Reciprocal regulation of pulmonary and cardiac angiotensin-converting enzyme in rats with severe left ventricular hypertrophy

Michael Pfeifer a, Günter Bruckschlegel a, Stephan R. Holmer a, Martin Paul b, A.J. Gunter Riegger a, Heribert Schunkert a,∗

a Klinik und Poliklinik für Innere Medizin II, Klinikum der Universität, Franz-Josef Strauß Allee 11, D-93053 Regensburg, Germany
b Department of Clinical Pharmacology and Toxicology, Free University of Berlin, Benjamin Franklin Medical Center Hospital, Berlin (M.P.), Germany

Received 22 July 1997; accepted 6 November 1997

Abstract

Objective: Numerous studies support the concept that cardiac angiotensin-converting enzyme (ACE) is involved in the pathophysiology of left ventricular hypertrophy. However, the pulmonary vasculature is considered to be the most prominent site of ACE expression. We thus examined the tissue specificity of ACE regulation in rats with severe cardiac pressure overload hypertrophy in transition to cardiac failure with secondary pulmonary hypertension. Methods and Results: Rats were studied 12 weeks after banding of the ascending aorta LVH, n = 20 that resulted in a 1.7-fold increase in left ventricular (LV) to body weight ratio. In addition, as compared to sham-operated rats (n = 20), we observed in LVH rats a 1.6-fold increase in right ventricular (RV) to body weight ratio, the development of pulmonary hypertension, and elevated plasma renin activities. Moreover, ACE mRNA and activity levels were more than 2-fold higher in both hypertrophied ventricles (P < 0.01, each). In contrast, pulmonary ACE mRNA and activity levels were markedly decreased in animals with LVH (more than 30%, respectively, P < 0.05 vs. sham). Interestingly, LV and RV ACE activity, as well as systolic pulmonary artery pressure and plasma renin activity, were all inversely related to pulmonary ACE activity. In order to differentiate the potential role of elevated renin in the down-regulation of pulmonary ACE, additional rats (n = 12) were treated with furosemide that resulted in a 8-fold rise in plasma renin activity, but only in a marginal decrease of pulmonary ACE mRNA levels and activity (−10% vs. sham, n = 8, P-value n.s.). Conclusions: The data indicate tissue specific reciprocal regulation of pulmonary and cardiac ACE in rats with cardiac pressure overload hypertrophy and pulmonary hypertension, a phenomenon that may potentially result in a partial shift of angiotensin II formation from the pulmonary to the cardiac circulation. © 1998 Elsevier Science B.V.

Keywords: Angiotensin-converting enzyme; Renin–angiotensin system; Pressure overload hypertrophy; Pulmonary hypertension

1. Introduction

The lung, with its large vascular bed and high angiotensin converting-enzyme (ACE) activity, is regarded to be the major site of angiotensin II release [1]. More recently, this concept was challenged by evidence for angiotensin II generation in various extra-pulmonary tissues that may be facilitated by locally regulated ACE activity [2]. In particular, pressure overload of the heart or the aorta has been shown to result in the activation of ACE mRNA and ACE activity and subsequently enhanced local angiotensin II generation [2–5].

The growing recognition of tissue specifically regulated extra-pulmonary ACE also recalled attention to the role of pulmonary ACE [6–8]. Interestingly, previous studies revealed suppressed ACE levels in the lung under certain conditions, such as hypoxic pulmonary hypertension or chronic angiotensin II infusion [7,9–11]. In parallel, rats with decreased pulmonary ACE activity displayed an impairment of pulmonary angiotensin I to angiotensin II
Table 1
Weights, systolic and RV pressure, as well as biochemical parameters of rats with chronic pressure overload by aortic banding and sham control

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>LVH</th>
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<tbody>
<tr>
<td></td>
<td>(n = 20)</td>
<td>(n = 20)</td>
</tr>
<tr>
<td>BW weight (g)</td>
<td>596 ± 119</td>
<td>520 ± 110b</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>0.99 ± 0.14</td>
<td>1.54 ± 0.30b</td>
</tr>
<tr>
<td>RV weight (g)</td>
<td>0.228 ± 0.04</td>
<td>0.322 ± 0.12b</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>1.67 ± 0.13</td>
<td>1.96 ± 0.7</td>
</tr>
<tr>
<td>Lung water (%)</td>
<td>77.6 ± 1.3</td>
<td>78.2 ± 2.3</td>
</tr>
<tr>
<td>Indirect systolic BP (mmHg)</td>
<td>132 ± 10</td>
<td>128 ± 14</td>
</tr>
<tr>
<td>Systolic pulmonary artery pressure (mmHg)</td>
<td>26.0 ± 10.3</td>
<td>37.9 ± 17.1a</td>
</tr>
<tr>
<td>Serum ACE (nmol/ml/min)</td>
<td>44.7 ± 20</td>
<td>62.3 ± 35a</td>
</tr>
<tr>
<td>Renin (ng ANG I/ml/h)</td>
<td>4.7 ± 1.8</td>
<td>8.0 ± 2.6a</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>163 ± 96</td>
<td>312 ± 180a</td>
</tr>
</tbody>
</table>

Values are means ± s.d.

aP < 0.05 vs. sham control.
bP < 0.01 vs. sham control.

Thus, down-regulation of pulmonary ACE may result in a decrease of pulmonary angiotensin I consumption and subsequently in an increase of arterial angiotensin I levels, specifically in situations when renin levels are high [8]. The pathophysiological significance of down-regulated pulmonary ACE may be further amplified by reciprocal regulation of ACE in other tissues, potentially resulting in an augmentation of extra-pulmonary release of the short-lived angiotensin II. Since angiotensin II has vasoactive, positive inotropic and growth-promoting properties [13–16], reciprocal regulation of pulmonary and cardiac ACE may be of specific significance in cardiac hypertrophy or failure and secondary pulmonary hypertension. We therefore studied the regulation of pulmonary and cardiac ACE in rats with chronic left ventricular pressure overload.

Fig. 1. RT-PCR of ACE mRNA. Top section displays a representative RT-PCR of left ventricular ACE mRNA (ACE target) of a sham-operated animal (upper panel) and a rat with left ventricular hypertrophy (lower panel). In the same reaction, increasing amounts of an exogenous truncated ACE competitor DNA were amplified. As can be seen, left ventricular ACE mRNA (ACE target) of the LVH rat gave substantially enhanced signals. Bottom section displays semi-quantitative analysis with bars representing the mean ± s.d. of 10 control and 12 LVH hearts.
2. Methods

Protocols were approved by the local standing committee on animal research. Normotensive, male Wistar rats (100 g body weight) were obtained from Charles River Wiga Breeding Laboratories (Sulzfeld, Germany). Forty animals were randomly assigned to aortic banding or sham operation. Aortic stenosis was created in anesthetized weanling rats by placing a stainless-steel clip of 0.6 mm internal diameter on the ascending aorta [17–19]. Sham-operation was performed in age-matched controls that underwent a left thoracotomy. Animals were individually housed in a 12-h dark/light cycle controlled room, fed a standard rat chow (H1003, Alma KG, Germany) with water ad libitum, and used after 12 weeks for experimentation. In order to investigate the effect of elevated renin levels on pulmonary ACE regulation, additional rats were treated with intraperitoneal injections of furosemide 10 mg/kg for 3 days [20]. The furosemide-treated animals received a low sodium diet (altromin C 1036, Na+ < 0.02%).

2.1. Blood pressure measurements

Indirect systolic blood pressure was determined by the tail-cuff method using an automated cuff inflator–pulse detection system (BP recorder No. 8005, W + W Electronic AG, Hofheim, Germany). Unanesthetized rats were placed in a restraining holder from which the tail protruded. Vasodilation was achieved by local warming of the tail. Cuff and transducer were placed around the tail, inflated, and slowly deflated until the pulse reappeared indicating the systolic blood pressure [17].

2.2. Measurement of pulmonary artery pressure

The measurement was carried out by the method described by Hampl et al. [21] with minor modifications. The rats were anesthetized with pentobarbital sodium (15 mg, i.p). Using a rodent ventilator (Animal respirator TSE, Kronberg, Germany) animals were artificially ventilated with a nose mask. The chest was opened at the midline and the pulmonary artery was directly cannulated with a 22-gauge needle. The needle was connected to a catheter that was attached to a TP 120 Statham pressure transducer. The system was filled and flushed with < 2 ml of heparin solution (1000 ml IU/ml). The pulmonary artery pressure was recorded after stabilization, usually within 4 min of puncture of the pulmonary artery.

2.3. Tissue preparation

After recording the pulmonary pressure, 1–3 ml blood were drawn from the right ventricle and collected in chilled EDTA tubes for quantification of plasma renin activity and Eppendorf cups for measurements of serum ACE activity and serum aldosterone. Hearts and lungs were quickly removed, rinsed with saline, and blotted dry. The right ventricle was isolated by dissection along its septal insertion. Weights of both ventricles and lungs were measured using a precision balance [17]. For determination of the relative water content (to exclude pulmonary edema in rats with left ventricular hypertrophy) a lung of each
animal was balanced before and after overnight baking at 80°C.

2.4. Biochemical measurements

Plasma and tissue ACE activity was measured by a modified fluorometric method as previously described in detail [2]. Plasma renin activity and serum aldosterone were determined using commercially available kits (Renk, No. 2714; ALDO-CTK-2, No. 2714, Sorin Biomedica AG). Protein content was assayed by the method of Lowry [22] such that ACE activity is displayed as nmol/mg protein/min.

2.5. RNA measurements

Total RNA extraction from both ventricles and lung was carried out using standard procedures [2]. To determine lung ACE mRNA Northern blot analyses of 20 μg pulmonary ACE mRNA from a sham-operated rat and a rat with left ventricular hypertrophy and pulmonary hypertension. For comparison, differentially spliced testicular ACE mRNA is included. Bottom section shows semi-quantitative analysis with bars representing the mean ± s.d. of lungs of 20 control animals and lungs of 20 rats with LVH.
Northern blots were hybridized with a control cDNA probe (glyceraldehyde-3-phosphate dehydrogenase; GAPDH) [17]. Autoradiograms generated by Northern blots were scanned with a microdensitometer (Pharmacia LKB Inc., Piscataway, NJ) with background set to zero for each autoradiograph. The signal for ACE mRNA was divided by signal for the GAPDH mRNA for each sample and results were expressed as ACE/GAPDH mRNA ratio.

2.6. Competitive ACE RT-PCR

The methods for reverse transcription of RNA, amplification of DNA in the presence of increasing concentrations of a deletional mutant have recently been described in detail [24]. In brief, 1 μg of total RNA was reversely transcribed to cDNA for 45 min at 42°C. Next, temperature was set to 95°C for 1 min before samples were chilled. Twenty-six amplification cycles consisted of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 26 cycles. The sense primer for the detection of ACE cDNA spanned bases 492–512 (GCCTCCCCCAACAAGACTGCCA) and the antisense primer spanned bases 860–880 (CCACATGTCTCAGCCAGATG). The deletion mutant was produced as previously described [25]. Increasing concentrations (1–100 pg) of mutant ACE cDNA were used for competition with primers [24]. Amplification products were electrophoretically separated on 1% agarose gels. The dilution curve of the mutant ACE cDNA was reflected as increasing intensity of the ethidium bromide signal. Optical densities were determined and regression analysis of the OD values were calculated in dependence of the corresponding amounts of internal (mutant) standard. Using μg of total RNA as reference basis, the pg of ACE mRNA per mg total RNA were obtained [24].

2.7. Statistical analysis

All data are presented as mean ± standard deviation (s.d.). Parameters obtained from LVH and sham control rats were directly compared by Student’s unpaired t-tests. Rank correlation analysis was employed to investigate the correlation between pulmonary ACE activity and other biochemical and functional parameters. The correlations were related to the whole group of animals (n = 40). Significance was accepted for P < 0.05.

3. Results

As compared to sham-operated animals, rats subjected to 12 weeks of aortic banding (LVH rats) were characterized by slightly lower body weights (Table 1). In contrast, left and right ventricular weights were markedly elevated in LVH rats (Table 1) resulting in a 1.7-fold increase in left ventricular to body weight ratio (LV/BW) and a 1.6-fold increase in right ventricular to body weight ratio (RV/BW). Furthermore, LVH rats presented with similar arterial blood pressure, whereas measurements of pulmonary artery pressure were substantially elevated suggesting the development of pulmonary hypertension (Table 1). Biochemical determinations revealed significantly elevated plasma renin activity and serum aldosterone levels as well as slightly elevated plasma ACE activity in rats with LVH (Table 1).

Fig. 6. Pulmonary ACE activity related to different renin plasma levels, LV/BW ratios and pulmonary pressure. Each bar represents the mean ± s.d. A significant suppression is observed in the groups that display most pronounced elevation of renin plasma levels, LV/BW ratios and increased pulmonary pressure.

3.1. Cardiac ACE mRNA and activity levels

As compared to sham-operated animals (Fig. 1, top section, upper panel), RT-PCR of left ventricular ACE mRNA gave markedly enhanced signals in rats with left ventricular hypertrophy (Fig. 1, top section, lower panel). Employing defined amounts of truncated ACE competitor DNA as internal standard, signals of LVH and sham hearts were quantified and compared statistically (Fig. 1, bottom section), revealing a 2-fold increase in left ventricular ACE mRNA in rats with LVH.

In parallel to the induction of cardiac ACE mRNA, we observed an increase of cardiac ACE activity in both the left and right ventricle of rats with LVH (Fig. 2). When individual rats were compared, levels of LV ACE mRNA and activity were highly correlated ($r = 0.77; P < 0.005$). Furthermore, the increase of cardiac ACE activity was proportional to the increase in LV/BW ($r = 0.75; P < 0.0001$) and RV/BW ratios ($r = 0.53; P < 0.0001$).

3.2. Pulmonary ACE mRNA and activity levels

Fig. 3, top section, displays a representative Northern blot of pulmonary ACE mRNA. Quantitative analysis of respective animal groups is shown in the bottom section of Fig. 3, demonstrating a significant down-regulation of pulmonary ACE mRNA levels in rats with LVH. In parallel, rats with aortic banding displayed a 30% decrease in pulmonary ACE activity (Fig. 4). Pulmonary ACE activity was inversely related to LV ($r = -0.46; P < 0.0001$), systolic pulmonary artery pressure ($r = -0.37; P < 0.001$), and serum ACE ($r = -0.29; P < 0.01$) activity in these animals (Fig. 5). Thus, the data strongly suggest differential mechanisms of ACE regulation in respective tissue and circulating systems. The most pronounced decrease in pulmonary ACE activity was observed in rats with severe left ventricular hypertrophy (LV/BW ratio > 3 mg/g), markedly elevated pulmonary arterial pressure (> 30 mmHg) or plasma renin activity (> 10 ng ANG1/ml/h) (Fig. 6).

3.3. Effects of renin stimulation by diuretic treatment on pulmonary ACE

In order to further delineate the effect of a stimulated renin angiotensin system on pulmonary ACE regulation,
additional rats were treated with subcutaneous injections of furosemide (Table 2). Diuretic treatment resulted in an elevation of renin levels that was even higher than that observed in rats with severe LVH ($P < 0.005$ vs. control). However, neither pulmonary nor cardiac ACE activity levels were significantly affected by furosemide treatment (Table 2).

4. Discussion

The present finding of a marked induction of left ventricular ACE activity and mRNA levels in the hypertrophied left ventricle is consistent with previous studies on experimental rats or patients with aortic stenosis [2,4,17,26]. Furthermore, we observed an increase in right ventricular ACE activity that occurred in parallel with the development of right ventricular hypertrophy. The novel feature of the present study is a decrease in pulmonary ACE activity in rats with cardiac hypertrophy and secondary pulmonary hypertension. Thus, the present study documents differential and tissue-specific regulation of pulmonary and cardiac ACE activity.

The decrease of pulmonary ACE activity is best explained by a decrease of local biosynthesis of the enzyme since mRNA levels decreased in parallel and to the same extent. However, we cannot excluded that these rats with sever aortic stenosis developed endothelial damage in the pulmonary vasculature that may contribute to the decrease in pulmonary ACE and (via shedding of the ectoenzyme) to the increase in serum ACE. Tissue edema is an unlikely explanation for the decrease in pulmonary ACE activity since the water content in lungs of sham-operated animals and rats with left ventricular hypertrophy was similar. Moreover, the difference in ACE activity between the groups persisted after correction for tissue protein content.

The increase in pulmonary artery pressure seems to be an attractive pathophysiological mechanism to explain the decrease in pulmonary ACE activity and mRNA levels in rats with left ventricular hypertrophy and subsequent backward failure of the left ventricle. In fact, heart failure may particularly increase the venous and capillary pressure, i.e. the most prominent sites of ACE expression. Similar observations have been made in lungs with pulmonary hypertension secondary to chronic hypoxia or monocrotaline treatment, albeit these forms of pulmonary hypertension may have more pronounced effects on precapillary vessels [6,8–11]. Furthermore, Huang and coworkers reported a selective decrease of ACE activity in lungs of rats with severe heart failure secondary to myocardial infarction [6]. Although pulmonary arterial pressure was not measured in that study, it is remarkable that the decrease in pulmonary ACE was confined to rats with severe heart failure that most likely developed pulmonary hypertension as well [6]. Similarly, we made the observation that the decrease in pulmonary ACE is only detectable in rats with severe hypotrophy or systolic pulmonary artery pressures higher than 30 mmHg.

An alternative explanation for the suppression of pulmonary ACE levels might be feedback down-regulation via an activated renin–angiotensin system in rats with severe left ventricular hypertrophy and failure. In particular, several investigators, including our group, provided evidence that high angiotensin II levels may suppress pulmonary ACE [7], whereas administration of ACE inhibitors had the opposite effect [27,28]. We thus studied rats treated with a loop diuretic that presented with markedly stimulated renin levels. Interestingly, pulmonary ACE activity and mRNA levels were not altered significantly by furosemide treatment. It seems to be noteworthy that acute infusion of angiotensin II causes pulmonary vasoconstriction and hypertension [29], whereas in contrast chronic application of loop diuretics does not increase pulmonary artery pressure. Thus, in retrospect, previous data on feedback down-regulation of pulmonary ACE in rats infused with angiotensin II might also be explained by vasoactive angiotensin II effects resulting in pulmonary hypertension and subsequent ACE down-regulation.

Irrespective of the cellular mechanism, down-regulation of pulmonary ACE may have significant functional implications. For example, Jackson et al. demonstrated that the pulmonary conversion of angiotensin I to angiotensin II is impaired in rats with low pulmonary ACE levels secondary to chronic hypoxia [12]. It has to be pointed out, however, that pulmonary ACE down-regulation in rats with chronic hypoxia may be a regional phenomenon and confined to the endothelium of alveolar capillaries [8]. In contrast, elevated ACE levels were found locally in muscularized small pulmonary arteries of rats exposed to chronic hypoxia [8]. In the present study, we observed such regional ACE up-regulation in hearts of rats with aortic banding. This regional augmentation of ACE activity in conjunction with ACE down-regulation on the wide-spread surface of alveolar capillaries may, hypothetically, result in a shift of angiotensin II generation towards regions with high ACE levels. Interestingly, reciprocal tissue-specific regulation of ACE has also been noted by Morell et al. [30] who reported that ACE is induced in the hypertrophied right ventricle of rats with chronic hypoxia, but down-regulated in the left ventricle of these rats. On the other hand, ACE inhibitors are highly efficacious in these models [17,31]. In particular, these drugs ameliorate the hypertrophy of muscularized pulmonary arteries or cardiac myocytes in rats with pulmonary hypertension or chronic aortic banding, respectively [17,31]. In conjunction, the data may allow one to speculate that ACE inhibitors may be specifically successful in countering the functional and morphologic alterations at sites of up-regulated tissue ACE.
Acknowledgements

We wish to thank Dr. F. Soubrier for providing the ACE cDNA probe. This work was supported by the Deutsche Forschungsgemeinschaft (DFG Schu 617/3-1, 9-1 and 10-1), the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (KBF 01 GB 9403) and an Astra Award for Cardiovascular Research (H.S.).

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