Physiological Parameters Affecting the Modulatory Role of Airway Epithelium on Airway Smooth Muscle Responsiveness

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ABSTRACT

Numerous studies have revealed the significant action of airway epithelium as a non-specific defence mechanism in airways. In addition, epithelial cells release biologically active agents, which modulate airway tone. Importantly, airway epithelium function is influenced by physiological parameters, including the release of endogenous factors, age, gender, load, and bronchi size. The primary goal of this review is to summarize knowledge concerning the effect of the aforementioned parameters on the modulatory role of airway epithelium on airway smooth muscle responsiveness. These effects may be implicated in the pathophysiology of airway diseases like asthma and Chronic Obstructive Pulmonary Disease (COPD).

KEYWORDS: Airways; Epithelium; Nitric oxide.

INTRODUCTION

Respiratory epithelium belongs to the class of ciliated pseudostratified columnar epithelium due to the arrangement of the columnar epithelial cells. Airway epithelium functions as a barrier to potential pathogens and foreign objects and prevents infection by the action of the ciliary escalator. It acts as a non-specific upper airways defence mechanism, that entraps particles and other inhaled material in mucus, and transports them away from the lungs. Efficient mucociliary transport is the result of the co-ordination of three airway epithelial functions, i.e. mucus secretion, ciliary beat and ion and fluid transport. Another important function of airway epithelium is its ability to produce endogenous biologically active substances like Nitric Oxide (NO), prostanoids, and endothelin.1-3,4 It is also worth mentioning that the function of airway epithelium is influenced by physiological parameters, like age, gender, load, and bronchi size.

Airway Smooth Muscles (ASM), whether contracted or relaxed, affect airway diameter and thus air flow to alveoli where gas exchange occurs. Excessive responsiveness of ASM to contractile agents is often characteristic of chronic respiratory diseases, with asthma being a typical example. This over-responsiveness results in airway obstruction and decline of airway flow. Remarkably, a common finding in asthma is epithelium damage, inflammation and in some cases airway remodelling. Moreover, epidemiological data suggest that the incidence of asthma becomes higher in females than males with the onset of puberty, and that this tendency prevails throughout the reproductive years.5

These observations triggered research interest toward the modulatory role of airway epithelium on ASM, in connection with the action of NO, prostanoid, cholinergic agents, mediators of inflammation and growth factors, but also in connection with airway size, animal age or gender and the initial load applied on airway smooth muscle.

In the following paragraphs, we discuss the impact of the above factors on the modulatory role of airway epithelium on ASM, as well as the possible implications on the pathophy-

Endogenous factors like insulin, histamine or acetylcholine cause airway contraction. On the other hand, they act on epithelial cells and cause the release of biologically active mediators, especially NO from epithelial cells. Epithelium responds to stretch by modulating epithelial NO release and swelling of mucosa. Furthermore, NO release in an epithelium-dependent way relaxes precontracted ASM.

**Table 1**: The effects of different physiological parameters on the modulatory role of epithelium on Airway Smooth Muscle (ASM) and the possible implication of these effects in airway diseases.

<table>
<thead>
<tr>
<th><strong>Endogenous factors</strong></th>
<th><strong>Effect on epithelium</strong></th>
<th><strong>Possible implication in airway diseases</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Promotes the survival of epithelial cells</td>
<td>Respiratory system is considered an alternative route for insulin administration for the treatment of type 1 diabetes mellitus. Low incidence of asthma in patients with diabetes mellitus.</td>
</tr>
<tr>
<td>Histamine</td>
<td>Causes: H₂O₂ production from epithelial cells, mucus release and swelling of mucosa and NO release from epithelial cells</td>
<td>Possible contribution to the increased airways responsiveness observed in asthma due to epithelium damage and inflammation</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Stimulates NO release from epithelial cells</td>
<td>Possible contribution to the increased airways responsiveness observed in asthma due to epithelium damage and the increased acetylcholine release</td>
</tr>
<tr>
<td>Age</td>
<td>Affects the capacity of airway epithelium to produce NO</td>
<td>Epithelium-derived NO has an important role in the regulation of airway tone in early newborn life.</td>
</tr>
</tbody>
</table>
| Gender                 | In rabbit trachea testosterone relaxes precontracted ASM in an epithelium-dependent way | Gender differences in the incidence of asthma. Testosterone serum levels are depressed in patients with respiratory failure, 
| Airway size            | Variations in the distribution of acetylcholinesterase | Regional differences in ASM responsiveness to contractile agents. Non-homogeneous distribution of bronchoconstriction observed in COPD and asthma. |
| Load                   | Epithelium responds to stretch by modulating epithelial NO synthase activity, NO production and ASM responsiveness to acetylcholine at increased load | Loss of the protective effect of deep inspiration in asthmatics and patients with COPD. |

**ENDOGENOUS FACTORS: INSULIN, HISTAMINE, ACETYLCHOLINE**

Endogenous factors like insulin, histamine or acetylcholine cause airway contraction. The effects of histamine on ASM contraction is mediated via M3 muscarinic receptors. Histamine further affects airway epithelium as it promotes H₂O₂ production from epithelial cells of bronchi, mucus release and swelling of mucosa. In fact, the effect of histamine on ASM contraction is mediated via the release of biologically active molecules like NO from epithelial cells and thus, depends on epithelium integrity. Histamine stimulates endothelial cell H1 receptors by increasing epithelial NO synthase phosphorylation and activity.

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Acetylcholine, the neurotransmitter released from postganglionic parasympathetic vagus nerves, induces airways contraction via Ca²⁺ release from intracellular stores and Ca²⁺ entry from extracellular space. Additionally, acetylcholine is also released from epithelial cells and can promote the chemotaxis of monocytes and neutrophils. Acetylcholine, as well as other muscarinic agonists, may induce proliferation of ASM cells that
depends on the activity of the MAPK and PI3K pathways, either alone\textsuperscript{28} or in combination with growth factors.\textsuperscript{29} In vitro studies demonstrate that the airway epithelium stimulates the breakdown of acetylcholine.\textsuperscript{30} In addition, acetylcholine seems to stimulate NO release from the epithelium and its mechanical removal increases airway responsiveness to acetylcholine.\textsuperscript{31}

**AGE**

Animal studies suggest that increased age decreases airway responsiveness to contractile agents,\textsuperscript{32} and increases their capability to relax.\textsuperscript{33,34} These age-dependent alterations in airway responsiveness are attributed to changes in airway architecture and organization,\textsuperscript{35,36} to the maturation of the non-adrenergic non-cholinergic system\textsuperscript{37} and to increased activity of acetylcholinesterase.\textsuperscript{38}

Evidence suggests that age may affect the release of biologically active factors, mainly NO, from the airway epithelium. The three NO synthase isoforms are expressed in airways, but their levels remain unchanged during life.\textsuperscript{40} However, the capacity of airway epithelium to produce NO seems to increase with age. Namely, in rabbits (Figure 1, our unpublished results) and pigs\textsuperscript{39} age affects the acetylcholine-induced NO production. Specifically, contractility studies were performed on tracheal strips obtained from young (four weeks old) or adult (eight weeks old) rabbits in the presence of 10^{-9} to 10^{-3} M of acetylcholine. In adult rabbits epithelium damage increases acetylcholine-induced contractions.\textsuperscript{22} On the contrary, these experiments revealed that in young rabbits the mechanical removal of epithelium (Figure 1A, our unpublished results) as well as the treatment of preparations with the inhibitor of NO synthase NG-nitro-L-arginine methyl ester (L-NAME), the precursor of NO formation L-arginine or the inhibitor of cyclooxygenase indomethacin had no effect on ACh-induced contractions (Figure 1B, our unpublished results).

![Figure 1: The effect of epithelium removal (A) or the presence of NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), NO precursor, L-arginine and cyclooxygenase inhibitor, indomethacin (B) on acetylcholine (ACh)-induced contractions of tracheal strips obtained from young rabbits (<4 weeks).](image)

Data are means, vertical lines show SE. N refers to the number of animals studied.

Histological studies reveal that the expression of M\textsubscript{3} receptors, which are involved in acetylcholine mediated NO release from the epithelium, increases with age.\textsuperscript{40}

**GENDER**

Epidemiological data indicate a role of sex hormones in the etiology of some chronic airway diseases, in particular, asthma. Gender differences in the incidence of asthma are attributed mainly to differences in the immune response, as testosterone is considered to be immunosuppressant while female sex steroids proinflammatory. Moreover, studies on blood vessels have provided evidence that testosterone may exert direct effects on smooth muscle.

Immunohistochemistry studies show that ASM obtained from male rabbits express classical androgen\textsuperscript{41} and estrogen receptors.\textsuperscript{42} Similarly, immunofluorescence experiments performed in our laboratory revealed that rabbit ASM cells express Androgen Receptors (ARs). In the majority of cells ARs are cytoplasmic. However, in a few ASM cells ARs are also present in the cell nucleus (Figure 2, our unpublished results).

During embryonic life, sex hormones contribute to growth and maturation of the respiratory system.\textsuperscript{43-46} Androgens seem to delay the maturation of embryonic lungs\textsuperscript{47} and might be involved in the pathogenesis of the Acute Respiratory Distress Syndrome (ARDS) that has increased incidence in male newborn. *In vitro* studies revealed that both androgens and estrogens may affect, via classical receptors, the proliferation of ASM.\textsuperscript{48,49} Also, sex hormones modulate directly the responsiveness of airway smooth muscle to contractile agents via a non-genomic pathway. Namely, in rabbit trachea 17β-estradiol relaxes precontracted airways in an epithelium independent way.\textsuperscript{41,50} On the other hand, testosterone may increase vagal activity and thus contract rab-
of epithelium intact airways is independent of airway size. In contrast, the mechanical removal of epithelium affects mainly the responsiveness of 3rd and 4th order airways to acetylcholine. This difference seems to be attributed to the capability of epithelial cells to produce NO along the tracheo-bronchial tree.

LOAD

During the tidal action of breathing load fluctuations are imposed continuously on ASM that undergo shortening and lengthening. Stress and strain can both be the mechanical signals involved in mechanosensitive modulation of ASM activation\(^{66,67}\) dependent on the applied contractile stimulus.\(^ {66,68}\) The mechanisms involved comprise ASM stiffness and extensibility, alterations in intracellular Ca\(^{2+}\) concentration and regulation of molecules involved in contractile protein activation. Despite the above involved mechanisms, the epithelium may also have a modulatory role in the mechanosensitive modulation of ASM responsiveness. To be precise, studies suggest that airway epithelium modulates ASM responsiveness to acetylcholine at increased load. This effect is mediated at least in part via NO release from epithelial cells.\(^ {58}\) The involved pathway comprises the calcium dependent activation of epithelial NO synthase.\(^ {52}\) As epithelium responds to stretch by modulating epithelial NO synthase activity, and thus NO production with a consequent reduction of airway responsiveness, this protective mechanism could be impaired in epithelium damage seen in airways diseases in particular asthma.\(^ {4}\)

CONCLUSION

In conclusion, airway epithelium has a significant modulatory role in ASM responsiveness to contractile agents. This role depends on age, gender, bronchi size and load. Also, endogenous released substances like hormones (insulin, sex hormones), inflammatory factors (histamine) and neurotransmitters (acetylcholine) act on airway epithelial cells, induce NO release and limit airway contraction. These factors may contribute to the pathophysiology of some airway diseases like asthma and Chronic Obstructive Pulmonary Disease (COPD).

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