Review

CellPress

Acetylcholine beyond bronchoconstriction: roles in inflammation and remodeling

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Acetylcholine is the primary parasympathetic neurotransmitter in the airways, where it not only induces bronchoconstriction and mucus secretion, but also regulates airway inflammation and remodeling. In this review, we propose that these effects are all primarily mediated via the muscarinic M_3 receptor. Acetylcholine promotes inflammation and remodeling via direct effects on airway cells, and via mechanical stress applied to the airways sequential to bronchoconstriction. The effects on inflammation and remodeling are regulated by both neuronal and non-neuronal acetylcholine. Taken together, we believe that the combined effects of anticholinergic therapy on M_3-mediated bronchoconstriction, mucus secretion, inflammation, and remodeling may account for the positive outcome of treatment with these drugs for patients with chronic pulmonary obstructive disease (COPD) or asthma.

Acetylcholine: not only a contractile neurotransmitter

It is generally accepted that acetylcholine (see Glossary), released from parasympathetic nerve endings, induces airway smooth muscle contraction. For this reason, anticholinergics are used as bronchodilators in obstructive airway diseases [1]. However, recent research has revealed that the (patho)physiological role of acetylcholine exceeds smooth muscle contraction, because resident and inflammatory cells that control inflammation and remodeling produce acetylcholine and express muscarinic receptors [2]. Moreover, anticholinergics reduce neutrophilia and small airway remodeling in animal models of COPD [3,4], and eosinophilia and airway remodeling in animal models of allergic asthma [5–7].

Despite these recent developments, anticholinergics are still primarily used for their bronchodilatory effects. Selectivity for the muscarinic M_3 receptor subtype is considered beneficial, because this is the primary muscarinic receptor subtype mediating the contractile effects of acetylcholine. Until recently, it was not known whether M_3 subtype selectivity of anticholinergics was a desired property for anti-inflammatory and remodeling effects, and whether the selective focus on the bronchodilatory capacities of anticholinergics is justified. Here, we synthesize the recent literature on the pro-inflammatory and pro-remodeling actions of acetylcholine in humans, human cell systems, and animal models of airways inflammation and remodeling, focusing on three key questions: (i) what is the role of individual muscarinic receptor subtypes in these responses; (ii) what is the role of the bronchoconstrictor effects of acetylcholine via the M_3 receptor in inflammation and remodeling; and (iii) what is the role of neuronal and non-neuronal acetylcholine?

Anticholinergics as bronchodilators in COPD and asthma

COPD and asthma are chronic obstructive airway diseases, and the incidence of both has increased over the past decades [8]. COPD is a leading cause of morbidity and mortality worldwide and is expected to become the third leading cause of death in 2020, which results in a substantial economic and social burden [9]. With 180 000 deaths worldwide each year, mortality from asthma is

Glossary

Acetylcholine: a primary parasympathetic neurotransmitter in the airways, where it induces bronchoconstriction and mucus secretion via muscarinic receptors, and a hormone released from non-neuronal cells.
Airway remodeling: structural changes that occur in both large and small airways in airway diseases, including asthma and COPD.
Anticholinergics: muscarinic receptor antagonists used for the treatment of COPD and, to a lesser extent, asthma, to inhibit the effects of acetylcholine.
Asthma: a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing.
COPD: a chronic inflammatory disorder of the airways associated with airflow limitation that is usually progressive.
Eosinophilia: increase of the number of eosinophils, which are leukocytes involved in the allergic asthmatic response.
Forced expiratory volume in 1 s (FEV_1): the volume of air that can forcibly be blown out in 1 s after full inspiration.
Lipopolysaccharides (LPS): major component of the outer membrane of Gram-negative bacteria, which generates an inflammatory response.
Muscarinic receptors: G protein-coupled receptors that are expressed by almost all cell types in the airways and are target receptors for acetylcholine.
Neutrophilia: increase in the number of neutrophils, which are granulocytes involved in the inflammatory response in COPD.
St George's Respiratory Questionnaire: an index designed to measure and quantify health status in patients with chronic airflow limitation.

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Keywords: anticholinergics; M_3 receptor; muscarinic receptor subtypes; mechanical strain; non-neuronal acetylcholine.

0165-6147/
 http://dx.doi.org/10.1016/j.tips.2014.11.005

Box 1. Subtype selectivity of anticholinergics

Tiotropium was the first long-acting anticholinergic introduced to the market 10 years ago. Tiotropium is a potent muscarinic receptor antagonist; however, onset of action is slower compared with the short-acting anticholinergic ipratropium [80]. Although the steady-state affinity of tiotropium for M_1, M_2, and M_3 receptors is similar, dissociation from the M_3 receptor is slower compared with the other receptor subtypes, in particular M_2 receptors (Table I) [81,82]. Used as a bronchodilator, this is a desired property, because inhibition of M_3 receptors inhibits airway smooth muscle contraction, whereas antagonizing autoinhibitory M_2 receptors on vagal nerve terminals would enhance acetylcholine release and thereby enhance airway smooth muscle contraction. Moreover, this long duration of action at the M_3 receptor allows for once-daily dosing. Slow dissociation of tiotropium from the M_3 receptor is attributed to interactions at the binding site, which prevents rapid dissociation via a snap-lock mechanism [83]. Recently, inhaled glycopyrronium, umeclidinium, and aclidinium were also introduced to the market. As becomes clear from Table I, all these long-acting anticholinergics are kinetically selective for the M_3 receptor. However, the dissociation half-life from the M_3 receptor of these compounds is shorter compared with tiotropium [81].

Table I. Binding affinity and half-life time at the human M_1, M_2 and M_3 receptor for different anticholinergics[a]

Anticholinergic	Binding affinitiy (-log M)			$t_{1/2}$ (h)		
	M_1R	M_2R	M_3R	M_1R	M_2R	M_3R
Ipratropium	9.40	9.53	9.58	0.1	0.03	0.22
Tiotropium	10.80	10.69	11.02	10.5	2.6	27
Aclidinium	10.78	10.68	10.74	6.4	1.8	10.7
Glycopyrronium	10.09	9.67	10.04	2.0	0.37	6.1

[a]Binding affinity was determined in heterologous competition experiments against [^{3}H]NMS. Data represent pK_i values of at least three independent experiments performed in triplicate and the standard error was 0.1 or less. Dissociation half-life was determined by the dissociation constants, by analyzing competition kinetics curves in the presence of [^{3}H]NMS and different concentrations of antagonist. At least three independent experiments were performed in triplicate. Data from [81]. Available data for umeclidinium indicate comparable affinity and half-life to tiotropium [84].

considerably lower, but around 300 million people currently have asthma and its greatest burden lies in the morbidity it causes, including in children [10]. Inflammation and remodeling are hallmark features of both diseases, which contribute to the decline in lung function and the severity of the disease [11–13]. Acetylcholine is the primary parasympathetic neurotransmitter in the airways, and induces bronchoconstriction and mucus secretion via M_3 receptors [14]. The activity of the neuronal system is altered in COPD and asthma via several mechanisms, which can originate early in life or develop as an acute or chronic response after allergen challenge or stimuli, such as cigarette smoke. Enhanced activity of the neuronal system leads to exaggerated acetylcholine release and airway narrowing [15]. Strikingly, the increased cholinergic tone is the major reversible component of airflow limitation in COPD [16,17]. Therefore, anticholinergics are effective bronchodilators in this disease, and represent a first line of treatment [9]. In asthma, use of anticholinergics is most commonly limited to the treatment of exacerbations [18]; however, recent clinical trials indicate that they might also be beneficial for chronic treatment of patients with moderate or severe asthma [19,20]. Currently available long-acting anticholinergics are kinetically selective for M_3 receptors (Box 1).

Muscarinic receptor subtypes in the lung: is there a need for M_3 selective anticholinergics?

Increasing evidence suggests that the role of acetylcholine in the airways is not limited to bronchoconstriction and mucus secretion. In animal models of COPD and asthma, anticholinergics inhibit both airway inflammation and airway remodeling [14]. Multiple muscarinic receptor subtypes are expressed in the airways, which may have differential roles in regulating bronchoconstriction, mucus secretion, inflammation, and remodeling [14]. Muscarinic receptor agonists and antagonists have limited selectivity towards individual muscarinic receptors (Box 1), which hinders the interpretation of the effects of these agents on functional parameters. Therefore, muscarinic receptor-specific knockout animals have proven useful to investigate the role of individual muscarinic receptor subtypes in inflammation and remodeling in animal models of COPD and asthma.

COPD

It was shown in different animal models of COPD that neutrophilic inflammation induced by cigarette smoke or lipopolysaccharides (LPS) can be prevented by pretreatment with anticholinergics, including tiotropium [3,4], glycopyrrolate [21], and aclidinium [22]. Studies in muscarinic receptor subtype-deficient mice support a role for the M_3 receptor in inflammation in response to cigarette smoke [23]. Knock-out of the M_3 receptor or inhibition using the M_3 receptor preferring antagonist 4-DAMP prevented the inflammatory response induced by cigarette smoke in mice, characterized by reduced numbers of neutrophils and reduced expression of the neutrophil chemotactic factor KC [interleukin (IL)-8 in humans] [23]. Interestingly, this inflammatory response was enhanced after knock out of the M_2 receptor. Loss of this autoinhibitory receptor results in enhanced acetylcholine release. With acetylcholine acting as a pro-inflammatory mediator, increased levels of acetylcholine in M_2 receptor subtype-deficient ($M_2R^{-/-}$) animals can explain the observed aggravated inflammatory response. Inflammation was also enhanced after knock out of the M_1 receptor. This might be explained via the role of the M_1 receptor on the airway epithelium, where it contributes to mucus production, by controlling electrolyte and water secretion. Therefore, impaired clearance of detrimental smoke particles from the airways after knock out of the M_1 receptor could underlie the enhanced inflammatory response observed in $M_1R^{-/-}$ animals. This is further supported by a marked induction in the release of the cytokines IL-6 and monocyte chemoattractant protein-1 (MCP-1) in $M_1R^{-/-}$ animals compared with wildtype animals [23] (Figure 1).

The fact that acetylcholine has a pro-inflammatory effect on neutrophilic inflammation via M_3 receptors is supported by various *in vitro* studies. Muscarinic receptor stimulation enhances the release of IL-6 and IL-8 in combination with cigarette smoke extract from airway smooth muscle cells [24]. Tiotropium and the M_3 selective antagonists 4-DAMP and DAU5884 inhibit this response, whereas no effect of the M_2 antagonist gallamine is observed [24]. Furthermore, acetylcholine can induce IL-8 release from bronchial epithelial cells, which can be inhibited by