic arterial pressure; Hct = hematocrit; CI = cardiac index. * P < 0.05 compared to normoxic group.

An aliquot of crude homogenate was centrifuged at 1000 × g for 15 min at 4°C, and the resulting supernatant was submitted to another centrifugation at 16000 × g for 30 min at 4°C to yield the particulate fraction containing cell membranes. The pellet was washed superficially with 5 ml cold assay buffer, then resuspended in 500 μl of fresh buffer containing the detergent, nonidet P-40 (1%), sonicated 2 × 10 s, centrifuged at 300 × g for 10 min and the supernatant assayed for ACE activity. The membrane preparation and crude homogenates were diluted 1:4, then 10 μl was added to 200 μl of 0.5 mM Z–Phe–His–Leu substrate, 50 mM Tris, pH 7.5, 150 mM NaCl at 37°C for 45 min. This reaction was stopped by the addition of 1.5 ml 0.28N NaOH. O-Phthalaldehyde (1 mg in 100 μl methanol) was then added for 10 min before terminating this reaction with 200 μl 2 N HCl. The fluorescence of the samples was measured with a fluorescence spectrometer (Perkin-Elmer 650-10S) at an emission wavelength of 500 nm and an excitation wavelength of 363 nm. Samples were run in duplicate and included blanks (consisting of all reagents apart from homogenate), His–Leu standards and samples incubated in the presence of the ACE inhibitor, captopril. The dilution of membrane preparation and crude homogenate chosen (1/4) gave an optimal fluorescent signal whilst maintaining initial reaction rate conditions [17]. The results were expressed as mU/mg of protein, where 1 mU represents the generation of 1 nmol His–Leu/min. Protein concentration was determined in the homogenates with a Bio-Rad protein assay kit Bio-Rad Laboratories, Hercules, CA and quantitated by comparison with a standard curve for bovine serum albumin.

The specificity of the assay for ACE activity was confirmed by the absence of fluorometric signal when 10−6 M captopril was added to the initial incubation of substrate with sample.

2.5. Immunohistochemistry

A monoclonal antibody to ACE (9B9) was used [18,19] to investigate the distribution of ACE antigen by immunohistochemistry [20] in the right and left ventricles before and during the development of hypoxic pulmonary hypertension. Cryostat sections of frozen tissue were cut (4 μm thick) and fixed in acetone for 10 min at room temperature. Sections were stored at −20°C for up to 1 week prior to staining.

ACE antigen was visualized by the alkaline phosphatase anti-alkaline phosphatase technique (APAAP) technique as previously described [21]. Briefly, sections were incubated with 10% rat serum in Tris (50 mM, pH 7.6) buffered saline to reduce non-specific binding of antibodies, then with the primary anti-ACE monoclonal antibody (0.5 μg/ml) for 1 h. This was followed by an incubation with
Fig. 3. Serial frozen sections of an intramural coronary artery and myocardium in the right ventricle of a control rat demonstrating higher level of ACE expression in the endothelium of muscular coronary arteries compared to myocardial capillaries. ACE immunostaining is seen in the endothelium of both coronary arteries (star) and capillaries (arrows) at higher concentrations of anti-ACE monoclonal antibody (A, 0.5 μg/ml), but at lower concentrations ACE is detected only in the endothelium of the coronary arteries (B, 0.25 μg/ml; C, 0.125 μg/ml). The specificity of staining was demonstrated by the absence of signal in sections incubated with control mouse IgG in place of the monoclonal antibody (D). Bar = approx. 100 μm.
Fig. 4. Regional differences in ACE expression in the normal rat endocardium. ACE immunostaining was prominent in the valvular endocardium, in this instance the mitral valve shown (mv) (A). In the parietal endocardium (arrows) ACE expression was highly localized, being observed in the endocardium overlying prominent trabeculations in the free wall of the left ventricle (LV) (B), but absent from the septal wall of the left ventricle (LVS) (C). In addition, ACE was present in the endocardium of the interventricular septal (IVS) wall of the right ventricle (D). Bar = approx. 100 μm.
rabbit anti-mouse ‘link’ IgG (1:40), then with monoclonal APAAP complex (1:50) for 30 min each. Incubations with rabbit anti-mouse IgG and APAAP were then repeated for 10 min each. All incubations were supplemented with 10% pooled rat serum. Samples were thoroughly washed in Tris-buffered saline between steps. Bound alkaline phosphatase was visualized by a 20 min incubation with new fuchsin (100 μg/ml) plus levamisole (400 μg/ml) to yield a red reaction product. Sections were then counterstained with hematoxylin, rinsed in distilled water, mounted in aqueous mounting medium (Aqua-mount®, Lerner Labs, Pittsburgh, PA) and examined on a Nikon Optiphot photomicroscope. Sections treated with all steps except the primary antibody, or control mouse IgG in place of the primary antibody, served as controls.

We used a semi-quantitative analysis to differentiate degrees of ACE expression with the APAAP technique, as suggested by F. Franke (Institute of Pathology, Giessen, Germany) [22]. A range of doubling dilutions of ACE antibody was found which allowed cardiac ACE to be detected with maximum signal at one end of the range and complete extinction of signal at the other. The range of dilutions of antibody was found empirically to be from 5 to 0.03 μg/ml. Serial sections of hearts from normoxic and 8 or 14 day hypoxic rats were processed together and stained with the range of primary antibody dilutions to allow comparison of signal between groups. Thus ACE expression in any vessel could be defined in terms of the dilution of antibody at which signal could still just be detected.

Preliminary observations gave the impression of a reduction in the number of capillaries which expressed ACE in the chronically hypoxic left ventricle. Thus we compared the number of myocardial capillaries immunoreactive for ACE in the free walls of left and right ventricles in normoxic chronically hypoxic rats. For this analysis, transverse sections of rat heart were stained with anti-ACE monoclonal antibody as above using a fixed antibody concentration of 0.5 μg/ml. After counterstaining, the sections were viewed under a high-power objective (×40) and all ACE-positive capillaries visible in 10 randomly selected fields were counted. The counts were expressed as ACE-positive capillaries per field.

2.6. Histochemistry

To assess the distribution of collagen in the normal and hypertrophied right ventricle, 4-μm-thick frozen sections, adjacent to those stained with anti-ACE monoclonal antibody, were stained with Pentachrome solution using standard histological techniques [23].

2.7. Statistical methods

All results are expressed as means ± s.e. One factor analysis of variance was used to compare means between groups with Duncan’s new multiple range test to establish significant differences. A P-value of < 0.05 was taken to indicate that conventional statistical significance had been achieved.

2.8. Reagents and antibodies

Z–Phe–His–Leu was obtained from Crescent Chemical Co. (Hauppauge, NY); O-phthaldehyde from Serva (Heidelberg, Germany); rabbit anti-mouse IgG, monoclonal APAAP complex from DAKO Corporation (Carpinteria, CA); new fuchsin substrate from BioGenex (San Ramon, CA); His–Leu, captopril, levamisole and control mouse ascitic fluid from Sigma Chemical Co. (St. Louis, MO).

3. Results

3.1. Confirmation of pulmonary hypertension and right ventricular hypertrophy

In subgroups of control rats and rats exposed to chronic hypoxia for 8 and 14 days the mean pulmonary artery pressure and mean pulmonary vascular resistance were measured to confirm the presence of pulmonary hypertension in hypoxic rats (Table 1). Although the cardiac output was unaffected by chronic hypoxia, the systemic blood pressure was moderately depressed in hypoxic rats (Table 1). The RV/LV + S ratio and RV/BW were significantly elevated in chronically hypoxic rats, indicating the presence of right ventricular hypertrophy (Table 1). There were no changes in the weight of the left ventricle (LV/BW) during hypoxia. Chronic hypoxia caused an expected increase in the mean hematocrit (Table 1).

3.2. Changes in ventricular ACE activity

In crude ventricular homogenate we found no difference between the ACE activity measured in the left and right ventricles of control animals: 1.12 ± 0.09 vs. 1.18 ± 0.06
mU/mg, respectively. Furthermore, ACE activity in the crude homogenate was not significantly altered by exposure to chronic hypoxia: at 8 days of chronic hypoxia left and right ventricular ACE activity was 0.95 ± 0.05 and 1.1 ± 0.16 mU/mg, respectively, and at 14 days 1.05 ± 0.06 and 1.06 ± 0.05 mU/mg, respectively.

In contrast, when the effect of contaminating serum was removed by measurement of ACE activity in membrane preparations from control ventricles, ACE activity was higher in the left ventricle plus septum (5.53 ± 0.75 mU/mg) than in the right ventricle (1.08 ± 0.15 mU/mg) ($P < 0.01$). Chronic hypoxia for 8 or 14 days caused a 2.6- and 3.4-fold increase, respectively, in specific membrane-bound right ventricular ACE activity compared to normoxic control rats (Fig. 1). In contrast, left ventricular ACE activity was significantly reduced during chronic hypoxia to less than half that measured in control rats (Fig. 1).

Since we found a marked increase in membrane-bound ACE activity in the hypertrophied right ventricle, we looked for a correlation between the degree of hypertrophy and the specific ACE activity measured in the same animal. Fig. 2 shows that a strong correlation existed between right ventricular ACE activity and the index of right ventricular hypertrophy, $RV/LV + S$ ($r = 0.83$, $P < 0.001$). A similar correlation existed between right ventricular ACE activity and $RV/BW$ ($r = 0.78$, $P < 0.001$).

### 3.3. Localization of ACE expression

Having found a differential effect of chronic hypoxia on membrane-bound right ventricular and left ventricular plus septal ACE activity, we sought to localize the sites of ACE expression in the normal and chronically hypoxic heart by immunohistochemistry. In the normoxic rat ACE immunoreactivity was prominent in the endothelial cells of muscular coronary arteries and capillaries (Fig. 3A–D). Serial dilutions of anti-ACE antibody demonstrated that the highest levels of ACE expression in the normoxic rat were found in the endothelium of small muscular intramyocardial coronary arteries, since endothelial staining in these arteries was still visible at antibody dilutions as low as 0.125 μg/ml (Fig. 3C), whereas ACE immunoreactivity in myocardial capillaries could not be detected at this antibody concentration. Specificity of staining was demonstrated by the absence of signal in sections incubated with control mouse IgG1 (same isotype as ACE monoclonal antibody) in place of anti-ACE antibody (Fig. 3D).

ACE expression was consistently demonstrated in the endothelium of valvular endocardium in both the right and left heart (Fig. 4A). ACE immunostaining was also observed in endothelial cells of the parietal endocardium, but interestingly was non-uniform in its distribution. In the left ventricle, ACE expression in the parietal endocardium was restricted to the endocardial endothelium overlying prominent trabeculations in the free wall (Fig. 4B), no ACE expression being visible in the septal wall of the left ventricle (Fig. 4C). However, in the right ventricle, ACE was detected mainly in the endocardium of the septum (Fig. 4D).

In chronically hypoxic rats, the free wall of the right ventricle appeared thicker than that in the normoxic rats, consistent with right ventricular hypertrophy. In chronically hypoxic rats locally increased ACE expression was seen at the following sites: (1) in all hypoxic animals ACE expression appeared in the epicardium (visceral pericardium) of the right ventricle from which it was entirely absent in normoxic animals (Fig. 5A,C); (2) increased ACE immunostaining was observed associated with areas of interstitial fibrosis of varying degrees within the right ventricular myocardium in all hypoxic rats (Fig. 5C,D); (3) the most dramatic change seen in hypoxic rats was the appearance of ACE-positive cells in regions of sub-pericardial fibrosis (Fig. 5E). These changes were observed in 3 animals exposed to 14 days of hypoxia. The presence of fibrosis was confirmed by Pentachrome staining in sections adjacent to those stained with anti-ACE monoclonal antibody, demonstrating that collagen deposition was localized to areas of increased ACE expression (Fig. 5E,F). No collagen was seen in the myocardium of the normal right ventricle (Fig. 5B).

In the free wall of the chronically hypoxic left ventricle none of the above changes was seen. However, there appeared to be fewer myocardial capillaries which expressed ACE compared to normoxic controls. To confirm this impression, we counted the number of capillaries immunoreactive for ACE in the free walls of right and left ventricles in normoxic rats and rats exposed to hypoxia for 8 and 14 days. Fig. 6 shows that the number of capillaries which expressed ACE in the left ventricle was reduced over 50%, while the number of ACE positive capillaries in the right ventricle was unchanged. It is also interesting to note that in control rats the number of ACE-positive capillaries per high-power field in the wall of the right ventricle (9.2 ± 0.9) was less than in the left ventricular wall (17.3 ± 0.5) ($P < 0.05$).
4. Discussion

This study has shown that membrane-associated ACE activity is increased in the hypertrophied right ventricle of chronically hypoxic rats and that the degree of right ventricular hypertrophy was positively correlated with right ventricular ACE activity. Furthermore, we have demonstrated by immunohistochemistry that the sites of increased right ventricular ACE expression during chronic hypoxia are highly localized, involving the visceral pericardium and areas of myocardial fibrosis. Since we have previously found that inhibitors of ACE and an angiotensin II type 1 receptor antagonist significantly attenuate right ventricular hypertrophy in the chronically hypoxic rat [11], it is likely that some of these effects may be due to inhibition of locally increased expression of right ventricular ACE and angiotensin II. Ventricular ACE activity is known to increase in the pressure-overloaded left ventricle [8,24,25] and in the right ventricle in rat models of congestive cardiac failure [24,25]. However, our observations are novel because changes in cardiac ACE expression and activity during chronic hypoxia are clearly not predictable from the results of previous studies in congestive cardiac failure (e.g., our finding of reduced left ventricular ACE activity and expression in chronically hypoxic rats). In addition, our results may be relevant to the clinical condition of cor pulmonale where right ventricular hypertrophy and dilatation occurs with minimal effects on left ventricular function.

In the normal heart we found by immunohistochemistry that ACE expression was prominent in endothelial cells of myocardial capillaries and the endothelium of coronary vessels. A semi-quantitative analysis using serial dilutions of anti-ACE antibodies confirmed a previous observation [26] that the highest levels of ACE expression are found in small muscular arteries of the myocardium (Fig. 3). We have also observed a similar gradient of ACE expression along the longitudinal axis of the vasculature in the rat lung [21], and this observation appears to have general application to other organs [27].

Localized regions of the endothelium of the parietal and valvular endocardium demonstrated ACE immunostaining in the present study (Fig. 4). The relatively high levels of ACE expression by valvular endothelial cells has been noted previously [28,29]. Binding of ACE to cultured parietal endocardial endothelial cells has been described [30], but previous reports of heart ACE localization in vivo either do not mention this site [26,28] or have used autoradiographic techniques with a lower cellular resolution than immunohistochemistry [29]. The site-specific expression of ACE by endothelial cells of the parietal endocardium noted in our study may represent regional differences in endocardial function within the ventricle [31].

The sites of increased right ventricular ACE expression in hypoxic rats were highly localized and involved the epicardium, regions of subepicardial fibrosis, as well as focal areas of fibrosis within the right ventricular myocardium (Fig. 5). Although we did not definitively identify ACE-expressing cells in areas of fibrosis as fibroblasts, induction of ACE expression in cardiac fibroblasts has been noted previously within the fibrotic reaction following myocardial infarction [26,25]. In congestive cardiac failure following myocardial infarction in rats, ACE expression has also been reported in the right ventricular pericardium and within regions of myocardial fibrosis associated with increased production of type I collagen [25]. One possible effect of locally increased ACE expression in the hypertrophied ventricle would be to increase the local production of angiotensin II from angiotensin I [8], though the amount of ACE necessary to degrade peptides locally remains unknown. Angiotensin II is known to cause hypertrophy of cardiac myocytes in culture [32], a process mediated through the type 1 receptor [33]. The observation that ACE expression can be induced in cardiac fibroblasts is especially interesting since angiotensin II is also mitogenic for cardiac fibroblasts [34,35].

One of the other important actions of ACE is the degradation of bradykinin. In the hypertrophied, pressure-overloaded left ventricle a majority of the antihypertrophic effects of ACE inhibitors are thought to be due to the potentiation of bradykinin [36]. However, we have previously found that although captopril partly prevents the development of right ventricular hypertrophy during chronic hypoxia, this antihypertrophic effect of captopril is not reversed by the co-administration of a bradykinin type 2 receptor antagonist [11]. Thus in the pathogenesis of hypoxia-induced right ventricular hypertrophy, increased local AII production by locally upregulated ACE expression may be more important than enhanced degradation of bradykinin.

Our observation that there is no difference between right and left ventricular ACE activities when measured in crude ventricular homogenates is in agreement with other recent reports which have shown minimal differences in ACE activity between the ventricles [8,37]. However, membrane-bond ACE activity is likely to be a more accurate index of tissue ACE activity than enzyme activity assayed in the crude homogenate, because unbound and serum ACE activity is excluded. Moreover, our data regarding ACE activity are consistent with the finding of a lower density of capillaries which express ACE in the free wall of the right ventricle (Fig. 6).

A surprising finding in the present study was reduced left ventricular ACE activity during chronic hypoxia. This reduction in ACE activity can be accounted for by the reduced number of left ventricular capillaries which expressed ACE in hypoxic rats (Fig. 6), though the present study does not differentiate between a reduction in capillary density or reduced ACE expression by a structurally unaltered capillary bed. Presumably, the regulation of ACE expression during chronic hypoxia is under different control mechanisms in the left and right ventricles. We have...
previously shown that lung ACE activity is also reduced during chronic hypoxia, suggesting that hypoxia may have a direct effect on capillary ACE expression in some organs [21]. The differential effect of chronic hypoxia on right and left ventricular ACE expression suggests the involvement of local factors in the regulation of right ventricular ACE expression, rather than circulating factors, which would be expected to affect both ventricles similarly. We feel it is unlikely that the modest reduction in mean systemic arterial pressure (less than 10%) in chronically hypoxic rats (Table 1) could account for the marked reduction in left ventricular ACE activity and capillary ACE expression. Indeed, the slight reduction in mean systemic arterial pressures in hypoxic rats may have been an artefact of general anaesthesia in these animals, since systemic blood pressure measured in conscious rats is unaffected by chronic hypoxia [11]. Moreover, left ventricular weight was not significantly changed by chronic hypoxia (Table 1). Conceivably, reduced left ventricular capillary ACE activity during chronic hypoxia may serve to limit myocardial oxygen consumption during prolonged hypoxia, analogous to the downregulation of β-adrenergic receptors in the chronically hypoxic rat heart [38].

In conclusion, this study has demonstrated that chronic hypoxia differentially affects ventricular ACE activity. Hypertrophy of the right ventricle during chronic hypoxia is associated with increased ACE activity, and ACE expression in the visceral pericardium and areas of myocardial fibrosis. In contrast, left ventricular ACE activity is decreased, probably due to a reduction in the number of myocardial capillaries which express ACE. Our results suggest that increased local production of Ang II in the chronically hypoxic right ventricle may contribute to the pathogenesis of cor pulmonale.

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References


