Airway epithelium: more than just a barrier!

Airway epithelium: source of non-neuronal acetylcholine and modulator of neurotransmission

We appreciate the recent review by Folkerts and Nijkamp outlining the functional importance of the airway epithelium[1], but we feel that two aspects need more consideration.

First, the authors stated that acetylcholine is synthesized by airway epithelium without giving a reference to this timely finding which contrasts with the present textbook view of acetylcholine being a neurotransmitter. Here, we wish to acquaint the critical reader with some details about the expression and function of the non-neuronal cholinergic system in human airways[2–5].

Human bronchial epithelial cells – either *in situ*, freshly isolated or cultured (24 h) – express the synthesizing enzyme choline acetyltransferase (ChAT) which was demonstrated by anti-ChAT immunohistochemistry using poly- and monoclonal antibodies and by biochemical measurement of ChAT enzyme activity[3–5]. In particular, ciliated cells express prominent anti-ChAT immunoreactivity at the rootlet of cilia, indicating an involvement in the regulation of ciliary activity[3–5].

The synthesis and presence of acetylcholine in human airway epithelial cells has been shown by specific HPLC measurements using substrate-specific bioreactor columns[2–5]. It has become increasingly clear that acetylcholine represents a phylogenetically extremely old 'cytomolecule'[6] which is widely distributed in pro- and eukaryotic cells and is also synthesized in the vast majority of human cells[3–7].

Although we are just beginning to understand the biological role of non-neuronal acetylcholine, experimental evidence shows that it is involved in the regulation of several basic cell functions. For example, antagonists of nicotinic and muscarinic receptors, as well as bromoacetylcholine (an inhibitor of ChAT) inhibited the proliferation of cultured human bronchial epithelial cells, indicating an involvement in cell growth[3]. Exposure of confluent epithelial-cell monolayers (keratinocytes) of human skin to the acetylcholine nicotinic or muscarinic receptor antagonists causes cell–cell detachment and a shrinkage of these epithelial cells[6], and a similar observation has been reported for rat tracheal epithelial monolayers exposed to tubocurarine[8]. This observation supports the view that acetylcholine is involved in the organization of the cytoskeleton[4,6].

Acetylcholine, via stimulation of nicotinic receptors, promotes the release of pro-inflammatory cytokines such as granulocyte-macrophage colony stimulating factor (GM–CSF) from primary culture of human bronchial epithelial cells[9], thus presenting evidence for its involvement in the modulation of immune responses. Additional support for such a function comes from experiments in which the stimulated histamine release from human isolated bronchi was measured[10]. Applied acetylcholine (in the picomolar range), as well as a low concentration of the cholinesterase inhibitor physostigmine, strongly inhibited the stimulated histamine release from human bronchial mucosa[10]. Thus, endogenous acetylcholine can control the secretory activity of mucosal mast cells in isolated human airways. Taken together, increasing experimental evidence indicates that non-neuronal acetylcholine can contribute to controlling the multiple functions of the airway epithelium.

Second, we would like to point to the modulatory effects of the airway epithelium on the release of autonomic neurotransmitters (neuro-epithelial interaction). For example, epithelial prostanoids and additional unidentified epithelial factors modulate the release of noradrenaline and acetylcholine from rodent airways[11–13]. Removal of the airway epithelium largely facilitated the release of the newly synthesized neurotransmitter [^{3}H]acetylcholine in guinea-pig trachea[11]. In visceral organs, including the lung (airways), the release of both these classical autonomic neurotransmitters is inhibited via prejunctional muscarinic receptors[14,15]. Thus, epithelial acetylcholine might represent a candidate to mediate an epithelium-dependent inhibition of neurotransmitter release. In addition, prostaglandin E_2 released from the airway epithelium (rat, guinea-pig) via stimulation of muscarinic receptors or β-adrenoceptors inhibits the release of both autonomic neurotransmitters. Therefore, the cross-talk between parasympathetic and sympathetic neurones can, at least in part, be mediated via the release of epithelial prostanoids[16–18]. Neuro-epithelial interactions also occur at the level of the sensory afferent neurones whose activity can be modulated by the epithelial cell layer, for example by its metabolic activity (neutral endopeptidase, cholinesterase) as mentioned by Folkerts and Nijkamp[1].

To conclude, the regulatory network in which the epithelium participates as a paracrine organ to control airway functions is very complex. Acetylcholine appears to be an important player in the cross-talk between the different airway cells, that is epithelial, glandular, smooth muscle, immune and neuronal cells. It is important to elucidate the various roles of acetylcholine in the airways in more detail and to highlight the role of non-neuronal acetylcholine in chronic airway diseases.

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Folkerts and Nijkamp reply

Airway epithelium: more than just a source for epithelium-derived relaxing factors!

We thank Ignaz Wessler, Charles Kirkpatrick and Kurt Racké for their interest in our review[1]. We apologize for not including the references of these authors when mentioning that acetylcholine can be released by the epithelium and that the epithelium contains the synthesizing enzyme choline acetyltransferease[2–5]. However, as pointed out in the abstract, our aim was to focus on the pharmacological relevance of the putative epithelium-derived relaxing factors prostaglandin E_2 and NO in the modulation of airway tone. Further, special attention was paid to the role of both epithelium-derived relaxing factors in the development of airway hyperresponsiveness in animal models and in patients with asthma.

Acetylcholine, although released by the epithelium, is a contractile factor – if it modulates airway function in a positive way, it does so by an indirect action[5]. By contrast, our review highlighted the direct actions of prostaglandin E_2 and NO on smooth muscle[1]. This was also the reason why the action of epithelium-derived relaxing factors on the release of autonomic neurotransmitters (neuro-epithelium interaction) was not discussed. Moreover, we were limited in the amount of text and the number of references that we could include. Nevertheless, the timely finding that epithelium can synthesize and release acetylcholine was so interesting that we presented it as a fact together with the ability of the epithelium to release mucus, cytokines, and chemokines as well as the ability of the epithelium to metabolize histamine and neuropeptides (all without references).

For clarity, it should be pointed out that acetylcholine is probably not the only neurotransmitter, nor is it the only contractile mediator, released by the epithelium. In rat trachea, removal of the epithelium reduced the tissue content of noradrenaline by about 30%. Moreover, the overflow of noradrenaline evoked by electrical stimulation of epithelium-denuded tracheas was reduced by 70–80%, which suggests that the epithelium was involved in the release of noradrenaline[6]. It is well recognized that arachidonic acid can be metabolized by airway epithelial cells[7,8] and that these products can influence airway tone and reactivity[9]. Recent evidence showed that the inherent tone of the human bronchus was maintained by a balance between 5-lipoxygenase and cyclooxygenase products formed by the epithelium[10]. Vasopressin and substance P immunoreactivity in cultured and intact epithelium from rabbit tracheae has also been demonstrated[11]. In addition, endothelin can be produced by human and animal airway epithelial cells[12,13].

A number of investigations have pointed to a role for endothelin in modulating airway responsiveness and asthmatic reactions[14–16]. As described in the review, endothelin induces a potent contraction in epithelium-denuded tissues. However, it appears that a number of agents that have a contractile action on smooth muscle cause a relaxation when applied to the mucosal side of the airways[1]. Indeed, endothelin induces a NO-dependent relaxation of tracheae that contain epithelium[17]. In a recent study, airway hyperresponsiveness to methacholine was observed in endothelin knock out-mice[18]. Interestingly, NO-synthesis inhibition in endothelin 1 (+/+) wild-type mice significantly enhanced methacholine responsiveness, whereas this pretreatment did not modulate airway responsiveness in endothelin knockout mice[18]. These results support the hypothesis presented in our review that, in asthmatic patients, the airway hyperresponsiveness to a variety of contractile agents might be explained by a diminished production of epithelium-derived relaxing factors[1].

Finally, it should be concluded that the airway epithelium is more than just a source of epithelium-derived relaxing factors!

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