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Review

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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-

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-siology of airway diseases like asthma and Chronic Obstructive Pulmonary Disease (COPD) (Table 1).

	Effect on epithelium	Possible implication in airway diseases
Endogenous factors		
Insulin	Promotes the survival of epithelial cells[11] Participates in epithelium restoration integrity after its damage [12] Stimulates NO release from epithelial cells[13] Relaxes precontracted airways[13]	Respiratory system is considered an alternative route for insulin administration for the treatment of type 1 diabetes mellitus[71] Low incidence of asthma in patients with diabetes mellitus[72]
Histamine	Causes: H_2O_2 production from epithelial cells,[15] mucus-release and swelling of mucosa[16] and NO release from epithelial cells[14,17,18] Relaxes precontracted airways[18]	Possible contribution to the increased airways responsiveness observed in asthma due to epithelium damage and inflammation
Acetylcholine	Stimulates NO release from epithelial cells[22,68]	Possible contribution to the increased airways responsiveness observed in asthma due to epithelium damage and the increased acetylcholine release[73]
Age	Affects the capacity of airway epithelium to produce NO[39,40]	Epithelium-derived NO has an important role in the regulation of airway tone in early newborn life[74,75]
Gender	In rabbit trachea testosterone relaxes precontracted ASM in an epithelium-dependent way[41]	Gender differences in the incidence of asthma Testosterone serum levels are depressed in patients with respiratory failure,[76] cystic fibrosis,[77] hypoxic pulmonary fibrosis[78] and COPD[79]
Airway size	Variations in the distribution of acetylcholinesterase[58, 59] Depends on animal species. E.g. in canine airways, epithelium mainly modulate the responsiveness of the large airways while sheep airways, epithelium integrity affects mainly the responsiveness of small airways[65]	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[68,22]	Loss of the protective effect of deep inspiration in asthmatics[80-82] and patients with COPD[83]

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.

ENDOGENOUS FACTORS: INSULIN, HISTAMINE, ACETYLCHOLINE

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, mainly NO, that relax ASM and as a result limit excessive airway contraction.

Insulin is the major modulator of blood glucose levels exhibiting also growth factor activity on many cell types including ASM cells. Namely, it promotes ASM cell proliferation, *via* activation of the Phosphatidylinositol 3-kinase (PI3K) pathway,[6] switches the ASM phenotype to contractile,[7-9] induces the release of contractile prostanoids from sources other than epithelium[10] and increases the responsiveness of ASM to contractile agents.[7] Moreover, available data suggest that in airways, insulin promotes the survival of epithelial cells[11] and participates in epithelium restoration integrity[12] after its damage. Additionally, insulin may stimulate NO release from epithelial cells and thus cause relaxation of precontracted airways.[13]

Histamine, a classical inflammatory agent, induces airway contraction.[14] Histamine further affects airway epithelium as it promotes H_2O_2 production from epithelial cells of bronchi,[15] mucus release and swelling of mucosa.[16] In fact, the effect of histamine on ASM contraction is mediated by the release of biologically active molecules like NO from epithelial cells and thus, depends on epithelium integrity.[14,17,18] Studies on vessels demonstrate that histamine stimulates endothelial cell H_3 receptors[19] by increasing epithelial NO synthase phosphorylation and activity.[20]

Acetylcholine, the neurotransmitter released from postganglionic parasympathetic vagus nerves, induces airways contraction *via* Ca^{2+} release from intracellular stores and Ca^{2+} entry from extracellular space.[21,22] Additionally, acetylcholine is also released from epithelial cells[23] and can promote the chemeotaxis of monocytes and neutrophils[24,25] *via*, at least in part, the release of interleukin-8.[26] ASM express mainly M_2 and M_3 muscarinic receptors.[27] Acetylcholine, as well as other muscarinic agonists, may induce proliferation of ASM cells that

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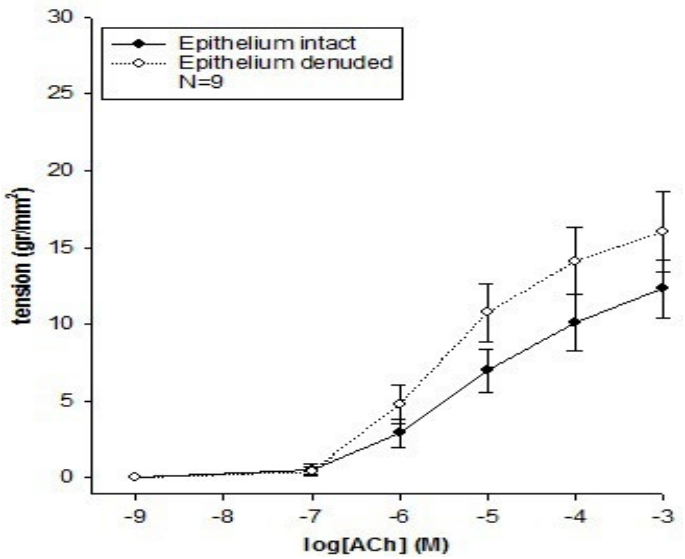
depends on the activity of the MAPK and PI3K pathways, either alone[28] or in combination with growth factors.[29] *In vitro* studies demonstrate that the airway epithelium stimulates the breakdown of acetylcholine.[30] In addition, acetylcholine seems to stimulate NO release from the epithelium and its mechanical removal increases airway responsiveness to acetylcholine.[31]

AGE

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

A



B

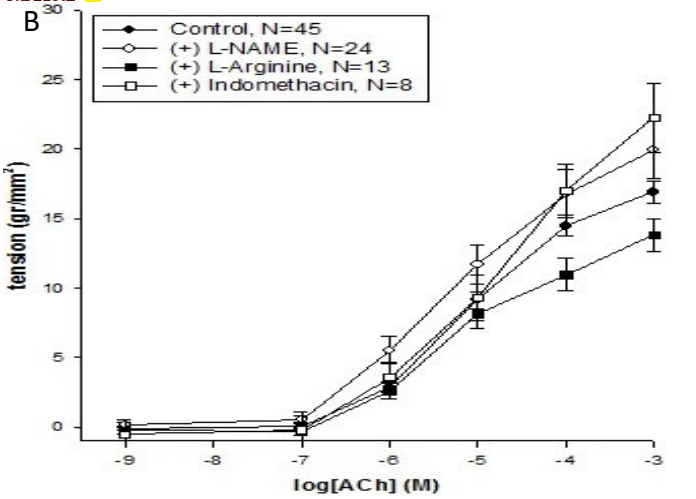


Figure 1: The effect of epithelium removal (A) or the presence of NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), NO precursor, L-arginine and cyclooxygenase inhibitor, indomethacin (B) on acetylcholine (ACh)-induced contractions of tracheal strips obtained from young rabbits (<4 weeks).
Data are means, vertical lines show SE. N refers to the number of animals studied.

Histological studies reveal that the expression of M_3 receptors, which are involved in acetylcholine mediated NO release from the epithelium, increase with age.[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[41] and estrogens receptors.[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.[43-46] Androgens seem to delay the maturation of embryonic lungs[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.[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.[41,50] On the other hand, testosterone may increase vagal activity and thus contract rab-

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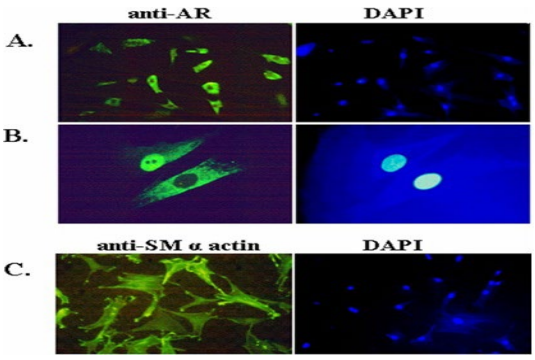

Figure 2: Airway smooth muscle cells express classical Androgen Receptors (ARs). Airway smooth muscle cells (passages 3-6) were analyzed by immunofluorescence using an anti-human classical Androgen Receptor (AR) mouse monoclonal antibody (G122-25, 1:200, BD Transduction laboratories). ARs are located mainly in the cytoplasm (magnification 20 X) (A). However, in a few ASM cells ARs are also present in the cell nucleus (magnification 40 X) (B). The smooth muscle origin of cells was confirmed by immunofluorescence with the anti SM α-actin mouse monoclonal antibody A104 (1:400, Sigma) (magnification 20 X) (C). The position of the cell nuclei was visualized by DAPI. The fluorescent signal was analyzed with an Optiphot-2 microscope and UFX-DX camera system (Nikon) at the indicated magnification.

bit[41] or guinea pig airways.[51] This effect of testosterone depends on animal gender as it is present only in male animals.[41] On the contrary, testosterone relaxes precontracted ASM[52,53] in an epithelium-dependent way.[52] Also, androgens seem to act synergistically with salbutamol.[54] Although these studies are limited, they suggest that in guinea pigs testosterone acts directly on airway smooth muscle and inhibits Ca^{2+} entry *via* voltage dependent channels, while in rabbits its action requires epithelium integrity and is, at least in part, mediated *via* NO release from epithelial cells.

AIRWAY SIZE

Several studies demonstrated that the responsiveness of smooth muscle to contractile agents varies in different parts of the bronchial tree and is influenced by airway innervation, receptor density, mechanical properties,[55,56] airway wall anatomy[57] and the distribution of acetylcholinesterase.[58,59] In addition, structural and functional regional differences in airway epithelium have also been described.[60-63] Regional differences in airway smooth muscle contractility are of physiological importance because smooth muscle contraction in central airways determines the resistance to airflow and gas distribution, whereas in peripheral airways it mainly controls the regional ratio of perfusion to ventilation. The regional differences in smooth muscle responsiveness to contractile agents may also be the basis of the non-homogeneous distribution of bronchoconstriction observed in pathological conditions such as Chronic Obstructive Pulmonary Disease (COPD) and asthma.

There are a few studies investigating the impact of airway size on the modulatory role of airway epithelium. Studies on 2nd and 3rd order of canine airways revealed that epithelium mainly modulate the responsiveness of the large airways.[64] On the contrary, we demonstrated that on sheep airways, epithelium integrity affects mainly the responsiveness of small airways.[65] Specifically, acetylcholine or KCl-induced contraction 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] depended on the applied contractile stimuli.[68-70] 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.[68] The involved pathway comprises the calcium dependent activation of epithelial NO synthase.[22] 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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