Effect of angiotensin II on histamine-induced bronchoconstriction in the human airway both in vitro and in vivo

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The renin–angiotensin system is activated in acute severe asthma. Angiotensin II causes bronchoconstriction in mild asthmatics and potentiates methacholine-evoked bronchoconstriction both in vitro and in vivo.

To evaluate the effect of angiotensin II on histamine-induced bronchoconstriction, human bronchial rings (n=6) were obtained from lung tissue at thoracotomy and were prepared in organ baths. Contractions were measured isometrically and cumulative concentration–response curves obtained to angiotensin II alone and to histamine in the presence and absence of threshold concentrations of angiotensin II.

Eight asthmatic patients with bronchial hyper-reactivity to histamine were challenged with histamine during intravenous infusion of placebo, angiotensin II 1 ng kg⁻¹ min⁻¹ and angiotensin II 2 ng kg⁻¹ min⁻¹ administered in a randomized, double-blind fashion. FEV₁ was measured prior to, during the infusion and during the histamine challenge.

Angiotensin II (3 x 10⁻⁷ M and 10⁻⁶ M) alone evoked small contractions (<0.25 g) of human bronchi in vitro, but pre-incubation with threshold concentrations of angiotensin II (10⁻⁷ M, 3 x 10⁻⁷ M and 10⁻⁶ M) had no effect on histamine-evoked contractions. In asthmatic patients, angiotensin II alone had no effect on baseline FEV₁ at the low levels infused and did not affect the response to nebulized histamine as measured by the PC₂₀ histamine: Geometric mean (range) PC₂₀ histamine (mg ml⁻¹) screening day 3.58 (1.26–7.75), placebo infusion 2.67 (0.89–9.57), angiotensin II 1 ng kg⁻¹ min⁻¹ 2.45 (0.42–6.97) and angiotensin II 2 ng kg⁻¹ min⁻¹ 3.09 (0.88–10.78).

It is concluded that, in contrast to its potentiating effect on methacholine-induced bronchoconstriction, angiotensin II has no effect on histamine-evoked bronchoconstriction in human bronchi in vitro or in vivo.
both in vitro and in vivo (7), and endothelin-1-evoked bronchoconstriction of bovine airways in vitro, by a type-1 angiotensin II receptor-specific effect (8). The mechanism of this potentiating effect remains obscure but the possible interaction with other spasmogens in the airway exists. Histamine is a potent bronchoconstrictor which is released from mast cells in asthmatic airways and therefore is an important spasmogen and any potential interaction with angiotensin II would be relevant.

Therefore, the current study examined the effect of angiotensin II at subthreshold doses on histamine-induced bronchoconstriction in human airways in vitro and in vivo.

Materials and Methods

IN VITRO

Tissue Collection and Preparation

Macroscopically normal human bronchial tissues (3rd to 6th order) were obtained from patients undergoing thoracotomy for bronchial carcinoma. All patients were smokers but information about pulmonary function was unavailable; none suffered from asthma. Tissues were dissected free of connective tissue and fat, and stored overnight at 4°C in oxygenated Krebs-Henseleit solution of the following composition; NaCl 118.4 mM, KCl 4.7 mM, CaCl₂ 2.5 mM, MgSO₄ 1.2 mM, NaHCO₃ 24.9 mM, KH₂PO₄ 1.2 mM and glucose 11.1 mM. Published data has shown that overnight storage of this tissue does not alter its reactivity (9).

Measurement of Contractile Responses

Contractile responses were measured from rings of human bronchi (3-5 mm) in vertical organ baths (10 ml) at 37 ± 0.5°C in oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solution. Tension (2 g) was applied via two platinum wires into the lumen. One wire was anchored and the other attached to a force displacement transducer (Grass FT03T). Tissues were allowed to equilibrate for 2 h during which time applied tension was readjusted to the initial level.

Cumulative concentration–response curves were constructed to angiotensin II to ascertain the threshold for contraction to this hormone. Angiotensin II produced small (≤0.25 g maximum), concentration-dependent contractions of human bronchi, with the threshold for contraction occurring between 10⁻⁷ M and 10⁻⁶ M (Fig. 1). This compared with the maximum contraction of mean (SEM) 1.45 (0.28) g with 3 × 10⁻⁴ M histamine.

In subsequent experiments, cumulative concentration-response curves were constructed to histamine (10⁻⁹–3 × 10⁻⁴ M) in the presence and absence of angiotensin II (10⁻⁷ M, 3 × 10⁻⁷ M and 10⁻⁶ M). Drugs were added directly to the organ bath and angiotensin II was added 15 min before histamine concentration–response curves.

IN VIVO

Patients

Eight mild asthmatic volunteers (five males) were recruited with mean (range) age 41 (27-69) years and baseline FEV₁ 88 (78-106) % of predicted. Bronchial hyper-responsiveness to inhaled histamine was demonstrated by challenge testing according to the method of Cockcroft et al. (10). Individuals found to be hypertensive, pregnant or taking antihistamines, diuretics or angiotensin-converting enzyme inhibitors were excluded. Two subjects were current and one was a former smoker. Seven subjects were taking inhaled salbutamol as required, four were taking additional low dose (<1000 µg daily) inhaled budesonide dipropionate, one was taking additional salmeterol and one was on no treatment (Table 1). Prior to each study day, subjects were instructed to withhold short-acting β-agonists for 8 h and long-acting β-agonists for 12 h but inhaled corticosteroids were continued unchanged.

Ethical approval for the study was obtained from the Glasgow West Ethical Committee and written, informed consent was obtained from the study volunteers prior to commencing the study.
Table 1. Study patient demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (years)</th>
<th>FEV\textsubscript{1} absolute value (l) (% predicted)</th>
<th>Histamine PC\textsubscript{20} (mg ml\textsuperscript{-1})</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>27</td>
<td>4.80 (106%)</td>
<td>1.84</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>49</td>
<td>3.56 (88%)</td>
<td>4.21</td>
<td>nil</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>32</td>
<td>2.75 (82%)</td>
<td>3.24</td>
<td>S/BDP</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>35</td>
<td>2.83 (98%)</td>
<td>1.26</td>
<td>S/BDP</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>56</td>
<td>2.14 (79%)</td>
<td>2.61</td>
<td>S/BDP</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>30</td>
<td>4.37 (97%)</td>
<td>7.75</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>30</td>
<td>2.46 (80%)</td>
<td>5.32</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>68</td>
<td>2.46 (78%)</td>
<td>6.33</td>
<td>S/BDP/Sm</td>
</tr>
<tr>
<td>Mean age:</td>
<td></td>
<td>40.9 (14.99)</td>
<td>3.18 (0.97) (10-97) (10.58)</td>
<td>88.5%</td>
<td>3.48*</td>
</tr>
</tbody>
</table>

S, salbutamol (pm); BDP, beclomethasone dipropionate (<1000 \mu g day\textsuperscript{-1}); Sm, salmeterol; *geometric mean.

Study Protocol

The study design was randomized, double-blind and placebo controlled. The subjects attended the laboratory on four separate occasions; initially for a screening visit comprising a physical examination followed by a histamine bronchial provocation test. This was performed using a Wright's nebulizer, calibrated to give an output of 0.12 ml min\textsuperscript{-1} at a flow rate of 8 litres min\textsuperscript{-1} air, to define the concentration required to cause a 20% fall in FEV\textsubscript{1} from baseline (the PC\textsubscript{20} histamine). On the three subsequent visits, an intravenous cannula (Venflon, Viggio AB, Helsinborg, Sweden) was inserted into a vein on each forearm; after 30 min rest, a blood sample was extracted to measure baseline plasma renin and angiotensin II levels. Baseline spirometry, pulse, blood pressure and pulse oximetry were measured then an intravenous infusion commenced via a 50-ml syringe driver (Perfusor Secura E, B.B. Braun, Melsunger AG, Germany). Subjects received either placebo (5% dextrose) or angiotensin II at subthreshold doses for bronchoconstriction of 1 or 2 ng kg\textsuperscript{-1} min\textsuperscript{-1} over 1 h. After 30 min, steady state was deemed to have been reached and a further blood sample was extracted to measure baseline plasma renin and angiotensin II levels. Baseline spirometry, pulse, blood pressure and pulse oximetry were measured then an intravenous infusion commenced via a 50-ml syringe driver (Perfusor Secura E, B.B. Braun, Melsunger AG, Germany). Subjects received either placebo (5% dextrose) or angiotensin II at subthreshold doses for bronchoconstriction of 1 or 2 ng kg\textsuperscript{-1} min\textsuperscript{-1} over 1 h. After 30 min, steady state was deemed to have been reached and a further blood sample was taken from the arm opposite to the infusion and the FEV\textsubscript{1} measured. A histamine bronchial provocation test was then performed and the PC\textsubscript{20} noted. At the completion of the test, a further blood sample was taken, then the bronchoconstriction was reversed with nebulized salbutamol (Ventolin, Allen and Hanburys, U.K.). Throughout the study pulse, blood pressure and oxygen saturation were monitored at 15-min intervals.

Measurements

FEV\textsubscript{1}. Spirometry was measured using a dry wedge spirometer (Vitalograph Model S, Buckinghamshire, U.K.), the best of three measurements being taken at each time point.

Pulse and Blood Pressure. A semi-automatic sphygmomanometer (Dinamap, 1846SX Vital Signs Monitor, Critikon, Florida, U.S.A.) was used to measure pulse and blood pressure from the opposite arm to the infusion at each time point.

Oxygen Saturation. Oxygen saturation was measured by transcutaneous pulse oximetry (Oxy Pulse Oximeter, Datex, Helsinki, Finland) at each time point.

Histamine Bronchial Provocation Test. Baseline spirometry was measured then repeated after a 2-min inhalation of 0.9% saline, administered via a Wright's nebulizer driven by compressed air, calibrated to give an output of 0.12 ml min\textsuperscript{-1} at a flow rate of 8 litres min\textsuperscript{-1}. Doubling doses of histamine were subsequently administered starting with a dose of 0.0625 mg ml\textsuperscript{-1}. The FEV\textsubscript{1} was measured at 30, 90 and 180 s following each inhalation until a 20% fall was achieved. The histamine concentration causing a 20% fall in FEV\textsubscript{1} was calculated by linear regression and taken as the PC\textsubscript{20}.

Angiotensin II Assay. Blood samples were collected into iced 10-ml plastic tubes containing EDTA and O-phenanthroline inhibitor. They were placed on ice.
until separation by centrifugation at 3000 g for 15 min, then the plasma was frozen at -20°C until analysis. The assay for angiotensin II is a modified radioimmunoassay which uses C_{18} cartridges (Sep-Pak; Waters, Milford, MA, U.S.A.) to extract angiotensin II from plasma (11). The intra-assay coefficient of variation is 6.4% and inter-assay variation 10%. The reference range for the authors’ laboratory is 3–12 pg ml⁻¹ (2.9–11.5 × 10⁻⁹ M).

Renin Assay. Blood samples for renin were collected in 5-ml glass tubes containing EDTA then placed on ice and frozen at -20°C until analysis. Plasma renin concentration was measured using an antibody trapping technique (12). The intra-assay coefficient of variation is 5.5% and the inter-assay variation 11% (13). The reference range for the authors’ laboratory is 9–50 μLU ml⁻¹.

Drugs. The drugs used were histamine (Fluka Chemicals, Gillingham, U.K. for in vivo studies: Sigma Chemicals, Poole, U.K. for in vitro studies) and angiotensin II (Sigma Chemicals, U.K.). For the in vitro studies, both histamine and angiotensin II were prepared as a stock solution in distilled water, then serial dilutions were prepared in Krebs-Henseleit solution. For in vivo studies, serial dilutions of histamine were prepared in phosphate-buffered saline ranging from 0.0625 to 8.0 mg ml⁻¹. Angiotensin II was prepared in 5% dextrose under sterile conditions.

Statistical Analysis. Statistical significance was determined by two-way analysis of variance (ANOVA) for in vitro studies and one-way ANOVA for in vivo studies with a probability level of P < 0.05 considered significant. Histamine PC_{20} values were transformed logarithmically prior to statistical analysis.

Results

IN VITRO STUDIES

Pre-incubation with angiotensin II (10⁻⁷ M) evoked contraction in only two of the six tissues and in both cases the contraction was less than 0.1 g compared with the control maximum contraction of mean 1.45 (SEM 0.28) g with histamine (3 × 10⁻⁴ M). No significant enhancement (P > 0.05) of histamine-evoked contractions (10⁻⁹ M–3 × 10⁻⁴ M) was seen in any of the tissues following pre-incubation with angiotensin II at 10⁻⁷ M, 3 × 10⁻⁷ M and 10⁻⁶ M (Fig. 2).

Fig. 2. Cumulative concentration–response curves to histamine alone (control, ■) and in the presence of angiotensin II at 10⁻⁷ M (□), 3 × 10⁻⁷ M (○), and 10⁻⁸ M (▲), showing no significant potentiation or attenuation of bronchoconstriction. Number of observations (n) = 6 in each case. Responses are expressed as a percentage of methacholine (10⁻⁴ M) evoked contraction.

IN VIVO STUDIES

On each of the three study days, there was no significant difference between baseline pulse, blood pressure, oxygen saturation, FEV₁, plasma renin and plasma angiotensin II measurements (Table 2). Prior to histamine challenge and during the infusion, there was no significant change in FEV₁ noted from baseline values on each study day; mean (SEM) change in FEV₁ (1) from baseline: placebo 0.08 (0.05), angiotensin II 1 ng kg⁻¹ min⁻¹ 0.12 (0.04), angiotensin II 2 ng kg⁻¹ min⁻¹ 0.08 (0.04).

Following histamine bronchial provocation testing on each study day, there was no significant difference in histamine PC_{20} (geometric mean (range)) between screening [3.48 (1.26–7.75) mg ml⁻¹, placebo [2.67 (0.89–9.57), mg ml⁻¹, angiotensin II 1 ng kg⁻¹ min⁻¹ [2.45 (0.42–6.97) mg ml⁻¹] and angiotensin II 2 ng kg⁻¹ min⁻¹ [3.09 (0.88–10.78) mg ml⁻¹] study days (Fig. 3). A significant rise (P < 0.05) in both systolic and diastolic blood pressure occurred following infusion of angiotensin II 1 ng kg⁻¹ min⁻¹ and 2 ng kg⁻¹ min⁻¹ compared to placebo [systolic BP mean (SEM) mmHg 121.4 (4.57), 139.4 (6.72) and 140.5 (5.84), and diastolic BP mean (SEM) mmHg 72.0 (2.40), 81.4 (2.54) and 82.8 (2.43) on placebo, angiotensin II 1 ng kg⁻¹ min⁻¹ and angiotensin II 2 ng kg⁻¹ min⁻¹ study days, respectively]. However,
### Table 2. Baseline measurements

<table>
<thead>
<tr>
<th>Study day</th>
<th>Ang. II 1 ng kg⁻¹ min⁻¹</th>
<th>Ang. II 2 ng kg⁻¹ min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>3.18</td>
<td>3.07</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>62.2</td>
<td>58.5</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139.8</td>
<td>132.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.4</td>
<td>73.8</td>
</tr>
<tr>
<td>O₂ saturation (%)</td>
<td>96.9</td>
<td>97.1</td>
</tr>
<tr>
<td>Renin (μU ml⁻¹)</td>
<td>—</td>
<td>16.9</td>
</tr>
<tr>
<td>Angiotensin II (pg ml⁻¹)</td>
<td>—</td>
<td>7.6</td>
</tr>
<tr>
<td>Angiotensin II (molar concentration)</td>
<td>7.3 x 10⁻⁹ M</td>
<td>7.0 x 10⁻⁹ M</td>
</tr>
</tbody>
</table>

Results are expressed as means (SEM).
No significant difference was found for any of the above parameters between study days.

No significant changes in pulse rate or oxygen saturation were noted throughout each study day.

Plasma angiotensin II was measured at baseline, steady state (30 min into the infusion) and following completion of the infusion on each study day. On the placebo day, angiotensin II levels remained unchanged; mean (SEM) [mean molar concentration] baseline 7.6 (1.6) pg ml⁻¹ [7.3 x 10⁻⁹ M], steady state 5.7 (1.1) pg ml⁻¹ [5.5 x 10⁻⁹ M] and post histamine challenge 5.4 (0.6) pg ml⁻¹ [5.2 x 10⁻⁹ M]. On both angiotensin II study days, plasma levels increased significantly (P<0.05) after 30 min compared to baseline and placebo, more so on the 2 ng kg⁻¹ min⁻¹ day and remained elevated until the end of the infusion; 7.3 (1.5) pg ml⁻¹ [7.0 x 10⁻⁹ M], 23.8 (3.7) pg ml⁻¹ [2.3 x 10⁻⁸ M] and 22.0 (4.3) pg ml⁻¹ [2.1 x 10⁻⁸ M] after angiotensin II 1 ng kg⁻¹ min⁻¹ and 8.2 (1.9) pg ml⁻¹ [7.9 x 10⁻⁹ M], 46.2 (4.2) pg ml⁻¹ [4.4 x 10⁻⁸ M] and 36.0 (3.2) pg ml⁻¹ [3.5 x 10⁻⁸ M] after angiotensin II 2 ng kg⁻¹ min⁻¹ (Fig. 4).

No adverse effects were reported by the subjects on any of the study days.

**Discussion**

The results of this study show that angiotensin II does not affect histamine-induced bronchoconstriction in human bronchi either *in vitro* or *in vivo*. 

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**Fig. 3.** The PC₂₀ histamine (mg ml⁻¹) on the screening day, following placebo infusion and following infusion of angiotensin II 1 ng kg⁻¹ min⁻¹ and 2 ng kg⁻¹ min⁻¹. Angiotensin II had no effect on the PC₂₀ histamine.

There was no statistically significant difference in either systolic or diastolic blood pressure following histamine challenge on each study day.
The intravenous infusion of angiotensin II in these in vivo studies resulted in an elevation of plasma angiotensin II to a maximum of median (interquartile range) [molar concentration] 43.9 (36.9-52.4) pg ml$^{-1}$ [4.2 x 10$^{-8}$ M] compared to maximum levels measured by Millar et al. in asthmatic patients of 56 (12-109) pg ml$^{-1}$ [5.4 x 10$^{-8}$ M] during acute severe asthma (1). Therefore, the levels achieved by the infusion of angiotensin II are physiologically relevant.

The rise in systolic and diastolic blood pressure during the 1 and 2 ng kg$^{-1}$ min$^{-1}$ angiotensin II infusions confirms its physiological effect. The plasma levels of angiotensin II measured during this study were similar to those found in the previous study by Millar et al. (7), where angiotensin II potentiated methacholine-induced bronchoconstriction.

As there was no significant effect on spirometry following 30 min of the infusion on each study day, it can be concluded that the dose of angiotensin II administered did not cause bronchoconstriction directly.

The reason for angiotensin II potentiating methacholine-induced bronchoconstriction (7) but not histamine-induced bronchoconstriction is undoubtedly complex and may be multi-factorial. The two studies have heterogeneous groups of subjects although four subjects took part in both studies. The current study group were older [mean (sd) age 41 (3-3) years compared to 27 (8) years in the study by Millar et al. (7)], but both groups were mild asthmatics with mean (sd) FEV$_1$ 88 (11) % predicted in the current study and 82 (9) % predicted in the study by Millar et al. (7). Bronchial reactivity during placebo infusion was similar in both groups with geometric mean (range) PC$_{20}$ histamine 2.67 (0.89-9.57) mg ml$^{-1}$ in the current study and PC$_{20}$ methacholine 3.09 (1.15-6.0) mg ml$^{-1}$ in the study by Millar et al. (7). Therefore, it is unlikely that variation in patient selection between these two studies has accounted for the different effect of angiotensin II on histamine- and methacholine-induced bronchoconstriction in vivo.

It is possible that, in asthmatics with greater bronchial reactivity the effect of angiotensin II on histamine-induced bronchoconstriction may be different due to more local inflammation, the presence of higher levels of mediators or a greater volume of airway smooth muscle. However, it is established that in mild asthmatics angiotensin II can potentiate the effect of methacholine, thus it appears that there is a fundamental difference between the interaction of angiotensin II with methacholine and histamine.

The lack of interaction between angiotensin II and histamine in the human airway could be explained by the absence of functional angiotensin II receptors in the bronchi. However, although no direct evidence exists demonstrating tissue-specific angiotensin II receptors in the lungs, Curnow et al. (14) have extracted type-1 angiotensin II (AT$_1$) receptor mRNA from human lung tissue. Both type-1 (15) and type-2 (16) angiotensin II receptor mRNA has also been identified in foetal rat lung.

Studies on bronchial reactivity in vitro in bovine bronchial rings have shown an AT$_1$ receptor-mediated potentiating effect of angiotensin II on endothelin-1-evoked bronchoconstriction (8). Although the mild bronchoconstrictor effect of angiotensin II on asthmatic airways in vivo (1) and its potentiating effect on methacholine-induced bronchoconstriction in human bronchi both in vitro and in vivo (7) have not been proven to be receptor-specific phenomena, it is likely that functional angiotensin II receptors do exist in the human airway.

The reason for the lack of potentiating effect of angiotensin II in relation to histamine may relate to differing second messenger intracellular pathways and varied cross-talk between pathways. The effect of angiotensin II on intracellular pathways in airway smooth muscle remains to be characterized. However, in vascular tissue, the type-1 angiotensin II receptor is coupled to a G-protein activating phospholipase-C and liberating diacylglycerol (DAG) and inositol.
triphosphate (IP₃), resulting in a rise in intracellular calcium concentration (17). In some tissues, the angiotensin II receptor is also coupled to an inhibitory G-protein which inhibits adenylate cyclase (18).

Histamine exerts its effects on airway smooth muscle via the H₁ receptor which is also coupled to a G-protein activating phospholipase C and liberating DAG and IP₃ (19). The increase in IP₃ is inhibited by cyclic adenosine-monophosphate (cAMP), attenuating the histamine-induced increase in intracellular calcium in canine airway smooth muscle (19).

Cholinergic muscarine receptors in the airways are also coupled to membrane phospholipid hydrolysis to form IP₃ but this occurs by a different pathway which is insensitive to cAMP (19).

Therefore, cross-talk at the second messenger level between angiotensin II intracellular pathways and histamine and acetyl choline/methacholine second messenger pathways could have different end results.

The final site of potential interaction is at the receptor level itself. Angiotensin II uncovers previously silent α₂-adrenoceptors in the rabbit distal saphenous artery (5) and if a similar interaction occurred between receptors for different spasmogens in airway smooth muscle this could lead to potentiation.

In conclusion, the role of the renin-angiotensin system in asthma is still incompletely understood. It seems that angiotensin II interacts diversely with different bronchoconstrictors potentiating the effects of methacholine and endothelin-1 but not histamine in the airway.

Acknowledgements

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References


