Eur Respir J 1997; 10: 2766–2771
DOI: 10.1183/09031936.97.10122766
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Investigations on the renin-angiotensin system in acute severe asthma

S.G. Ramsay*, K.D. Dagg*, I.C. McKay+, B.J. Lipworth#, C. McSharry+, N.C. Thomson*

Investigations on the renin-angiotensin system in acute severe asthma. S.G. Ramsay, K.D. Dagg, I.C. McKay, B.J. Lipworth, C. McSharry, N.C. Thomson. ©ERS Journals Ltd 1997.
ABSTRACT: The renin-angiotensin system is activated in acute severe asthma. The precise mechanism of activation is at present unknown, but may involve, β_2-agonists, catecholamines or proteases released in airway inflammation.

This study aims to identify potential factors involved in the activation of the renin-angiotensin system in acute asthma.

Forty asthmatics with severe exacerbations of asthma, assessed by measurement of peak expiratory flow rate (mean (SD) 35 (18)% predicted), oxygen saturation (94 (4)%) and pulse rate (108 (16) beats·min^{-1}) were recruited. Nineteen (48%) asthmatics had elevated plasma angiotensin II levels (median (interquartile range) 10.9 (4.3–23.5) pg·mL^{-1} (normal range 3–12 pg·mL^{-1})) and 10 (25%) had elevated plasma renin concentration (22.0 (10.0–50.0) μU·mL^{-1} (normal range 9–50 μU·mL^{-1})). Plasma renin and angiotensin II correlated strongly, implying renin-dependent angiotensin II for-mation. No correlation was found between plasma salbutamol, adrenaline, nor-adrenaline, endothelin-1, histamine, eosinophilic cationic protein, serum angio-tensin-converting enzyme (ACE) activity, total immunoglobulin E (IgE), urea and electrolytes, indicators of the severity of the attack, atopic status, blood pressure and renin or angiotensin II levels.

We conclude that although a subpopulation of asthmatics appear to have raised renin and angiotensin II during attacks of acute, severe asthma, the mechanism of activation of the renin-angiotensin system remains unclear.
Eur Respir J 1997; 10: 2766–2771.

Depts of *Respiratory Medicine and +Immunology, Western Infirmary, Glasgow, Scotland, UK. #Dept of Clinical Pharmacology and Therapeutics, Ninewells Hospital, Dundee, Scotland, UK.

Correspondence: S.G. Ramsay
Dept of Respiratory Medicine
West Glasgow Hospitals University
NHS Trust
1053 Great Western Road
Glasgow G12 0YN
Scotland
UK

Keywords: Angiotensin
asthma
endothelin-1
renin
salbutamol

Received: March 27 1997
Accepted after revision September 3 1997

The renin-angiotensin system is activated in acute severe asthma, although not in all asthmatics [1, 2]. The mechanism of this activation is unclear, but recent evidence has shown elevation of renin and angiotensin II in response to nebulized and intravenous salbutamol [3, 4]. A wide variation in plasma β_2-agonist levels has been found in acute asthmatics in previous studies [5], and could, therefore, account for the variation in renin and angiotensin II levels.

However, the degree of activation of the renin-angiotensin system following administration of β_2-agonists is less than that seen in acute asthma, suggesting an additional mechanism. The generation of angiotensin II *via* an "alternative" pathway, through the action of inflammatory proteases [6] either circulating or locally within the airways, could occur in addition to the "classical" activation of the renin-angiotensin system. Endogenous catecholamines released during an acute attack of asthma [7] could stimulate adrenoceptors on juxtaglomerular cells in the kidney [8] resulting in renin release, and may act synergistically with systemically absorbed albuterol.

Serum angiotensin-converting enzyme (ACE) levels vary between individuals and correlate with a genetic polymorphism of the ACE gene [9]. Homozygotes for the deletion polymorphism have a higher ACE activity [10], and, therefore, the capacity to produce greater levels of angiotensin II in response to activation of the renin-angiotensin system. Thus, serum ACE activity could contribute to the variation seen in angiotensin II levels in acute asth-ma.

We hypothesize that activation of the renin-angiotensin system in acute severe asthma is partly due to high levels of circulating β_2-agonists in some asthmatics, and that other factors, as suggested above, may also contribute, possibly with a synergistic effect. Thus, we have examined the associations between renin, angiotensin II and multiple factors during acute attacks of asthma, with particular emphasis on the mechanisms outlined above.

Materials and methods

Patients

Forty adult asthmatic patients (26 females and 14 males; mean (SD) age 45 (17) yrs) were recruited into the study. All presented nonconsecutively at the accident and emergency department, with acute exacerbations of asthma unresponsive to their regular medication and requiring hospital admission for treatment. Admission parameters were measured with peak expiratory flow rate (PEFR) 35 (18) % predicted, oxygen saturation 94 (4) %, cardiac frequency 108 (16) beats·min^{-1}, systolic blood pressure 139.5

Table 1. – Characteristics of patients studied on admission to hospital

Age yrs	45 (17)
PEFR % pred	35 (18)
FEV_1 % pred (n=19)	52 (28)
Cardiac frequency beats·min^{-1}	108 (16)
Oxygen saturation %*	94 (4)
P_{a,O_2} kPa*	11.3 (4.6)
P_{a,CO_2} kPa*	4.9 (1.4)
SBP mmHg	139.5 (22.8)
DBP mmHg	86.0 (16.0)
Sex M/F	16/26
Smokers n	21

Results are presented as mean and SD in parenthesis with n=40 unless otherwise stated in text. *: values obtained when patients receiving supplementary oxygen. PEFR: peak expiratory flow rate; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension; SBP: systolic blood pressure; DBP: diastolic blood pressure; M: male; F: female.

(22.8) mmHg, and diastolic blood pressure 86.0 (16.0) mmHg. Spirometry was not measured during the acute admission but premorbid values were available for 19 of the 40 subjects studied (table 1). Arterial blood gas values were measured, with mean arterial oxygen tension (P_{a,O_2}) 11.3 (4.6) kPa and arterial carbon dioxide tension (P_{a,CO_2}) 4.9 (1.4) kPa; however, due to the acute nature of the admissions, 12 of the 40 patients were already receiving supplementary oxygen at concentrations ranging 24–100%. The values for P_{a,O_2} therefore, probably overestimate the true P_{a,O_2} on breathing air alone.

All patients received treatment with nebulized salbutamol, 31 received oral prednisolone (dose range 15–40 mg), 13 received intravenous hydrocortisone, one received intravenous salbutamol, and one intravenous aminophylline. Thirty three patients were taking regular inhaled corticosteroids, 11 were previously taking oral prednisolone, seven oral theophylline, nine inhaled salmeterol, and one was receiving no medication. Thirty nine patients used inhaled β_2-agonists as required (nine *via* a home nebulizer), seven inhaled ipratropium bromide, one inhaled sod-ium cromoglycate, and one was using oral β_2-agonists. Only two were receiving regular diuretic treatment and none were taking ACE inhibitors.

Ethical approval for the study was obtained from the Glasgow West Ethics Committee and written, informed consent was obtained from the study volunteers prior to entering the study.

Sample collection

All patients had blood samples taken within 24 h of admission (mean (SD) time postadmission 13.5 (9.6) h) for estimation of renin, angiotensin II, plasma salbutamol, serum ACE activity, adrenaline, noradrenaline, histamine, eosinophilic-cationic protein (ECP), endothelin-1, immunoglobulin E (IgE), sodium, potassium, urea and creatinine. Plasma salbutamol levels were measured 3.4 (1.9) h following the previous dose of salbutamol, and an estimation made of the cumulative dose of salbutamol administered in the previous 24 h. All samples were immediately placed on ice until separated by centrifugation at 4°C for 15 min at 3,000×g. Plasma or serum was then removed and kept frozen at -20°C until analysis.

Plasma assays

Renin. The plasma renin concentration was measured by an antibody-trapping technique [11]. The intra-assay coefficient of variation is 5.5% and interassay variation is 11%. The reference range for our laboratory is 9–50 μU·mL^{-1}.

Angiotensin II. The assay for angiotensin II was a modified radioimmunoassay (RIA), which uses C-18 cartridges (Sep-Pak; Waters, Milford, MA, USA) to extract angiotensin II from plasma [12]. The intra-assay coefficient of variation is 6.4% and interassay variation 10%. The reference range for our laboratory is 3–12 pg·mL^{-1}.

Catecholamines. The assays for adrenaline and noradrenaline used a liquid chromatography technique with electrochemical detection [13]. Both the intra- and interassay coefficients of variation are below 10%. The reference ranges for our laboratory are up to 0.4 nmol·L^{-1} for adrenaline and up to 5.0 nM for noradrenaline.

Endothelin-1. Endothelin-1 was measured by RIA using a commercially available kit (Nichols Institute, Saffron Walden, UK). The intra-assay coefficient of variation is 4.5% and the interassay variation 6.8%. The reference range for our laboratory is 1.6–4.9 pg·mL^{-1}.

Salbutamol. The salbutamol assay was a high performance liquid chromatography (HPLC) with solid phase extraction using a mobile phase of 39.4% methanol, 59.1% acetonitrile and 1.5% ammonium acetate [14]. Salbutamol was extracted from plasma using Bond-Elite silica cartridges (Jones Chromatography Ltd., Mid Glamorgan, UK) and then HPLC (10cm Scherisorb S3W-Silica, Hichrom, Berkshire, UK) was performed and the drug detected in the effluent by an LS-1 Fluorescence Detector (Perkin-Elmer, Beaconsfield, UK). The intra-assay coefficient of variation is 3.9% and the interassay variation is 4.1%.

Angiotensin-converting enzyme activity. Serum ACE activity was determined by a continuous monitoring spectrophotometric method based on the hydrolysis of N-(3-(2furyl)-acryly)-L-phenylalanyl-glycyl-glycine [15]. The intra-assay coefficient of variation is 1.7–6.3% and the interassay variation is 3.7–9.7%. The normal range for our laboratory is <88 U·L^{-1}.

Histamine. The assay for plasma histamine was a RIA using a kit (Immunotech S.A., Marseille, France) with high-affinity monoclonal antibodies to acylated histamine, following an acylation step on the sample. The sensitivity of the assay is 0.2 nM. The intra-assay coefficient of variation is 3.4–8.9% and the interassay variation is 6.4–13.0%. The normal range is <10 nM.

Eosinophilic cationic protein. The ECP assay was a double antibody radioimmunoassay using a commercially available kit (Pharmacia UK Ltd, Milton Keynes, UK). The sensitivity of the assay is <2 μg·L^{-1}. The intra-assay coefficient of variation is 4.8–10.9%. The normal range for the assay is <20 μg·L^{-1}.

Immunoglobulin E. Total serum IgE was measured with a Phadebas radioallergosorbent test (RAST) kit (Pharmacia, UK) according to the manufacturer's recommendations. Quality control of the assay was continually assessed by the satisfactory performance of daily internal control samples and monthly national control samples. An estimate of error was made at 20%, based on the coefficient of variation of the measurements of the same internal quality control samples over 50 consecutive weeks. The normal range for our laboratory is <120 International kilo Units $(kU)\cdot L^{-1}$.

Electrolytes. The sodium and potassium assays used standard ion selective electrodes. The normal range for our laboratory is 135–144 mM for sodium and 3.5–5.1 mM for potassium.

Renal function. The assay for urea used a standard urease method. The normal range for our laboratory is 2.5–7.5 mM. The assay for creatinine used the Jasse method measuring in infra-red. The normal range for our laboratory is 60–110 μM.

Statistical analysis. Spearman's Rank correlation coefficient was used to analyse correlations between the variables and, as multiple tests were performed a p-value less than 0.001 was considered significant.

Results

The results of the assays measured are shown in figure 1. Although the levels of renin and angiotensin II were not elevated in the study group as a whole, a subpopulation of asthmatics had raised levels of renin and angiotensin II above the normal range for our laboratory assays, suggesting activation of the renin-angiotensin system in these patients (fig. 2). Nineteen (48%) of the patients had elevated angiotensin II levels, and 10 (25%) had elevated plasma renin. There was a strong correlation between renin and angiotensin II levels (Spearman's rank correlation coefficient (ρ)=0.942; p<0.001).

Adrenaline and noradrenaline correlated strongly with each other (ρ =0.794; p<0.001), although all except one patient had adrenaline levels within the normal range. Noradrenaline levels were elevated in only three of the 40 asthmatics. Histamine correlated positively with ECP (ρ=0.412; p=0.008), and negatively with oxygen saturation (ρ= -0.473; p=0.002). Sixteen patients (40%) had raised plasma endothelin-1 levels which correlated negatively with PEFR (ρ= -0.37; p=0.02). The plasma salbutamol level measured correlated with the estimated dose of salbutamol administered in the previous 24 h (ρ=0.327; p=0.045).

No association was found between renin or angiotensin II levels and plasma levels of salbutamol, adrenaline, noradrenaline, endothelin-1, serum total IgE, histamine, ECP, serum ACE activity, renal function (urea, creatinine) or electrolytes (sodium, potassium), measures of the clinical severity of the asthma attack (oxygen saturation, PEFR) or the cumulative doses of salbutamol or steroids administered (table 2).

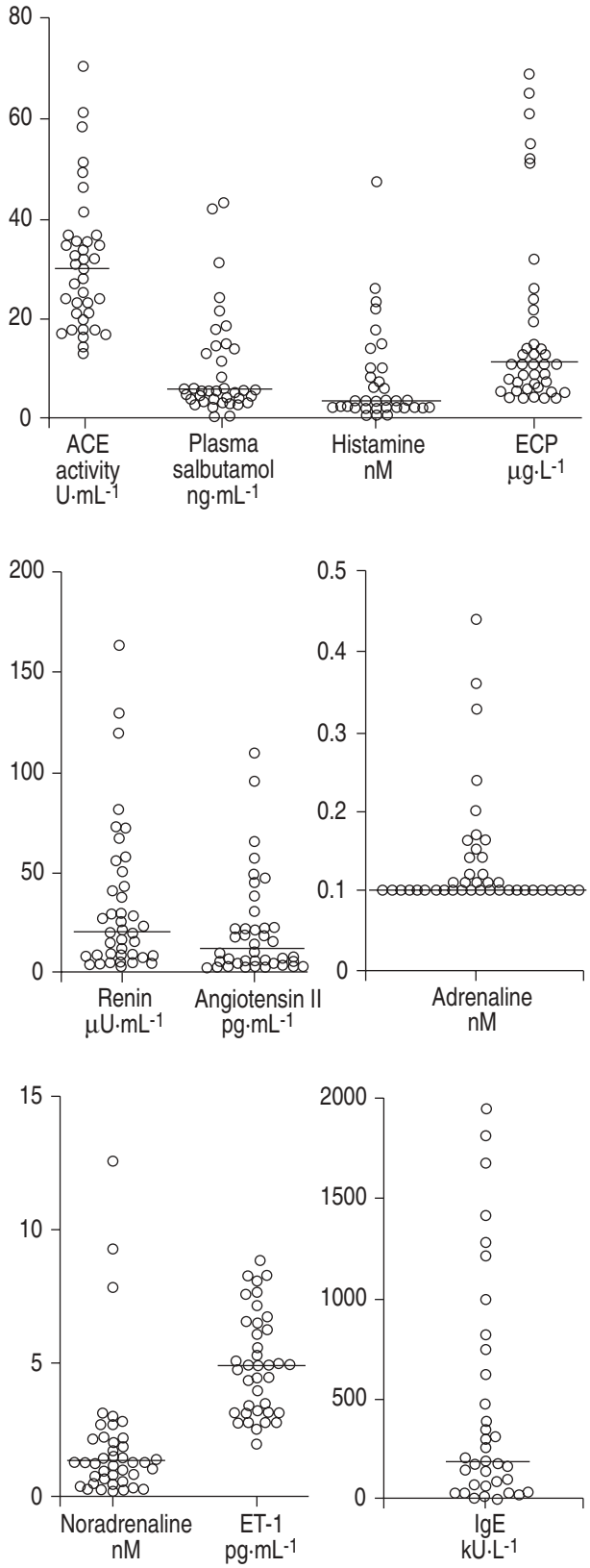

Fig. 1. – Scatter plot illustrating the plasma levels measured in asthmatics during acute exacerbation. Bars indicate median values. Reference ranges are stated in the text. IgE: immunoglobulin E; ECP: eosinophil cationic protein; ACE: angiotensin-converting enzyme; ET-1: endothelin-1.

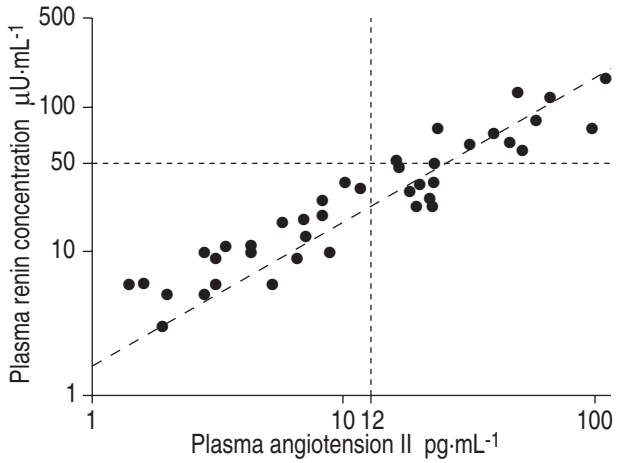

Fig. 2. – Correlation between plasma renin and angiotensin II levels (ρ=0.94). Dotted lines indicate the upper limit of the normal range for plasma renin and angiotensin II in our laboratory. The line is the linear regression line

Table 2. – Spearman's rank collection coefficient (ρ) for the correlations between renin and angiotensin II and the parameters measured

	Renin	Angiotensin II
Renin	-	0.94***
Angiotensin II	0.94***	-
Plasma salbutamol	0.26	0.11
Adrenaline	0.29	0.35
Noradrenaline	0.17	0.22
Histamine	-0.02	-0.07
Endothelin-1	0.13	0.09
Serum ACE Activity	-0.10	0.03
Eosinophilic cationic protein	0.11	0.10
Immunoglobulin E	0.15	0.16
Urea	-0.10	-0.08
Creatinine	0.23	0.26
Sodium	-0.07	-0.04
Potassium	-0.01	-0.03
PEFR	0.11	0.20
Oxygen saturation	0.33	0.40
Age	-0.24	-0.20

ACE: angiotensin-converting enzyme; PEFR: peak expiratory flow rate. ***: p<0.001.

Blood pressure as a group mean (SD) showed a systolic blood pressure of 139.5 (22.8) mmHg and diastolic blood pressure of 86.0 (16.0) mmHg. No difference was found in blood pressure between those with elevated plasma renin (mean (SD) 135 (16.5) mmHg systolic, and 81.1 (15.1) mmHg diastolic) and those without (140.9 (24.4) mmHg systolic, and 87.5 (16.2) mmHg diastolic), or in those with elevated plasma angiotensin II (135.5 (15.4) mmHg systolic and 82.6 (12.7) mmHg diastolic) and those without (143.0 (27.4) mmHg systolic and 89.0 (18.2) mmHg diastolic). There was no correlation between systolic or diastolic blood pressure measurements and either renin or angiotensin II levels.

Discussion

This study examines the mechanism of activation of the renin-angiotensin system in acute asthmatics, evidence of which has been identified in our previous work [1, 2]. It confirms activation of the renin-angiotensin system in some, but not all, individuals with acute severe asthma, in addition to examining the plasma levels of circulating inflammatory mediators, endogenous catecholamines and salbutamol. Angiotensin II is a weak bronchoconstrictor *in vitro* [16, 17] and *in vivo* [1], potentiates the effect of other bronchoconstrictor agents, such as methacholine both *in vitro* and *in vivo* [16], and endothelin-1 *in vitro* [17], but not histamine [18].

Endothelin-1, a potent vasoconstrictor and bronchoconstrictor, has been isolated from human respiratory epithelial cells [19] and contributes to bronchoconstriction in asthma [20]. Circulating levels of endothelin-1 are raised in some patients with acute asthma [2l] but not in others [22, 23]. In the present study, plasma endothelin-1 levels were raised in 16 patients (40%), correlating negatively with PEF suggesting involvement in bronchoconstriction.

Histamine, derived from mast cells, is involved both in the early and late allergic asthmatic responses [24]. In this study, plasma histamine was elevated in nine subjects (22%), correlating negatively with oxygen saturation possibly reflecting either histamine-induced bronchospasm or an increase in histamine release due to hypoxia.

Eosinophils are elevated in the blood and bronchoalveolar lavage (BAL) fluid of asthmatics and correlate with the severity of the disease [25]. ECP is released from activated eosinophils, is a marker of eosinophil activity and is elevated in serum from asthmatics, providing an indicator of inflammatory airflow obstruction [26]. In this study, serum levels of ECP were elevated in 10 subjects (25%).

Although there was elevation of the above inflammatory mediators in some asthmatics, exhibiting varying degrees of correlation with the severity of the attack, no relationship was identified with the plasma levels of renin and angiotensin II. These mediators do not appear to have a major role either directly or synergistically in activation of the renin-angiotensin system.

The findings of this and our previous work pose the question as to what purpose activation of the renin-angiotensin system serves in acute asthma. It is unclear whether this represents a pathological phenomenon or is a physiological response. In either case, the mechanism of activation is unclear, although several potential routes exist.

Pathological activation of the renin-angiotensin system

The renin-angiotensin system may be activated by direct stimulation of renin secretion through activation of renal juxtaglomerular α- and β_2-adrenoceptors [8] by either β_2-agonists, adrenaline or noradrenaline. Recently, the β_2-agonist, salbutamol, administered both intravenously and by nebulization, has been shown to cause activation of the renin-angiotensin system by a renin-driven, ACE-dependent mechanism [3, 4]. In acute asthmatic patients, there is a marked variation in the plasma levels of salbutamol [5], and also variation in the systemic absorption of nebulized salbutamol [27]. In acute asthmatic attacks, the high doses of β_2-agonists administered may reasonably be expected to stimulate renin release, since they are in excess of doses shown to cause elevation of renin and angiotensin II in a previous study. Our study shows a wide range of plasma

salbutamol levels, which correlate with the estimated dose administered over the preceding 24 h. However, we have been unable to demonstrate any correlation between serum levels of salbutamol and plasma levels of renin and angiotensin II.

Similarly, noradrenaline, which is raised in acute asthma [7] and following histamine-induced bronchoconstriction [28], induces renin release *in vitro*, a mechanism which is potentiated by theophylline [29]. In the present study, levels of noradrenaline were elevated in only three cases, in contrast to previous studies [7, 30], and no correlation was found with either renin or angiotensin II.

The lack of any significant elevation of catecholamines makes the possibility of an additive or synergistic effect with salbutamol on the juxtaglomerular adrenoceptors to stimulate renin release unlikely. As adrenaline is not elevated, the acute asthma attack and admission to hospital do not appear to cause sufficient stress to contribute to activation of the renin-angiotensin system.

The role of hypoxia in activation of the renin-angiotensin system is controversial; whilst it potentiates exercise-induced activation of the system [31], it does not directly increase renin and angiotensin II at rest [32]. In the current study, no correlation has been identified between oxygen saturation and P_{a,O_2} and renin or angiotensin II levels.

Variation in serum ACE activity is known to exist in the population as the result of an insertion/deletion polymorphism of the ACE gene [9]. Individuals who are homozygous for the deletion have a higher serum ACE activity [10], and achieve higher levels of angiotensin II following infusion of angiotensin I [33]. A higher ACE activity could potentially explain the elevation of angiotensin II in the subjects studied, but would not account for the raised renin levels. However, no correlation was found between serum ACE activity and angiotensin II levels.

Polymorphisms of the other components of the renin-angiotensin system, in particular the angiotensinogen gene [34], which is the rate-limiting element, could affect angiotensin II levels. Therefore, if a subgroup of asthmatics had the propensity to produce higher amounts of angiotensinogen, that, in combination with other stimuli such as exogenous β_2-agonists, could theoretically lead to the production of higher levels of angiotensin II for a given increase in renin release.

The strong correlation of plasma renin and angiotensin II levels indicates that the formation of angiotensin II is renin-dependent, presumably *via* the action of ACE. Thus, formation of angiotensin II by an alternative pathway, such as the action of nonspecific proteolytic enzymes [6], is unlikely. Although such alternative pathways do not appear to contribute to circulating levels in acute asthma, this does not preclude a role at a local tissue level in the airway.

Physiological activation of the renin-angiotensin system

Elevation of mean systolic blood pressure has been documented in acute asthmatics [7]. The absence of raised blood pressure in those asthmatics with elevation of renin and angiotensin II levels in the current study is, therefore, surprising. The release of renin and subsequent angiotensin II formation in acute asthma may be part of a physiological homeostatic regulatory mechanism to maintain an adequate blood pressure in the face of arterial vasodilatation. Histamine has vasodilator properties but no correlation was found with renin or angiotensin II levels. Bradykinin is a potent bronchoconstrictor and also causes arterial vasodilation and capillary leakage [35]. Plasma kinin is elevated in acute asthma and has been found in BAL fluid from asthmatics following allergen challenge [36]. Therefore, bradykinin-induced vasodilation could trigger activation of the renin-angiotensin system, but bradykinin levels have not been estimated in this study.

The renin-angiotensin system is also activated by a drop in renal perfusion pressure caused by a reduction in renal artery flow due to local factors or a reduction in systemic blood pressure. In acute severe asthma, raised intra-thoracic pressure produces mechanical impairment of cardiac diastolic ventricular filling (*pulsus paradoxus*) lead-ing to an intermittent drop in systemic blood pressure, which may stimulate the renin-angiotensin system. The levels of urea and creatinine measured in this study are normal, making a chronic disturbance of renal function unlikely, but no inferences can be made about acute changes in renal function.

Electrolyte imbalance, in particular hyperkalaemia, can stimulate renin release. Administration of salbutamol is known to cause a shift of potassium ions intracellularly resulting in hypokalaemia [37] and, thus, would antagonize renin release. No significant abnormality of sodium and potassium levels was found in the present study population.

In conclusion, the role of the renin-angiotensin system in asthma remains incompletely understood. Although raised levels of renin and angiotensin II are found in a subgroup of acute severe asthmatics, the mechanism of activation of the renin-angiotensin system and the underlying physiological role, if any, remain to be clarified. Various potential mechanisms of activation have been suggested above but our results do not support any single factor. The increased levels of many inflammatory mediators and cytokines, as well as exogenously administered medications, during acute asthmatic attacks may interact synergistically to activate the system, but our analysis has failed to provide evidence to support this. Of the parameters measured in our study, none have discriminated bet-ween the subgroup with elevated renin and angiotensin II levels and those without.

Acknowledgements: The authors would like to thank G. Clark for his assistance with the plasma salbutamol assays, I. Morton for performing the renin, angiotensin II and endothelin-1 assays, and R. Spooner for assistance with the ACE activity assays.

References

1. Millar EA, Angus RA, Hulks G, Morton JJ, Connell JMC, Thomson NC. Activity of the renin-angiotensin system in acute severe asthma and the effect of angiotensin II on lung function. *Thorax* 1994; 49: 492–495.
2. Thomson NC, Dagg KD, Ramsay SG. Humoral control of airway tone. *Thorax* 1996; 51: 461–464.
3. Millar EA, McInnes GT, Thomson NC. Investigation of the mechanism of β_2-agonist-induced activation of the renin-angiotensin system. *Clin Sci* 1995; 88: 433–437.
4. Millar EA, Connell JMC, Thomson NC. The effect of nebulised albuterol on the activity of the renin-angi-

otensin system in asthma. *Chest* 1997; 111: 71–74.
5. Janson C, Boe J, Boman G, Mossberg B, Svedmyr N. Bronchodilator intake and plasma levels on admission for severe acute asthma. *Eur Respir J* 1992; 5: 80–85.
6. Tonnesen MG, Klempner MS, Austen KF, Wintroub BU. Identification of a human neutrophil angiotensin II-generating protease as cathepsin G. *J Clin Invest* 1982; 69; 25–30.
7. Ind PW, Causon RC, Brown MJ, Barnes PJ. Circulating catecholamines in acute asthma. *Br Med J* 1985; 290: 267–269.
8. Reid IA, Morris BJ, Ganong WF. The renin-angiotensin system. *Ann Rev Physiol* 1978; 40: 377–410.
9. Teo KK. Angiotensin-converting enzyme genotypes and disease. *BMJ* 1995; 311: 763–764.
10. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86: 1343–1346.
11. Millar JA, Leckie BJ, Morton JJ, Jordan J, Tree M. A microassay for active and total renin concentration in human plasma based on antibody-trapping. *Clin Chim Acta* 1980; 101: 5–15.
12. Morton JJ, Webb DJ. Measurement of plasma angiotensin II. *Clin Sci* 1985; 68: 483–484.
13. Goldstein DS, Feuerstein G, Izzo JL, Kopin IJ, Keiser HR. Current concepts. II. Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. *Life Sci* 1981; 28: 467–475.
14. Lipworth BJ, Clark RA, Dhillon DP, *et al.* Pharmacokinetics, efficacy and adverse effects of sublingual salbutamol in patients with asthma. *Eur J Clin Pharmacol* 1989; 37: 567–571.
15. Maguire GA, Price CP. A continuous monitoring spectrophotometric method for the measurement of angiotensin-converting enzyme in human serum. *Ann Clin Biochem* 1985; 22: 204–210.
16. Millar EA, Nally JE, Thomson NC. Angiotensin II potentiates methacholine induced bronchoconstriction in human airway both *in vitro* and *in vivo*. *Eur Respir J* 1995; 8: 1838–1841.
17. Nally JE, Clayton RA, Wakelam MJO, Thomson NC, McGrath JC. Angiotensin II enhances responses to endothelin-1 in bovine bronchial smooth muscle. *Pulm Pharmacol* 1994; 7: 409–413.
18. Ramsay SG, Clayton RA, Dagg KD, Thomson LJ, Nally JE, Thomson NC. Effect of angiotensin II on histamine-induced bronchoconstriction in the human airway both *in vitro* and *in vivo*. *Respir Med* 1997; 91: 609–615.
19. Vittori E, Marini M, Fasoli A, de Franchis R, Mattoli S. Increased expression of endothelin in bronchial epithelial cells of asthmatic patients and effect of corticosteroids. *Am Rev Respir Dis* 1992; 146: 1320–1325.
20. Reddington AE, Springall DR, Ghatei MA, *et al.* Endothelin in bronchoalveolar lavage fluid and its relation to airflow obstruction in asthma. *Am J Respir Crit Care Med* 1995; 151: 1034–1039.
21. Nomura A, Uchida Y, Kameyama M, Saotome M, Oki K, Hasegawa S. Endothelin and bronchial asthma. *Lancet* 1989; 2: 747–748.
22. Mattoli S, Soloperto M, Marini M, Fasoli A. Levels of endothelin in the bronchoalveolar fluid of patients with symptomatic asthma and reversible airflow obstruction. *J Allergy Clin Immunol* 1991; 88: 376–384.
23. Kraft M, Beam WR, Wenzel SE, Zamora MR, O'Brien RF, Martin RJ. Blood and bronchoalveolar lavage endothelin-1 levels in nocturnal asthma. *Am J Respir Crit Care Med* 1994; 149: 947–952.
24. Horwitz RJ, Busse WW. Inflammation and asthma. *Clin Chest Med* 1995; 16: 583–602.
25. Bousquet J, Chanez P, Lacoste JY, *et al.* Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033–1039.
26. Wever AMJ, Wever-Hess J, Hensgens HE, Hermans J. Serum eosinophil cationic protein (ECP) in chronic asthma: relationship to spirometry, flow-volume curves, PC20 and exacerbations. *Respir Med* 1994; 88: 613–621.
27. Penna AC, Dawson KP, Manglick P, Tam J. Systemic absorption of salbutamol following nebulizer delivery in acute asthma. *Acta Paediatr* 1993; 82: 963–966.
28. Larsson K, Carlens P, Bevegard S, Hjemdahl P. Sympathoadrenal responses to bronchoconstriction in asthma: an invasive and kinetic study of plasma catecholamines. *Clin Sci* 1995; 88: 439–446.
29. Nolly HL, Reid IA, Ganong WF. Effect of theophylline and adrenergic blocking drugs on the renin response to norepinephrine *in vitro*. *Circ Res* 1974; 35: 575–579.
30. Emerman CL, Cydulka RK. Changes in serum catecholamine levels during acute bronchospasm. *Ann Emerg Med* 1993; 22: 1836–1841.
31. Milledge JS, Catley DM. Renin, aldosterone and converting enzyme during exercise and acute hypoxia in humans. *J Appl Physiol: Respirat Environ Exercise Physiol* 1982; 52: 320–323.
32. Colice GL, Ramirez G. Effect of hypoxia on the renin-angiotensin system in humans. *J Appl Physiol* 1985; 58: 724–730.
33. Ueda S, Elliot HL, Morton JJ, Connell JMC. Enhanced pressor response to angiotensin I in normotensive men with the deletion genotype (DD) for angiotensin converting enzyme. *Hypertension* 1995; 25: 1266–1269.
34. Katsuya T, Koike G, Yee TW, *et al.* Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. *Lancet* 1995; 345: 1600–1603.
35. Fuller RW, Barnes PJ. Kinins. *In*: Barnes PJ, Rodger IW, Thomson NC, eds. Asthma: Basic Mechanisms and Clinical Management. London, Academic Press, 1988; pp. 259–272.
36. Trifilieff A, Da Silva A, Gies J-P. Kinins and respiratory tract diseases. *Eur Respir J* 1993; 6: 576–587.
37. Scheinin M, Koulu M, Laurikainen E, Allonen H. Hypokalaemia and other nonbronchial effects of inhaled fenoterol and salbutamol: a placebo-controlled dose-response study in healthy volunteers. *Br J Clin Pharmacol* 1987; 24: 645–653.