Hydrocortisone abolishes the angiotensin II-mediated potentiation of endothelin-1 in bovine bronchi

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ABSTRACT

Angiotensin II potentiates methacholine-evoked bronchoconstriction both in bovine airways in vitro and in asthmatic patients in vivo. Angiotensin II also potentiates endothelin-1-evoked contractions in vitro, but fails to alter such contractions in vivo. One possible confounding factor in patients is their use of inhaled corticosteroids. Accordingly, the present study examined the effects of hydrocortisone (cortisol) on contractions evoked by methacholine and endothelin-1 in the presence and absence of angiotensin II. Contractions of rings of isolated bovine airways were measured isometrically in organ baths. Concentration–response curves were obtained for endothelin-1 or methacholine in the presence and absence of angiotensin II, hydrocortisone and a combination of angiotensin II and hydrocortisone. Hydrocortisone abolished the angiotensin II-mediated potentiation of endothelin-1-evoked, but not methacholine-evoked, contractions. Hydrocortisone alone evoked the enhancement of methacholine responses, similar to the effect produced by angiotensin II. While species differences may exist, our present results suggest that the use of corticosteroids can have a profound effect on the interaction between angiotensin II and endothelin-1. Accordingly, the presence of inhaled corticosteroids might explain the differences between the results obtained in vitro and in vivo.

INTRODUCTION

The octapeptide hormone angiotensin II is a weak bronchoconstrictor in isolated human [1] and bovine [2] bronchial rings, and also in human asthmatic subjects when given by infusion [3]. In addition, angiotensin II can exaggerate the contractions evoked by other agents present in the airways. For instance, in human and bovine airways, angiotensin II potentiates both methacholine-induced and endothelin-1-induced contractions in vitro [4]. In addition, infusion of angiotensin II in patients with mild asthma likewise potentiated the bronchoconstriction evoked by methacholine [1]. In contrast, Chalmers et al. [5] were unable to demonstrate any potentiating effect of angiotensin II on endothelin-1-induced bronchoconstrictions in vivo. It was unclear why there was a difference between the in vivo and in vitro results.

During their study, Chalmers et al. [5] excluded patients with a recent history of oral corticosteroid use. It seemed likely, however, that some of the patients involved in the study would have continued to use inhaled corticosteroids, and that this might have had a bearing on the results. Corticosteroids are commonly used in asthma therapy for their anti-inflammatory effects. Accordingly, the role of corticosteroids in the angiotensin II-mediated potentiation of both endothelin-1- and methacholine-evoked contractions was investigated in bovine airways in vitro. These agents are important in the airways, since endothelin-1 is a potent

Key words: angiotensin II, bovine airway, hydrocortisone (cortisol), endothelin-1, methacholine.
Abbreviations: $pD_{25}$, $-\log EC_{50}$; $EC_{200mg}$, concentration effecting a contraction of 200 mg wt.
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bronchoconstrictor which is present at elevated levels in the airways of patients with asthma [6], while methacholine is a poorly hydrolysable analogue of acetylcholine, the principal neurotransmitter released from parasympathetic nerves.

MATERIALS AND METHODS

Tissue collection and preparation
Bovine bronchial tissues were obtained from cattle within 30 min of slaughter. Tissues were dissected free of connective tissue and fat in oxygenated Krebs/Henseleit solution of the following composition (mM): NaCl 118.4, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, NaHCO$_3$ 24.9, KH$_2$PO$_4$ 1.2 and glucose 11.1.

Measurement of contractile responses
Contractile responses were measured isometrically from rings of bronchi (3–5 mm external diameter) in vertical organ baths (10 ml) at 37 ± 0.5 °C in oxygenated (95% O$_2$/5% CO$_2$) Krebs/Henseleit solution. Tension (2 g wt.) was applied via two stainless steel wires inserted into the lumen. One wire was anchored and the other was attached to a force displacement transducer (Grass FT03T). Cumulative concentration–response curves were carried out for angiotensin II (10 nM–10 μM) to ascertain the threshold for contraction, in the presence and absence of hydrocortisone (cortisol) (1 μM). In subsequent experiments, cumulative concentration–response curves were constructed for endothelin-1 (0.1 nM–0.3 μM) or methacholine (10 nM–30 mM) in the presence and absence of angiotensin II (3 μM). Additional curves were also constructed for endothelin-1 or methacholine in the presence of hydrocortisone (1 μM) or a combination of angiotensin II (3 μM) and hydrocortisone (1 μM). Drugs were added directly to the organ bath. Hydrocortisone was incubated with the bronchial tissue for 2.5 h and angiotensin II for 15 min prior to the concentration–response curves.

Materials
The following chemicals were used: acetyl-β-methylcholine chloride (methacholine; Sigma), human angiotensin II acetate (Sigma), endothelin-1 (Novabiochem) and hydrocortisone (Sigma). Stock solutions were prepared in distilled water, with the exception of hydrocortisone, which was prepared in ethanol. Where ethanol was used as a solvent, control concentration–response curves contained an equivalent concentration of ethanol (0.001%).

Analysis of results
Maximum responses to endothelin-1 could not be obtained, and hence pD$_2$ ($-\log{EC_{50}}$) values could not be calculated. Accordingly, endothelin-1 results are expressed in terms of the negative log of the EC$_{200mg}$ (the concentration effecting a 200 mg wt. contraction). This system of expressing results is similar to that used previously [7,8]. Enhanced responses at 0.3 μM endothelin-1 are expressed as mean percentage increase above the control contraction.

Methacholine results are expressed as a percentage of the control maximum response and, where appropriate, the pD$_2$. Enhanced maximum responses are expressed as mean percentage increase above the control contraction.

All results are expressed as means ± S.E.M. Statistical significance between data sets was tested by two-way ANOVA. Significance between single points was calculated by Dunnett’s post test. A probability level of $P < 0.05$ was considered significant. The number of observations ($n$) refers to the number of animals used.

RESULTS

Angiotensin II
Angiotensin II (10 nM–10 μM) produced small contractions in bovine bronchi, which were not concentration-dependent. Pre-incubation with 3 μM angiotensin II produced a small contraction in only one out of six tissues, and in those cases the level of contraction was

![Figure 1](image_url) Cumulative concentration–response curves evoked by endothelin-1 alone and in the presence of angiotensin II and/or hydrocortisone

- ■, Endothelin-1 alone (0.1 nM–0.3 μM); □, + angiotensin II (3 μM); ○, + hydrocortisone (1 μM); ●, + angiotensin II and hydrocortisone. Values are means ± S.E.M. ($n = 8$ in each case). Potentiation by angiotensin II of endothelin-1-mediated contractions ($P < 0.01$) was abolished by hydrocortisone. Hydrocortisone did not alter the contractions evoked by endothelin-1 in the absence of angiotensin II.
Effects of hydrocortisone on angiotensin II and endothelin-1 in bovine bronchi

Values are given for endothelin-1 alone (control) and in the presence of angiotensin II (3 μM), hydrocortisone (1 μM) and a combination of angiotensin II (3 μM) and hydrocortisone (1 μM) (n = 8 in each case). Statistical comparisons between control values for endothelin-1 and those in the presence of angiotensin II alone or in combination with hydrocortisone were calculated by Student’s t-test: **P < 0.01.

Table 1  Effects of angiotensin II and hydrocortisone on contractions evoked by endothelin-1 in bovine bronchi

<table>
<thead>
<tr>
<th>Additions</th>
<th>-log EC_{50} (mg wt.)</th>
<th>(% increase above control contraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1 controls</td>
<td>7.5 ± 0.2</td>
<td>513 ± 54</td>
</tr>
<tr>
<td>Endothelin-1 + angiotensin II</td>
<td>7.8 ± 0.3</td>
<td>994 ± 144**</td>
</tr>
<tr>
<td>Endothelin-1 + angiotensin II + hydrocortisone</td>
<td>7.2 ± 0.3</td>
<td>456 ± 100</td>
</tr>
<tr>
<td>Endothelin-1 + hydrocortisone</td>
<td>7.2 ± 0.4</td>
<td>413 ± 84</td>
</tr>
</tbody>
</table>

less than 10 mg wt. The short-term presence of hydrocortisone (1 μM) did not significantly alter contractions evoked by angiotensin II (results not shown).

**Endothelin-1**

Endothelin-1 (0.1 nM–0.3 μM) evoked concentration-dependent contractions of bovine bronchi (Figure 1). Contraction was initiated between 1 and 10 nM. In the presence of 3 μM angiotensin II, the responses to endothelin-1 were significantly enhanced (P < 0.01) compared with those in its absence (Figure 1), with a mean increase of 93.8% at 0.3 μM endothelin-1 (Table 1). The potentiation of the endothelin-1 response by angiotensin II was abolished by the additional presence of 1 μM hydrocortisone (Figure 1). When tissues were pre-incubated with 1 μM hydrocortisone alone, there was no significant change in the responses evoked by endothelin-1 in comparison with those in the absence of hydrocortisone (Figure 1).

**Methacholine**

Methacholine (10 nM–30 mM) evoked concentration-dependent contractions of bovine bronchi, and contraction was initiated between 3 μM and 30 μM (Figure 2). The contractions produced by methacholine were significantly potentiated (P < 0.001) by pre-incubation with 3 μM angiotensin II (Figure 2), with a mean increase of 49.5% in the maximum response (Table 2). The presence of the corticosteroid, hydrocortisone, also significantly enhanced (P < 0.001) the contractions evoked by methacholine (Figure 2), with a mean increase of 36.0% in the maximum response (Table 2). When tissues were incubated with a combination of 1 μM hydrocortisone and 3 μM angiotensin II, the methacholine-evoked contractions were significantly enhanced (P < 0.001) in comparison with the control (Figure 2), with a mean increase of 41.6% in the maximum response (Table 2).

There were no significant differences between the enhancement of methacholine-mediated contractions evoked by angiotensin II alone, hydrocortisone alone or a combination of these two agents.

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DISCUSSION

Angiotensin II has very low potency in bovine bronchi, producing small contractions, which, although reproducible, are not concentration-dependent. This is an unusual finding, since angiotensin II produces concentration-dependent contractions in a wide variety of tissues, including human airway [1]. These results may reflect the presence of more than one receptor type in bovine tissue. Angiotensin II at sub-threshold concentrations did, however, markedly enhance contractions evoked by methacholine and endothelin-1. This is in keeping with previous in vitro results reported in human [1] and bovine [2] bronchi. We have also shown previously that this does not reflect a non-selective potentiation of all contractions, since the action of histamine is unaffected by the presence of angiotensin II in human airway both in vivo and in vitro [9]. Such interactions may be of particular importance in asthmatic patients, since levels of both angiotensin II [3] and endothelin-1 [6] are raised in patients with acute severe asthma. In addition, angiotensin II can potentiate vagally mediated contractions in rabbit airways by pre-junctional enhancement of release [10]. This action is likely to occur in addition to post-junctional enhancement of contraction.

The short-term presence of hydrocortisone did not alter the small contractions evoked by angiotensin II in bovine airway. This finding is in contrast with the results of other studies; for example, corticosteroids potentiated the action of angiotensin II in rat aorta [11] and evoked synergy between adrenal steroids and angiotensin II in a liver epithelial cell line [12].

We have previously described the ability of antagonists of either 5-lipoxygenase or cyclo-oxygenase to abolish the interaction between endothelin-1 and angiotensin II in bovine bronchi, suggesting a role for eicosanoids in this interaction [13,14]. The presence of the corticosteroid hydrocortisone, over a relatively short period, abolished the angiotensin II-potentiated endothelin-1-induced contractions, while having no effect on the contractions evoked by endothelin-1 alone. The present results suggest that hydrocortisone abolishes the production of eicosanoids evoked in response to angiotensin II. This is in agreement with previous work, which suggested that corticosteroids inhibit eicosanoid synthesis [15].

Interestingly, when angiotensin II potentiates methacholine-evoked contractions, neither 5-lipoxygenase nor cyclo-oxygenase inhibitors abolish the angiotensin II-mediated effect (C. M. Pitt and J. E. Nally, unpublished work), suggesting a different mechanism of interaction between methacholine and hydrocortisone than that observed between endothelin-1 and hydrocortisone. It is unclear from the present study whether hydrocortisone alters the angiotensin II-induced potentiation of the methacholine response, due to the direct potentiating effect of hydrocortisone itself.

The potentiating action of methacholine by hydrocortisone in bovine tissue is perhaps surprising, since corticosteroids are routinely used to inhibit airway responsiveness in vivo. It is also in contrast with other studies that have shown corticosteroids reducing the contractions evoked by methacholine in rat airway [16], while having no effect on pulmonary resistance or dynamic compliance in dogs [17]. This may simply reflect a species difference; however, corticosteroids have been described as inducing a paradoxical early bronchoconstriction in a number of asthmatic patients [18].

The mechanism by which interaction occurs between angiotensin II and methacholine or endothelin-1 is not yet clear. A number of possibilities exist, including receptor–receptor interactions and synergy at the intracellular level. We have demonstrated that endothelin-1 [8,19], methacholine [20] and histamine (J. E. Nally, N. C. Thomson and M. J. O. Wakelam, unpublished work) each evoke hydrolysis of phosphatidylinositol 4,5-bisphosphate, and yet there are clear differences between the effects of angiotensin II on the action of each of these agonists, suggesting that the interaction does not occur at

### Table 2  Effects of angiotensin II and hydrocortisone on contractions evoked by methacholine in bovine bronchi

<table>
<thead>
<tr>
<th>Additions</th>
<th>$pD_2$</th>
<th>Mean maximum response (mg wt.)</th>
<th>(% increase above control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacholine controls</td>
<td>4.4 ± 0.1</td>
<td>2690 ± 430</td>
<td>-</td>
</tr>
<tr>
<td>Methacholine + angiotensin II</td>
<td>4.9 ± 0.1</td>
<td>4020 ± 480*</td>
<td>49.5</td>
</tr>
<tr>
<td>Methacholine + hydrocortisone</td>
<td>4.7 ± 0.2</td>
<td>3810 ± 600‡</td>
<td>41.6</td>
</tr>
<tr>
<td>Methacholine + angiotensin II + hydrocortisone</td>
<td>4.6 ± 0.2</td>
<td>3660 ± 340*</td>
<td>36.0</td>
</tr>
</tbody>
</table>
this level. In addition, we have shown that non-selective depolarization by KCl does not evoke any potentiation of endothelin-1-evoked contractions [19]. Receptor–receptor interactions remain a possibility, and such interactions have been postulated between angiotensin II and α-adrenoceptors [21]. This might explain the different interactions of angiotensin II and various spasmogens, as well as the inability of other agonists, such as U46619 [19] or histamine (J. E. Nally, N. C. Thomson, M. J. O. Wakelam and J. C. McGrath, unpublished work), to mimic angiotensin II.

While the present study needs to be repeated in human tissue, bovine airway has proved to be qualitatively similar to human bronchi in the past, and thus to represent a good in vitro model.

In conclusion, the presence of a corticosteroid can prevent the potentiation of the action of endothelin-1 by angiotensin II in bovine bronchi in vitro. Based on the suggestion that several of the patients in the in vivo study [5] were using inhaled corticosteroids, our present results may explain the discrepancy between the interactions of endothelin-1 with angiotensin II in vitro and in vivo, i.e. the use of corticosteroids would profoundly attenuate the angiotensin II–endothelin-1 interaction in vivo. We are currently repeating these experiments with beclomethasone, a corticosteroid more commonly used in inhaled corticosteroid therapy in asthmatic patients.

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REFERENCES


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