The interaction of acetylcholine and histamine on human bronchial smooth muscle contraction


ABSTRACT: The interaction of histamine (Hist) and acetylcholine (ACh) on human isolated bronchial smooth muscle (HIBSM) contraction, and the influence of the epithelium, was assessed using HIBSM obtained from 15 patients undergoing thoracotomy. Cumulative concentration effect curves for ACh and Hist, together with combinations of equipotent concentrations of both agonists, were generated using both epithelium-intact and epithelium-denuded HIBSM.

In epithelium-denuded HIBSM both ACh (p<0.05) and Hist (p<0.005) produced a significantly enhanced maximal response and a 2.1 fold increase in the potency of ACh (p<0.02, n=13). When ACh and Hist were added simultaneously, in equipotent concentrations, to epithelium-intact HIBSM, a significantly less (p<0.0005, n=13) than additive response occurred with only 60% of the predicted maximum response being observed. However, following epithelium removal, an additive interaction between the two agonists (n=8) occurred.

Using HIBSM from five of the original 15 patients, similar experiments were performed to determine the influence of the muscarinic receptor antagonist atropine (0.1 µM) and the H1 receptor antagonist mepyramine (10 µM). Both resulted in a significantly less than additive interaction (40-50% of predicted tensions). Similar experiments were also performed in the presence of the cyclo-oxygenase inhibitor indomethacin (5 µM) and these failed to reverse the inhibition observed in HIBSM contraction (n=5).

The inhibitory interaction between ACh and Hist appears to be epithelium dependent and is not mediated via the release of prostanoids. Thus, there appears to be a complex interaction between contractile agonists and the epithelium, which is not just a simple summation of the activation of individual receptors on HIBSM.


Airway smooth muscle contraction is recognized as a central component of the asthmatic response. A variety of endogenous agents have been shown to cause both bronchoconstriction in vivo and contraction of airway smooth muscle in vitro and include histamine (Hist), acetylcholine (ACh), prostaglandins, leukotrienes and tachykinins [1-3]. These mediators are present in a variety of cells resident in airways and are liberated after allergen-immunoglobulin E (IgE) interaction or neural stimulation [4]. The bronchoconstrictor response to inhaled agents such as histamine is characteristically greater in asthmatic patients than in non-asthmatic subjects [4]. The precise cause of this bronchial hyperreactivity or enhanced smooth muscle contraction is unknown. A variety of individual mediators are known to be released simultaneously following allergen challenge and it seems unlikely that an increased sensitivity to any one bronchoconstricting agonist is the primary cause. It is more likely that airway oedema, epithelial loss and/or the effect of mediator interaction contribute to the overall hyperreactiveness of the airway.

Studies of the role of the epithelium and of mediator interactions on airway smooth muscle responsiveness in man have been limited. Assessments of mediator interaction have primarily involved in vivo investigations using normal or asthmatic volunteers. These studies have assessed various interactions of histamine, histamine, leukotrienes and prostaglandins [5-11]. Similar studies have been performed in animal models both in vivo [12] and in vitro [13]. However, in vitro studies of the interaction between putative asthmatic mediators on human airway smooth muscle responses are lacking. Thus, the effect of the
concomitant addition of Hist and ACh on isolated human bronchi was investigated and the influence of the epithelium on this response assessed.

Methods

Human bronchi were obtained from lungs resected from 15 patients undergoing pneumonecctomy or lobectomy for the treatment of lung carcinoma. Macroscopically normal bronchi (4-10 mm ID) remote from the site of tumour were removed within 20 min of resection and placed in ice-cold Krebs-Henseleit solution previously aerated with 5% CO₂ in O₂ and of the following composition (mM) NaCl 118, KCl 5.4, NaHCO₃ 25, KH₂PO₄ 1.1, MgSO₄ 0.57, D-glucose 11.1, CaCl₂ 2.5. The bronchi were dissected free of all visible connective tissue and blood vessels and either used on the day of resection or stored at 4°C in Krebs-Henseleit solution for a maximum of 40 h. Bronchi were cut longitudinally along the cartilage plates and the resulting open segments (3-4 mm wide) of human isolated bronchial smooth muscle (HIBSM) were suspended in organ baths under 1 g tension in Krebs-Henseleit solution.

Preparations were allowed to equilibrate (60-90 min) under tension and the bath fluid exchanged every 15 min. Changes in isometric tension were measured using a Grass FT03C force displacement transducer and a Rikadenki L50 chart recorder. Any changes in resting tone were readjusted to a tension to 1 g. Following equilibration, all HIBSM strips were exposed to sub-maximal doses of ACh (10⁻⁵ M, final bath concentration) to determine the magnitude and reproducibility of contraction. In order to ensure that the mechanical properties of the muscle strips did not alter during the course of the experiments, length-tension relationships were also assessed at various time intervals, by determining the response to a maximal concentration of ACh using tensions of 0.5, 1.0 and 2.0 g.

Epithelium-intact experiments

HIBSM strips from 13 of the 15 patients were used. Independent cumulative concentration-effect curves (CCEC) were constructed for both Hist and ACh by the cumulative addition of the specific agonist (range 10⁻⁷ - 3x10⁻³ M) to the organ bath until a maximum affect (Emax) was obtained. Individual Emax values were determined when responses to two sequential doses of agonist were within 5% of each other. Hist and ACh were added so as to produce successive 1/2 log increases in agonist concentration. The initial agonist used to generate the first CCEC was randomly determined and the muscle strip was then allowed to return to baseline and re-equilibrate before generating CCECs with the second agonist.

Using the data obtained from each of the single agonist CCEC, the concentrations of each agonist required to produce the 10, 20, 30, 40 and 50% of Emax (EC₁₀⁻ - EC₉₀) were calculated. From these data, the individual equipotent concentrations (EC₁₀⁻ - EC₉₀) of ACh and Hist were tabulated and their responses summated to produce a predicted additive CCEC. After completing the single agonist CCECs, the same muscle strip was washed and allowed to return to baseline tension and then cumulatively stimulated with combined equipotent concentrations of ACh and Hist.

The above experiments were then repeated on HIBSM from five of the 15 patients but with the muscarinic antagonist atropine (0.1 μM), the H₁ receptor antagonist mepyramine (10 μM) or the cyclooxygenase inhibitor indomethacin (5 μM) being added to the organ baths 30 min prior to the combined addition of ACh and Hist.

Epithelium removed experiments

A similar set of experiments to those describe above were performed in parallel, using adjacent muscle strips from eight of the 15 subjects. In these experiments the epithelium was removed prior to any stimulation. This was achieved by gently rubbing the mucosal surface with a moist cotton swab. Verification of successful epithelium removal was obtained by light and scanning electron microscopy.

Analysis and statistics

At the end of the experiments, the strips were weighed and their length measured for determination of cross-sectional area and wet weight. Results were expressed as the active tension developed in g/g wet weight of tissue.

The predicted CCEC data and the experimentally generated CCEC were compared to determine whether interactions between ACh and Hist were supra-additive, additive or inhibitory. The mean (±SEM) tensions were calculated for each datum point and the statistical significance of differences between experimental and predicted values determined using two-way analysis of variance and Student’s paired and unpaired t-test. Data from the single agonist curves were also used to determine Emax and the pD₂ (-log EC₉₀) values. These were compared using one way analysis of variance and subsequent Student’s paired t-test.

Drugs and solutions

The following drugs were used: histamine diphosphate (British Drug Houses, Sydney, Australia); acetylcholine chloride, atropine sulphate, mepyramine maleate and indomethacin (Sigma Chemical Company Ltd, St Louis, USA).

Stock solutions of ACh, Hist, atropine and mepyramine were prepared by dissolving the reagents in distilled water. Solutions were stored in 2 ml aliquots at -20°C and used as required. For each experiment a stock solution of indomethacin was
prepared in 5% (w/v) Na$_2$CO$_3$ and Krebs-Henseleit solution was used for subsequent dilutions.

### Results

**Single agonist CCEC**

The CCEC for Hist was displaced to the left, relative to ACh, resulting in the pD$_2$ for Hist being significantly greater than ACh (p<0.05, n=13; table 1). Although ACh induced a greater maximal response (Emax) than Hist, the difference between the two values was not statistically different (p<0.375; table 1).

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Acetylcholine</th>
<th>Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>(+) Epi</td>
<td>(+) Epi</td>
</tr>
<tr>
<td>pD$_2$</td>
<td>4.46±0.1</td>
<td>5.16±0.1*</td>
</tr>
<tr>
<td>Emax</td>
<td>9.86±2.1</td>
<td>16.15±2.8*</td>
</tr>
<tr>
<td>g/unit tissue</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

The mean±SEM values for both pD$_2$ (-log EC$_{50}$) and Emax following cumulative addition of acetylcholine and histamine to isolated epithelium-intact and epithelium-denuded human bronchial smooth muscle. *: p<0.02 (epithelium intact compared to epithelium denuded preparations); **: p<0.005 (epithelium intact compared to epithelium denuded preparations); *: p<0.01 (Hist pD$_2$ compared to ACh pD$_2$).

Mechanical removal of the epithelium enhanced Emax for both ACh and Hist when compared to responses obtained from epithelium-intact preparations (p<0.005; table 1, fig. 1A and 1B). Removal of the epithelium also resulted in increases in pD$_2$ for both ACh and Hist although this was significant only for ACh (p<0.02; table 1).

**Combined agonist data**

The mean predicted additive CCEC and the subsequent experimentally generated CCEC data for epithelium intact preparations are shown in figure 2a. The combined addition of ACh and Hist resulted in a significantly inhibited response when compared to that predicted for an additive interaction. The apparent degree of inhibition was between 40–50% of the predicted response at each dosage increment (fig. 2A).

In contrast to these results, HBISM strips devoid of epithelium demonstrated significantly greater responsiveness to the combined addition of ACh and Hist than did the adjacent epithelium intact preparations (fig. 2B). As a consequence there was no statistical difference between predicted additive values and experimentally generated data for the epithelium removed preparations.

**The effect of selective antagonists**

The muscarinic antagonist, atropine (0.1 µM), had little effect on the response to the combined addition of ACh and Hist with there being a significantly reduced contraction, equating to approximately 40% of the predicted additive values (p<0.005 for Emax, n=5; table 2). Similarly, the addition of the H$_1$ receptor antagonist mepyramine (10 µM) failed to enhance the responses induced by combined addition of ACh and Hist with the responsiveness (Emax) not exceeding 45% of the predicted values (p<0.005; table 2).

**The effect of indomethacin**

Exposure of muscle strips to indomethacin (5 µM) failed to modify the inhibitory interaction between ACh and Hist. The magnitude of the responses obtained in the presence of this antagonist was approximately 50% of the predicted values for an additive interaction (p<0.01 for Emax, n=5; table 2).

**Control data**

In all of the experiments length-tension relationships were preserved. Similarly, responses to a maximal dose of ACh at the commencement and completion of each experiment were not statistically significantly different, suggesting that the results obtained were not influenced by alterations in muscle responsiveness during the course of the experiment. Histological
Fig. 1. A) The mean tension (±SEM) generated following cumulative addition of acetylcholine (ACh) to epithelium-intact (n=13) (■) and epithelium-denuded (n=8) (▲) human isolated bronchial smooth muscle. B) The mean tension (±SEM) generated following cumulative addition of histamine (Hist) to epithelium-intact (n=13) (■) and epithelium-denuded (n=8) (▲) human isolated bronchial smooth muscle.

Fig. 2. A) The mean tension (±SEM) generated following cumulative addition of equipotent concentrations (EC10-60) of acetylcholine and histamine in epithelium-intact, isolated human bronchial smooth muscle strips (■) compared to predicted curve (○) (n=13). *: p<0.05; **: p<0.01; ***: p<0.005. B) The mean tension (±SEM) generated following cumulative addition of equipotent concentrations (EC10-60) of acetylcholine and histamine in epithelium-denuded isolated human bronchial smooth muscle strips (▲) compared to predicted curve (○) (n=8).

Table 2. The observed mean (±SEM) response in epithelium intact preparations, obtained for the cumulative addition of equipotent concentrations (EC10-60) of acetylcholine and histamine in the presence of selective antagonists.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>EC value</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>72±17*</td>
<td>74±11*</td>
<td>66±7*</td>
<td>61±6**</td>
<td>57±4***</td>
</tr>
<tr>
<td>Atropine</td>
<td>n=5</td>
<td>28±15*</td>
<td>35±15**</td>
<td>36±15***</td>
<td>35±15***</td>
<td>36±12***</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>n=5</td>
<td>31±18*</td>
<td>27±10**</td>
<td>27±9***</td>
<td>41±8***</td>
<td>44±9***</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>n=5</td>
<td>45±16*</td>
<td>57±19*</td>
<td>58±14*</td>
<td>55±10*</td>
<td>57±9**</td>
</tr>
</tbody>
</table>

Data are expressed as a percentage of the predicted response for an additive interaction. Atropine (0.1 μM); mepyramine (10 μM); indomethacin (5 μM). *: p<0.05; **: p<0.01; ***: p<0.005 compared to predicted value unpaired t-test.
assessment of bronchial strips following experimentation revealed complete removal of epithelial cell layer, without obvious damage to underlying muscle.

**Discussion**

The combined addition of ACh and Hist to HIBSM bronchial smooth muscle strips resulted in a 40% reduction of expected smooth muscle contraction. In contrast, when the epithelium was removed, the combined effect of ACh and Hist on muscle contraction was essentially additive. These results suggest an interaction between Hist, ACh and the epithelium which results in the inhibition of smooth muscle contraction, although the underlying mechanism(s) is unclear.

Previous investigations of the interactive effects of ACh and Hist on bronchial smooth muscle contraction have been of varying design and results. In animals, in vivo studies in the guinea-pig and the dog [12, 14] have demonstrated synergism between Hist and methacholine. However, in the guinea-pig isolated trachea, graded doses of Hist did not enhance the CCEC to carbachol and indeed, as with the results reported here, at higher Hist concentrations there was a significant reduction in the potency of carbachol [15]. In man, in vivo studies have been limited and conflicting. MITCHELL and BOHURIS [10] reported an additive effect on forced expiratory volume in one second (FEV1) when non-asthmatic patients were challenged with single concentrations of Hist and ACh, and STEK et al. [11] reached a similar conclusion by demonstrating that Hist produced a small but additional contractile response following a maximum response to inhaled methacholine.

In vivo studies are difficult to interpret since a variety of tissue responses enhance airway obstruction and, thus, Hist and ACh as well as causing smooth muscle contraction can stimulate mucus production and airway wall oedema [16]. More importantly, the geometry of the airway is such that small changes in airway radius will have substantial effects on resistance to flow [17]. Therefore, a small dose of one agonist may appear to sensitize the airway to the effect of another, suggesting apparent pharmacological synergism. Equally, if the concentrations of agonist are chosen because of similar weight or molar concentration, it is difficult to interpret the results as each agonist may be acting at a different position on their respective CCEC. Ideally, similarly effective concentrations of each agonist should be compared to determine if true synergy exists.

In this study, it seems unlikely that the responses obtained were a reflection of de novo alteration in muscle responsiveness or fatigue. All strips could be maximally contracted by ACh at the conclusion of the experiment and this response was not statistically significantly different from the original responses to ACh. Additionally, although the experiments involved the same muscle strips being studied over 36 h, previous studies from our laboratory [18] and from others [19] have shown storage for this period of time does not alter tissue responsiveness. Similarly, removal of the epithelium did not appear to alter length-tension relationships.

Hist is currently thought to act via three receptor subtypes, H1, H2, and H3 [16]. H1 receptors cause bronchoconstriction in man and although H2 and H3 receptors have been demonstrated in lung parenchyma and airways, their role is unclear [20]. ACh may act on autonomic ganglia as well as directly via muscle receptors. Nine different muscarinic receptor subtypes have been identified but only three (M1–M3) have been described in human airways. M1 receptors are found on human airway smooth muscle and submucosal glands, whilst the presence of muscarinic receptors on human airway epithelium has yet to be confirmed [21, 22].

Despite the knowledge of different receptor subtypes, the relative effect of co-stimulating these receptors and the role of the epithelium is unknown. To determine the relative contributions of each agonist, the selective receptor antagonists atropine and mepyramine were employed to block ACh and Hist responses, respectively. The apparent contribution made by ACh in the presence of mepyramine, was approximately 35% of predicted response. For Hist, in the presence of atropine, a similar result was obtained with the average response also being approximately 35% of the predicted additive response. This would suggest that both ACh and Hist are being equally inhibited when HBISM is co-exposed to both agonists and that this is abolished when the epithelium is removed. Recently, several investigators have postulated the presence of epithelially-derived inhibitory factor(s) (EpDif) which may be protective against hyperstimulation of airway smooth muscle [23–28]. The regulation of EpDif release, its nature and its mode of action have yet to be defined. However, an enhanced generation of EpDif occurring only in response to multiple agonist challenge could provide a possible explanation for the data discussed above.

In some animal species, cyclooxygenase metabolites are thought to be at least partially responsible for regulating muscle tone. Hist stimulation generates the release of a variety of prostanooids from the airways of a number of species, including man [28, 30]. In human airways, inhibitory prostaglandins such as PGF2α and PGI2 appear to be preferentially released both under basal and agonist-induced conditions [29]. In this study, the cyclooxygenase inhibitor indomethacin did not significantly reverse the inhibited response observed following the simultaneous addition of ACh and Hist, suggesting that under these conditions prostaglandins were not significantly involved in modulating airway smooth muscle responses. These data are compatible with the data from HAYE-LEGRAND et al. [30], who suggested that although human airways generate significant quantities of prostaglandins following Hist challenge, they are not involved in modifying subsequent responses to Hist.
In this study, the interaction of Hist, ACh and the epithelium produced an inhibitory effect with all three components being required. Whether this reflects the possibility that both ACh and Hist synergistically enhance the release of a non-prostanoid epithelially-derived inhibitory factor is uncertain. However, recent in vivo studies indicate that tachyphylaxis to repeated airway stimulation with Hist in mild asthmatic patients can occur [31] and, thus, it is also possible Hist may be causing a down-regulation of its own receptor, which is modulated via the epithelium and thereby contributes to an apparent reduction in additive response.

Since asthmatic patients demonstrate increased sensitivity to inhaled stimuli when compared to normal subjects, and since epithelial damage and desquamation are histological features frequently seen in the asthmatic airway [32], the results from this study may be clinically significant. The difference between asthmatic patients and normal individuals may relate to the enhanced interactive responsiveness as seen with ACh and Hist when the epithelium is damaged, and it remains to be determined whether this is related to the postulated epitheliually-derived inhibitory factor(s). Nevertheless, it would appear that the regulation of smooth muscle contraction by epithelial factors and the interaction of contractile agonists is more complex than is currently accepted. Further investigation of the pathways and the mediators involved is required.

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References
28. Yen SS, Mathe AA, Dugan JJ. - Release of


RÉSUMÉ: L’interaction de l’histamine (Hist) et de l’acétylcholine (ACh) sur la contraction du muscle lisse bronchique humain (HIBSM) et l’influence de l’épithélium ont été appréciées en utilisant le muscle lisse bronchique isolé obtenu chez 15 patients ayant subi une thoracotomie. On a élaboré les courbes d’effets cumulatifs des concentrations pour ACh et Hist, en même temps que pour des combinaisons de concentrations équivalentes des deux agonistes, en utilisant à la fois du muscle lisse avec épithélium intact ou épithélium dénudé.

Dans le muscle lisse avec épithélium dénudé, tant l’ACh (p<0.05) que l’Hist (p<0.005) ont produit une réponse maximale significativement accrue et une augmentation de 2.1 fois dans la puissance de l’ACh (p<0.02, n=13). Quand l’ACh et l’Hist sont additionnées simultanément à des concentrations équivalentes au muscle lisse avec épithélium intact, une réponse significativement inférieure à la réponse additive (p<0.0005, n=13) est survenue: 60% seulement de la réponse maximale prédite étant observée. Toutefois, après ablation de l’épithélium une interaction additive des deux agonistes (n=8) survient.

En utilisant le HIBSM provenant de 5 des 15 patients originaux, des expériences similaires ont été conduites pour déterminer l’influence de l’antagoniste des récepteurs muscariniques (l’atropine: 0.1 µM) et de l’antagoniste des récepteurs H, (la mepyramine: 10 µM). Les deux produits ont entraîné une interaction significativement plus faible que l’interaction additive (40 à 50% seulement des tensions prévues). Des expériences similaires ont été également été conduites en présence de l’indomethacine (5 µM), inhibiteur de la cyclooxygénase, et celles-ci n’ont pas réussi à inverser l’inhibition observée dans la contraction de HIBSM (n=5). L’interaction inhibitrice entre ACh et Hist s’avère donc dépendante de l’épithélium et n’est pas modifiée par la libération de prostanoïdes. Donc, il semble qu’il existe une interaction complexe entre les agonistes constricteurs et l’épithélium, qui n’est pas une simple sommation de l’activation des récepteurs individuels de HIBSM.