Tissue expression of components of the renin–angiotensin system in experimental post-infarction heart failure in rats: effects of heart failure and angiotensin-converting enzyme inhibitor treatment

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1. It has been suggested that local tissue renin–angiotensin systems may be activated in heart failure and that effects on such systems may, at least partially, explain the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors in this syndrome. To investigate these hypotheses, we examined expression of renin–angiotensin system components in several tissues in a rodent model of post-myocardial infarction (MI) heart failure, and analysed whether such expression is modified by ACE inhibitor treatment.

2. Four groups of rats (n = 8–12 per group) were studied 30 days after surgery: (A) sham-operated rats with no treatment, (B) rats with post-MI heart failure induced by ligation of the left coronary artery, (C) sham-operated rats treated with the ACE inhibitor perindopril (1.5 mg day\(^{-1}\) kg\(^{-1}\)), and (D) rats as per B, but treated with perindopril. Expression of renin, angiotensinogen, ACE and angiotensin subtype 1 receptor was assessed by quantification of their respective mRNAs by Northern blotting.

3. Renal renin mRNA increased 2-fold in animals with MI (group B) compared with controls (group A) (P < 0.05) and between 50 and 100-fold after ACE inhibitor treatment (P < 0.001). No change in renin gene expression was found in any extra-renal site either following MI or after ACE inhibitor treatment. Hepatic angiotensinogen mRNA level was similar in all groups, but kidney angiotensinogen mRNA level was increased 1.6-fold (P < 0.01) in the groups receiving perindopril. ACE mRNA level in the lung was not affected by ACE inhibitor treatment but decreased by 50% following MI (groups B and D, P < 0.01). This was associated with a similar (50%, P < 0.01) fall in lung ACE activity and was correlated with the severity of heart failure. Angiotensin subtype 1 receptor mRNA level was not affected in any tissue by either MI or ACE inhibitor treatment.

4. We did not find a systematic activation of tissue renin–angiotensin systems, as assessed by steady-state mRNA levels of key components of the system in experimental post-MI heart failure, or a major effect of ACE inhibitor treatment on expression of these components. However, we observed tissue-specific changes in expression of selected components of the renin–angiotensin system in the kidney and the lung in post-MI heart failure and after ACE inhibitor treatment, which may be of relevance to the pathophysiology of the syndrome and the effects of ACE inhibition.

INTRODUCTION

Congestive heart failure is a common complication of myocardial infarction (MI) and is an important indicator of an adverse short- and medium-term prognosis [1, 2]. It usually occurs as a result of left ventricular dysfunction due to myocardial damage at the time of the MI, and is associated with activation of neurohumoral compensatory mechanisms including the sympathetic nervous system and the renin–angiotensin system (RAS) [3].

The RAS, through the action of its effector molecule, angiotensin II, on specific tissue receptors, plays an important role in cardiovascular homoeostasis, affecting both blood pressure and fluid volume. The classical view of the RAS is one of an endocrine system in which renin secreted by the kidney acts on circulating angiotensinogen produced by the liver to release angiotensin I; this is converted into the active angiotensin II by angiotensin-convert ing enzyme (ACE), mainly in the pulmonary circulation. Angiotensin II then mediates the two main roles of the RAS, i.e. blood pressure maintenance

Keywords: gene expression, heart failure, myocardial infarction, rat, renin-angiotensin system, angiotensin-converting enzyme inhibition.

Abbreviations: ACE, angiotensin-converting enzyme; AT1, angiotensin II subtype I; GAPD, glyceraldehyde-3-phosphate dehydrogenase; MI, myocardial infarction; PAC, plasma angiotensinogen concentration; PRC, plasma renin concentration; RAS, renin–angiotensin system.

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and body water and electrolyte balance, by actions on multiple-target organs, including the vascular wall, adrenal gland and kidney. There is overwhelming evidence that such an endocrine RAS, responsive to perturbations in blood pressure and body sodium content, participates in acute cardiovascular homeostasis [4].

However, in addition to an endocrine RAS, there is now substantial evidence for local RASs in several tissues subserving paracrine/autocrine functions, which may be complementary to or independent of those served by the endocrine RAS [5]. The molecular demonstration of gene expression of individual components of the RAS in tissues has provided strong support for the concept of tissue RASs [5-7].

Several recent large clinical trials have shown that treatment of individuals with post-infarction left ventricular dysfunction with an ACE inhibitor improves prognosis [1, 2, 8]. While the haemodynamic sequelae of inhibition of the circulating RAS and prevention of degradation of the vasodilator peptide bradykinin may play a role [9], the mechanisms underlying the beneficial effects of ACE inhibitors in heart failure remain to be fully elucidated. Specifically, there is current interest as to whether tissue RASs are activated in post-infarction heart failure and whether ACE inhibitor treatment modulates tissue expression of the genes for various components of the RAS [10]. Studies to date have reported increased cardiac ACE activity and mRNA level [11, 12] and angiotensinogen mRNA [13] in rodent models of experimental heart failure, which may play an important role in the ventricular remodelling that occurs after MI. Less attention has been focused on changes that may occur in other sites of RAS expression and which may be of relevance to cardiovascular homeostasis in heart failure, such as the adrenal gland, brain, lung, kidney and liver, and the effects of ACE inhibitor treatment on such expression. In this study we therefore examined expression of RAS components in several tissues in a well-described rodent model of post-infarction heart failure, and analysed whether such expression is modified by ACE inhibitor treatment. Circulating levels of components of the RAS and lung ACE activity were also measured to compare and contrast with the changes in the tissues.

METHODS

Animals

Male Wistar rats (20 weeks old, ~400 g) were used for all studies. MI was induced by ligation of the left coronary artery under general anaesthesia and positive pressure ventilation using the technique of Selye et al. [14]. Care was taken to place the ligature (6.0 silk) no more than 2-3 mm from the aorta to ensure that the left main coronary artery, rather than a distal branch, was ligated. Success of the procedure was immediately apparent by observation of the anterior and lateral left ventricular wall becoming pale and hypodynamic, and by dilatation of the left auricular appendage due to an acute rise in intracardiac pressures. After closure of the pectoral and skin incisions, and reversal of the anaesthesia, the rats were allowed to recover. Animals were given 0.1 g (intraperitoneal) of ampicillin prophylactically, to prevent infection, and 0.15 ml of buprenorphine (300 µg/ml) for two days post-operatively to reduce pain. Sham animals were operated on identically except for placement of the ligature.

All procedures were carried out in accordance with our institutional guidelines and the investigation conforms with the United States NIH guidelines for the care and use of laboratory animals.

ACE inhibitor treatment

The animals were allowed to recover for 24 h and survivors [100% (16/16) for sham-operated rats, 96% (23/24) for MI rats] were then randomly assigned to either receive or not receive the ACE inhibitor perindopril, 1.5 mg day⁻¹ kg⁻¹ given in drinking water, with adjustment of concentrations to ensure appropriate dosing on the basis of twice weekly assessment of fluid intake. Animals were housed singly for the duration of the experiments, with controlled temperature, humidity and light periods, and had free access to food and water.

Blood pressure measurement

Two days before being killed at 30 days, blood pressure was measured in conscious rats using the tail cuff technique as described [15].

Blood and tissue collection

Animals were killed by cervical dislocation. Samples of blood were rapidly collected in pre-chilled tubes containing EDTA (for measurement of renin, angiotensinogen and angiotensin II) and lithium heparin (for measurement of plasma ACE). The tubes were immediately placed on ice and the plasma was rapidly separated and stored in aliquots at -70°C until assays were carried out. Both kidneys, a lobe of liver, whole brain, both adrenals, both lungs and whole left ventricle were then rapidly removed and individually frozen in liquid nitrogen and again stored at -70°C pending RNA extraction. The lungs were weighed before freezing.

Biochemical measurements

Plasma renin concentration (PRC). Duplicate plasma samples (20 µl) were incubated with rat renin-free plasma (50 µl) in 0.1 mol/l Tris/acetate buffer (10 µl), pH 7.4, containing 2,3-dimercaptopropanol (16 mmol/l) and PMSF (0.5 mg/ml) for
120 min at 37°C. The reaction was stopped by chilling the sample and adding 400 µl of ice-cold 0.1 mol/l Tris-acetate, pH 7.4, containing human serum albumin (1 mg/ml). The angiotensin I generated was measured by RIA as described by Menard and Catt [16]. Results were expressed in pmol of angiotensin I generated h⁻¹ (ml of rat plasma)⁻¹.

**Plasma angiotensinogen concentration (PAC).** Duplicate plasma samples (10 µl) were mixed with mouse submaxillary gland renin (20 µl, 112 ng) and 30 µl of 1 mol/l Tes buffer, pH 7.2, containing EDTA (50 mmol/l), 2,3-dimercaptopropanol (8 mmol/l) and PMSF (0.5 mg/ml) for 0, 60 and 90 min at 37°C, the last time point being used to ensure that the reaction had reached a plateau. Reactions were stopped by chilling the sample and adding 300 µl of ice-cold 0.1 mol/l Tris-acetate, pH 7.4, containing human serum albumin (1 mg/ml). After dilution, the angiotensin I generated was measured by RIA as described by Menard and Catt [16]. PAC was calculated from the 90 min results, assuming that 1 mol of angiotensinogen generates 1 mol of angiotensin I.

**Plasma angiotensin II concentration.** Angiotensins were extracted from 1 ml of plasma using a Sep-Pak C₁₈ cartridge and the angiotensin II level was determined by RIA, as described previously [17]. The sensitivity of the RIA was 1 fmol of angiotensin II per fraction and cross-reactivity with angiotensin I was <0.1%.

**Plasma and tissue ACE activity.** Plasma samples (100 µl) were incubated in 50 mol/l Hepes buffer, pH 8.15, containing 5 mmol/l hippuryl-histidyl-leucine and a trace amount of ¹⁴C-labelled hippurylhistidyl-leucine (2 × 10⁵ c.p.m./tube) for 2.5 h at 37°C in a total volume of 500 µl. Each plasma sample was assayed in duplicate in the absence or presence of 12.5 µmol/l enalaprilat. The reaction was stopped by adding 500 µl of 1 mol/l HCl. Released [¹⁴C]hippuric acid was extracted from acidified samples by vigorous mixing and 1 h incubation with ethylacetate (1 ml) at 4°C. The two phases were separated by centrifugation at 5000 g for 20 min at 4°C. Aliquots of the organic phase (200 µl) were mixed with 5 µl of scintillation cocktail and counted in a Tri-Carb scintillation counter (Hewlett-Packard) for 5 min. Results were expressed in nmol of substrate hydrolysed (h of incubation)⁻¹ (ml of plasma)⁻¹. Tissue ACE activity was measured in a similar manner using freshly prepared homogenates prepared in ice-cold assay buffer containing Triton X-100, except that the substrate used was [phenyl-4(n)-³H]hippurilyglycylglycine at a pH of 7.5 [18]. Results were expressed in nmol of substrate (min of incubation)⁻¹ (mg of protein)⁻¹.

**RNA extraction and mRNA analysis**

Total tissue RNA was extracted using the LiCl/urea precipitation method [19]. For kidney, brain, liver and lung, RNA was prepared individually from five randomly chosen animals from each group. For adrenal and left ventricle, because of limited amounts of material, samples from two to four animals were pooled before RNA extraction. RNA concentrations were determined by spectrophotometry at 260 nm, and before Northern blot analysis substantial degradation of RNA during extraction was excluded by running aliquots on agarose gels stained with ethidium bromide.

Northern blotting and hybridization were performed using standard protocols [20]. Briefly, 60 µg of RNA per sample was electrophoresed through 1.2% (w/v) agarose gels containing 2.2 mol/l formaldehyde and transferred to Hybond N membrane (Amersham Ltd, Aylesbury, U.K.) as recommended by the membrane manufacturer. Membranes were then probed in random order to determine mRNA levels for renin, angiotensinogen, ACE, angiotensin II subtype 1 (AT1) receptor, and glyceraldehyde-3-phosphate dehydrogenase (GAPD) mRNA (internal control), using as probes a 1.4 kb rat kidney renin cDNA (pREnh4.ceb) [21], a 1.6 kb rat liver angiotensinogen cDNA (pRang6) [22] (both provided by Dr K. Lynch, University of Virginia, Charlottesville, VA, U.S.A.), a 3.3 kb human ACE cDNA [23] (provided by Dr F. Soubrier, INSEMR Unit 358, Paris, France), a 2.2 kb rat angiotensin subtype II IA receptor cDNA [24] (supplied by Dr K. Bernstein, Emory University, Atlanta, GA, U.S.A.) and a 29mer oligonucleotide complementary to GAPD mRNA [25] respectively. All cDNA probes (20 ng per reaction) were radioactively labelled with deoxy-[α³²P]CTP by the random-primer method [26] and had specific activities greater than 1 × 10⁶ c.p.m./µg of DNA. The GAPD probe (50 ng) was end-labelled using terminal deoxynucleotide transferase [20].

For all probes, prehybridization (4 h) and hybridization (14–16 h) were carried out at 45°C in a buffer containing 50% (v/v) formamide, 6 × SSPE (20 × SSPE = 3.6 mol/l NaCl, 0.2 mol/l sodium phosphate, pH 7.7, 20 mmol/l sodium-EDTA), 5 × Denhardt's [100 × Denhardt's = 2% (w/v) of each of BSA, Ficoll 400 and polyvinyl pyrollidine], 0.5% (w/v) SDS, 6% (w/v) polyethylene glycol 6000 and denatured salmon sperm DNA (200 µg/ml). After hybridization, membranes were washed to a stringency of 0.5 × SSPE and 0.1% SDS at 60°C. Autoradiography was carried out at −70°C using Kodak X-Omat AR film (Eastman Kodak, Liverpool, U.K.) and intensifying screens. Before reprobing, a previous probe was stripped from the membranes by immersion for 90 min at 65°C in two changes of a solution containing 10 mmol/l Tris/HCl, pH 8, 1 mmol/l EDTA and 1 × Denhardt's. The molecular masses of the bands seen on the membranes were estimated from a concurrently run RNA molecular-mass ladder (Life Technologies, Paisley, U.K.). The intensities of the autoradiographic signals were quantified by two-dimensional densitometry scanning using an LKB Ultrascan 2222-010 XL Scanner.
Statistical analysis

Survival differences were analysed by Fisher's exact test. Results for quantitative variables were first compared by analysis of variance for all groups. Where this proved significant, pair-wise comparisons were carried out using Tukey's test for multiple comparisons. Interactions between the effects of MI and ACE inhibitor treatment were examined using general linear modelling using Minitab version 7.0. Spearman's rank correlation was used to analyse the relationship between lung mass and lung ACE mRNA level. For data where the variability increased with the means of the values, analyses were carried out on log-transformed data. \( P < 0.05 \) was considered to be of statistical significance.

RESULTS

Four groups of rats were studied: A, sham-operated rats with no ACE inhibitor \((n = 8)\); B, MI rats with no ACE inhibitor \((n = 12)\); C, sham-operated rats with perindopril treatment \((n = 8)\); D, MI rats with perindopril treatment \((n = 11)\). Table 1 shows the survival of the rats in the different groups at 30 days. There was no mortality in either of the sham-operated groups, whereas mortality was 42\% (5/12) in untreated MI rats \((P = 0.05)\) compared with group A), which was reduced to 9\% (1/11) in MI rats treated with perindopril \((P = 0.09)\) compared with group B). The body mass of survivors was not different between the groups \((P = 0.362)\) (Table 1).

Blood pressure was however significantly different between all groups \((P < 0.01)\), with independent effects of both MI \((P < 0.001)\) and ACE inhibitor treatment \((P < 0.001)\). Lung masses were markedly increased, about 2.5-fold on average, in the MI groups \((P < 0.001)\), confirming the severity of the MI and the associated left-ventricular dysfunction. Although slightly reduced in the groups receiving perindopril, this was not statistically significant. The effects on circulating levels of components of the RAS in the different groups are shown in Table 2. Compared with sham-operated rats (group A), there was a 9-fold increase in PRC with MI alone (group B, \(P < 0.01\)). PRC was massively increased, more than 100-fold, by ACE inhibitor treatment in both groups C and D \((P < 0.001)\), but there was no significant interaction between MI and ACE inhibitor \((P = 0.18)\). PAC was slightly but not significantly decreased by MI alone (group B), but more than two-fold decreased in the groups receiving ACE inhibitor treatment \((P < 0.001)\). PAC was lowest in group D, and in pair-wise comparisons this was significantly different from all other groups. Across the groups there was a strong negative correlation \((r = -0.71, P < 0.001)\) between PRC and PAC levels. Plasma ACE level was unchanged with MI but, as expected, was markedly decreased \((>15\text{-fold}, P < 0.001)\) in both groups receiving perindopril (Table 2). Plasma immunoreactive angiotensin II showed a small but insignificant increase with MI. Despite the marked suppression of plasma ACE activity the angiotensin II level was not reduced in either group of rats treated with perindopril, and indeed the highest level was seen in group D rats (MI rats treated with ACE inhibitor). However, because of considerable inter-rat variability, there was no overall difference in plasma immunoreactive angiotensin II levels between the groups by analysis of variance \((P = 0.17)\).

Steady-state levels of mRNAs of components of the RAS in the kidney at 30 days in the different groups are shown in Figure 1 \((n = 5\) per group for RNA analysis, see Methods). There was a two-fold \((P < 0.01)\) increase in renin mRNA in animals with MI alone compared with sham controls, and an increase of 5-6-fold \((P < 0.001)\) with ACE inhibitor treatment (Figure 2). The highest levels were seen in animals with MI treated with perindopril. The

### Table 1. Characteristics of the four groups of rats studied. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Body mass, blood pressure and lung mass are given for animals that survived to the end of the study (30 days). Mean \(\pm\) SEM are shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Mortality n (%)</td>
<td>0 (0)</td>
<td>5 (42)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>426 ± 18</td>
<td>424 ± 22</td>
<td>437 ± 11</td>
<td>401 ± 9</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>125 ± 5</td>
<td>89 ± 4</td>
<td>105 ± 4</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>Lung mass (mg)</td>
<td>1895 ± 216</td>
<td>4638 ± 572</td>
<td>1714 ± 115</td>
<td>4441 ± 503</td>
</tr>
</tbody>
</table>

### Table 2. Circulating levels of components of the renin–angiotensin system in studied rats. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Means \(\pm\) SEM are shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>PRC (pmol angiotensin I h(^{-1}) ml(^{-1}))</td>
<td>2.0 ± 0.5</td>
<td>18.2 ± 7.2</td>
<td>215 ± 51</td>
<td>566 ± 125</td>
</tr>
<tr>
<td>PAC (pmol/l)</td>
<td>1.9 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>ACE activity (nmol/h ml(^{-1}))</td>
<td>308 ± 10</td>
<td>331 ± 4</td>
<td>19 ± 3</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Angiotensin II (fmol/ml)</td>
<td>123 ± 4.8</td>
<td>21.4 ± 6.3</td>
<td>9.7 ± 1.4</td>
<td>39.9 ± 16.0</td>
</tr>
</tbody>
</table>
Kidney angiotensinogen mRNA level was unchanged with MI, but there was a relatively small but highly significant ($P = 0.002$) 1.6-fold increase with ACE inhibitor treatment (compare groups C+D with A+B, Aogen panel, Fig. 1 and Fig. 2, middle graph). AT1 receptor mRNA level was unchanged by either MI or ACE inhibitor treatment. mRNA for ACE was not detectable in the kidney using Northern blotting. Messenger RNA levels in the liver, brain and adrenal gland are shown respectively in Figs. 3–5. Unlike in the kidney, there was no significant change in hepatic angiotensinogen mRNA level with ACE inhibitor treatment (or with MI). Likewise, there was no change in hepatic expression of AT1 receptor or renin in any of the groups (Figure 3). In the brain, only angiotensinogen and renin mRNAs were detectable, and there was no change in expression of either after MI or with perindopril treatment (Figure 4). In the adrenal gland, renin and AT1 receptor mRNAs were detectable and again there was no alteration in the expression of either of these genes (Figure 5).

In the lung, ACE mRNA level decreased by 50% ($P < 0.001$) after MI (Figs. 2 and 6). ACE inhibitor treatment had no effect either by itself or after MI (Figs. 2 and 6). In the MI group as a whole, there was a significant inverse relationship ($r = -0.68$, $P = 0.029$) between lung mass and lung ACE mRNA level (Figure 7). AT1 receptor mRNA was unchanged in all of the groups. Renin and angiotensinogen mRNA levels were not detectable.

To investigate whether the observed change in lung ACE mRNA level with MI was associated with a change in ACE activity, a further series of sham and infarct rats ($n = 5$ per group) were studied. Pulmonary ACE activity was significantly lower in animals with MI compared with sham-operated rats ($P < 0.01$) (Fig. 8). In contrast, in the kidney, ACE activity was much lower but unchanged after MI (Fig. 8).

Finally, in the left ventricle, no signals were detectable for ACE, angiotensinogen or angiotensin-1 receptor mRNAs in any of the experimental
expression of RAS components in the liver in post-infarction heart failure and after ACE inhibitor treatment. Northern blot of RNAs probed serially for angiotensinogen (Aogen), renin, AT1 receptor (AT1R) and GAPD mRNAs is shown. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Each track contains RNA prepared from an individual animal. Sizes of mRNA transcripts are as follows: renin (1.6 kb); Aogen (1.9 kb); AT1R (2.3 kb); GAPD (1.4 kb). Exposure times of autoradiographs were as follows: Aogen (4 h); renin (14 days); AT1R (4 days); GAPD (12 h).

Fig. 4. Expression of RAS components in the brain in post-infarction heart failure and after ACE inhibitor treatment. Northern blot of RNAs probed serially for angiotensinogen (Aogen), renin and GAPD mRNAs is shown. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Each track contains RNA prepared from an individual animal. Sizes of mRNA transcripts are as follows: renin (1.6 kb); Aogen (1.9 kb); GAPD (1.4 kb). Exposure times of autoradiographs were as follows: Aogen (1 day); renin (3 days); GAPD (8 h).

Fig. 5. Expression of RAS components in the adrenal in post-infarction heart failure and after ACE inhibitor treatment. Northern blot of RNAs probed serially for renin, AT1 receptor (AT1R) and GAPD mRNAs is shown. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Because of limiting amounts of tissue adrenals from 3–4 rats in each group were pooled before RNA extraction. The blots also contain kidney (K) and liver (L) RNA samples as positive controls. Exposure times of autoradiographs were as follows: renin (9 days); AT1R (14 days); GAPD (12 h).

Fig. 6. Expression of RAS components in the lung in post-infarction heart failure and after ACE inhibitor treatment. Northern blot of RNAs probed serially for ACE, AT1 receptor (AT1R), and GAPD mRNAs is shown. A, sham-operated rats with no ACE inhibitor; B, MI rats with no ACE inhibitor; C, sham-operated rats with perindopril treatment; D, MI rats with perindopril treatment. Each track contains RNA prepared from an individual animal. Sizes of the mRNA transcripts are as follows: AT1R (2.3 kb); GAPD (1.4 kb); ACE mRNA 4.2 kb. Exposure times of autoradiographs were as follows: ACE (7 h); AT1R (8 days); GAPD (12 h).

**DISCUSSION**

In this study we show tissue- and gene-specific changes in expression of components of the RAS in relation to experimental post-infarction heart failure and ACE inhibitor treatment. The findings are of potential clinical relevance because of the substantial evidence pointing to a detrimental effect of an activated RAS in this situation [3] and the demonstration that ACE inhibitor treatment improves both symptoms and prognosis [1,2,8].

**Comments on the model used**

Several studies [27–30] have demonstrated that the rat coronary ligation model is characterized by decreased cardiac output, regional blood flow redistribution, elevated left ventricular end-diastolic pressure and reduced survival. Thus, the haemodynamics of the model resemble the most common features of
Tissue renin systems in heart failure

In moderate to severe human congestive heart failure. In previous studies [27] we have shown that left coronary ligation as performed here is associated with a left ventricular infarct size of between 30 and 40%. Although we did not measure MI size in this study (to permit rapid freezing of tissues for RNA analysis), we observed a marked increase in lung mass, decreased blood pressure and a high mortality in our group of rats with MI, consistent with the predicted marked depression of left ventricular function.

To investigate the effects of ACE inhibition, we chose an agent, perindopril, known to significantly inhibit tissue ACE both acutely and chronically and with a prolonged duration of action [31]. The dose (1.5 mg day⁻¹ kg⁻¹) was chosen on the basis of a previous report showing a substantial anti-hypertensive effect in spontaneously hypertensive rats [32] and on preliminary studies showing that it was associated with >70% tissue ACE activity inhibition in a wide variety of tissues of both normal and infarct rats (O. Kahr, unpublished work). Although the choice of dose was somewhat empirical, the treatment was nonetheless associated with a reduction in mortality, a finding previously reported in the model with both perindopril [33] and other ACE inhibitors [29, 34, 35], and consistent with the observations in man [1, 2, 8]. However, interestingly, within the time-frame of the study the improvement in mortality was not associated with a significant reduction in lung mass in the surviving animals. The reason for this is unclear, but there are several possibilities: we may have studied the animals at too early a time point; the severity of the left ventricular dysfunction induced may have been such as to prevent much improvement in pulmonary oedema, despite a beneficial effect on survival (which may also depend on other ACE inhibitor effects); significant improvement in pulmonary oedema may require concomitant use of diuretics, which is usually the situation clinically [1, 2, 8] but was not the case here.

Changes in circulating RAS

The increase in PRC in animals with MI was anticipated and was consistent with the well-documented activation of the endocrine RAS in moderate to severe heart failure [3]. Several mechanisms probably contribute to the increased secretion of renin by the kidney in this situation, including reduced renal perfusion pressure (baroreceptor mechanism), reduced sodium delivery to the distal tubule (macula densa mechanism) and increased direct sympathetic stimulation of the renin-producing juxta-glomerular cells [36]. It is notable that the rise in PRC was several-fold greater than the increase seen in kidney mRNA levels. Such disproportionate changes in kidney renin mRNA and PRC have been reported previously in relation to other stimuli [37, 38] and indicate important post-transcriptional regulation of renin production and secretion by the kidney. With perindopril treatment, a further and marked increase in PRC was seen in both normal and MI animals. This is probably mainly due to the removal of the negative feedback effect of intrarenal angiotensin II on kidney renin synthesis and secretion [36] (see below).

In contrast with PRC, PAC was reduced both with MI and particularly with ACE inhibitor treatment. There was no significant change in liver angiotensinogen mRNA level in any of the groups, and the decrease in PAC probably reflects increased consumption by the raised PRC. Indeed, across the groups there was a strong negative correlation between PRC and PAC. In keeping with other similarities between this model and the human condition, PAC is also decreased in patients with severe heart failure [39].

In agreement with some [11, 12], but not all [40], previous reports, we found no change in plasma ACE level in post-infarction heart failure. Plasma ACE is believed to be mainly derived from endothel-
lial ACE released from tissue vascular beds [41]. Since the pulmonary vascular bed contains the highest concentration of endothelial ACE and makes a major contribution to plasma ACE, one might have expected plasma ACE to be decreased in rats with heart failure, given the changes found in lung ACE expression and activity in these animals. The reasons why this did not happen need to be identified, but it is possible that increased expression and release from other sites compensates for reduced production by the lung, or that turnover of plasma ACE is reduced in heart failure and a change was not detectable by the time the experiments were terminated. However, the findings emphasize the importance of looking at both circulating and tissue components of the RAS in pathological conditions.

The findings on plasma angiotensin II in animals treated with perindopril may at first sight appear inconsistent with ACE inhibition, and point to possible measurement problems. Indeed, there are limitations to our technique. First, despite a very low cross-reactivity of the antibody used with angiotensin I (0.1%), there could be a significant contribution from this peptide, especially in the groups treated with perindopril who would be predicted to have increased angiotensin I levels. Further, since we did not employ HPLC to separate angiotensin II from angiotensin degradation products such as angiotensin (2–8) and angiotensin (3–8), which are also recognized by the antibody used, we cannot exclude a significant contribution from these peptides. However, several studies which used HPLC [17, 42–47] have shown a similar lack of reduction of circulating angiotensin II level with chronic ACE inhibitor treatment. Two mechanisms have been advanced to explain the failure of angiotensin II level to fall with ACE inhibitor treatment. First, a partial 'escape' of ACE inhibition driven by the high level of angiotensin I consequent on the increase in PRC [48]. Thus, a combination of renin inhibitor and ACE inhibitor have been found to cause a reduction in plasma angiotensin II level [49]. Alternatively, it has been proposed that the persistent presence of angiotensin II may reflect its generation by alternative pathways not involving ACE [50]. Considerable evidence, especially in vitro, exists for the presence of such pathways [50], but their physiological significance remains unclear [5]. Some of these pathways may also depend on angiotensin I [50], thus providing a possible explanation for the finding of the highest angiotensin II levels in animals with the highest PRC (group D). Whatever, the mechanism involved, the findings on plasma angiotensin II are important for two reasons. First, they support the hypothesis that the beneficial actions of ACE inhibitors in heart failure are through mechanisms that extend beyond the simple suppression of circulating angiotensin II. Secondly, the findings provide a potential explanation for some of the tissue-specific changes seen in the expression of specific RAS components following ACE inhibition, as discussed below.

Changes in tissue RASs in heart failure

With regard to post-infarction heart failure, apart from an increase in kidney renin mRNA level, the main change we found in tissue expression of components of the RAS was in lung ACE. The ACE mRNA level was reduced by 50% and was accompanied by a similar reduction in ACE activity. The findings are very similar to those recently reported by Huang et al. [40] at 3 months after infarction in the same model, but contrast with the findings of Hirsch et al. [11] at 85 days. However, since the reduction in pulmonary ACE expression is significantly correlated with the severity of the heart failure as assessed by lung mass (Figure 7), and Hirsch et al. [11] studied rats with compensated (mild) heart failure, it is possible that the stimulus in their study was not sufficient to significantly affect pulmonary ACE expression. The mechanism(s) responsible for the change in lung ACE mRNA level and activity remain to be elucidated. The effect is tissue-specific, as we did not see a similar effect on kidney ACE (Figure 8). The vascular endothelium is the main site of pulmonary ACE expression, and one explanation to account for the decreased ACE activity post-MI could be remodelling and a relative decrease in the percentage of cells producing ACE. However, this seems unlikely to be the full explanation as in the study by Huang et al. [40] no concomitant changes were found in the activity of two other endothelially-expressed pulmonary ectoenzymes, neutral endopeptidase and aminopeptidase A. The effect also seems to be model-specific as no change in lung ACE expression was found in a volume-overload model of heart failure induced by creation of an aortocaval fistula [51]. Our findings suggest that the reduced activity is mainly due to decreased synthesis, although whether the reduction in ACE mRNA level is itself due to decreased transcription or increased transcript turnover remains to be determined. Multiple hormonal and physiological factors are known to influence pulmonary ACE activity. A potential factor that is relevant here is chronic hypoxia, a likely feature of severe heart failure, which has been shown to lead to a decrease in pulmonary ACE activity [52]. Alternately, it is being increasingly recognized that haemodynamic factors, and in particular shear stress, are important regulators of endothelial endocrine activity, and the down-regulation of pulmonary ACE in heart failure could therefore be a direct consequence of increased pulmonary pressure. Keane et al. [53] induced pulmonary hypertension in rats using two different (non-MI) methods. In both situations, they found lung ACE activity to be reduced and inversely related to the severity of the pulmonary hyper-
tension. The negative correlation found in our study between lung mass and pulmonary ACE mRNA level is consistent with this.

The physiological consequences of the reduction in pulmonary ACE activity in post-infarction heart failure remain to be determined. Since pulmonary ACE makes a major contribution to angiotensin I conversion [41], Huang et al. [40] postulated that its reduction in heart failure may be beneficial by damping the elevation in circulating angiotensin II that would have otherwise resulted from the increase in PRC. The substantial decrease in the ratio of angiotensin II/PRC in MI rats (group B) compared with control rats (group A) in our study (Table 2) is consistent with this hypothesis. At a local pulmonary level, decreased generation of angiotensin II may be a protective mechanism to limit the extent of elevation of pulmonary arterial pressure in heart failure.

Changes in tissue RASs with ACE inhibitor

With regard to ACE inhibitor treatment, we observed two main changes in mRNA levels, both in the kidney: a marked rise in renin mRNA and a more modest rise in angiotensinogen mRNA. As with PRC, the main reason for the increase in renin mRNA is probably a reduction in feedback inhibition by angiotensin II [36]. There is strong evidence that, despite a lack of effect on plasma angiotensin II level, angiotensin II concentrations are reduced in several tissues after chronic ACE inhibitor treatment [43–47]. Specifically, Campbell et al. [43] have shown kidney angiotensin II level to be reduced in the rat treated with perindopril when plasma level was unaffected. Thus, a local intra-renal reduction in the level of negative feedback inhibition by angiotensin II is still a plausible explanation for the increase in renin mRNA level, despite the unchanged, or even increased, plasma angiotensin II level.

An analogous scenario may explain the differential effect of ACE inhibition found on renal and hepatic angiotensinogen mRNA (Figs. 1 and 3). Angiotensinogen expression is known to be under regulation by angiotensin II [54, 55]. It is possible that hepatic expression is directly regulated by circulating levels of angiotensin II, and hence did not change significantly in our study, while renal expression is more responsive to local angiotensin II level, and hence, like renin mRNA, increased after ACE inhibitor. Renal angiotensinogen expression has been localized to the proximal tubule, which is also a key site of action for angiotensin II [56]. Thus, increased angiotensinogen synthesis with ACE inhibition could affect proximal tubular function, although functional correlates of such an effect remain to be identified.

Our findings with renal angiotensinogen expression contrast with those of Schunkert et al. [57]. They found an increase in renal angiotensinogen mRNA level in rats with either stable compensated or severe chronic heart failure, induced in a similar manner to that in our study. The increase ranged from 30 to 80% and was related to the severity of the MI. The increase was reversed by enalapril, but interestingly not by another ACE inhibitor captopril [58]. They concluded that renal angiotensinogen was under positive regulation by angiotensin II, which was reversed by enalapril. Since their experimental model was identical with ours, except that perhaps our rats had even more severe heart failure, judged by the increase in PRC, the discrepant findings are surprising and difficult to reconcile easily.

Other notable observations and limitations of the study

We found no obvious changes in RAS gene expression in heart failure in two important sites where tissue RASs have been implicated in cardiovascular control, i.e. the brain (through effects on the sympathetic nervous system) and the adrenal gland (through effects on aldosterone production and secretion) [5]. These are important negative findings, because both the SNS and aldosterone have been implicated in the pathophysiology and downward progression of heart failure [3, 59, 60]. Likewise, we found no systematic or tissue-specific up or down regulation in AT1 receptor mRNA level in response to either heart failure or ACE inhibitor treatment. The AT1 receptor mediates all the known cardiovascular effects of the RAS [61]. Thus, in so far as the steady state AT1 receptor mRNA level reflects the AT1 receptor density in a tissue, our study suggests that changes at this level do not influence the activity of the RAS in either chronic heart failure or after ACE inhibitor treatment. At least two subtypes (AT1A and AT1B) of the AT1 receptor are known to exist [62]. The precise physiological roles of the AT1 receptor subtypes remain to be established, although their expression is known to vary in different tissues [63]. It should be noted that because of strong homology between mRNAs for the AT1A and AT1B subtypes of the AT1 receptor [62, 63], our full-length AT1 receptor cDNA probe cloned from AT1A receptor subtype mRNA [24], could not distinguish between subtype transcripts by Northern blotting, and thus our study cannot exclude subtype specific changes in expression.

A somewhat disappointing finding was that apart from an additive effect of heart failure and ACE inhibitor treatment on kidney renin mRNA levels and PRC, we found no other effect, especially in tissue gene expression, that specifically resulted from a combination of both factors (i.e. in rats of group D). Clearly, we have only studied limited parameters of tissue RASs, but this finding leaves open the question of the importance of the role of tissue RASs in the pathophysiology of post-infarct heart failure and specifically their role in mediating the beneficial effects of ACE inhibitor in this condition.
An important limitation of our study is that we could not detect an mRNA species by Northern blotting in a particular tissue, which we did not proceed to a more sensitive technique such as PCR to see if changes, albeit at a much lower level, had occurred. This particularly relates to the heart itself where we could not detect any signals for ACE, angiotensinogen or AT1 receptor mRNAs in the left ventricle of any of the experimental groups, and only a weak unchanged signal for renin mRNA (results not shown), although previous studies have reported upregulation of ventricular ACE mRNA [11, 12, 51] analysed by PCR. There has been some debate [64] about the significance of RAS mRNAs only detectable after multiple cycles of PCR, but nonetheless this is a limitation of our study. A further shortcoming of our study is the fact that we only examined one time-point and therefore could have missed period-specific changes in tissue RASs following MI. Some evidence for such changes comes from the finding of Lindpaintner et al. [13] of early activation of cardiac angiotensinogen gene expression following MI at 5 days, which had reversed by 25 days. Finally, we cannot exclude the possibility that despite a lack of overall change in expression of a specific mRNA in a tissue, divergent changes (resulting in a net lack of change) could have occurred in different cell types in the tissue. This would require a technique such as quantitative in situ hybridization to identify. However, despite these limitations, our study provides novel information on changes that occur in circulating and several, hitherto unexamined, tissue RASs in relation to severe post-infarction heart failure and ACE inhibitor treatment, and suggest new aspects of pathophysiology that merit further investigation.

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