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Review

Acetylcholine: a novel regulator of airway smooth muscle remodelling?

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Abstract

Increased airway smooth muscle mass is a pathological feature that asthma and chronic obstructive pulmonary disease (COPD) have in common. This increase has gained renewed interest in view of recent developments showing that airway smooth muscle, instead of solely being a contractile partner, is capable of interacting dynamically with its environment, especially under inflammatory conditions. Airway smooth muscle cells are able to proliferate, to migrate, and to secrete chemokines, cytokines, extracellular matrix proteins and growth factors, and most importantly, to adapt to these functions by changing its phenotype from contractile to proliferative/synthetic. Conversely, switching to a (hyper)contractile phenotype may also occur. A vast number of inflammatory stimuli regulate these functions and exert their effects via excitatory G_q or G_i-coupled receptors. Since acetylcholine activates muscarinic M_2 and M_3 receptors in the airway smooth muscle cell membrane, which are coupled to G_i and G_q proteins, respectively, and since acetylcholine release may be enhanced in airway inflammation, a pathophysiological role of acetylcholine related to the above processes and exceeding contraction could be envisaged. In this review, evidence in favour of this hypothesis, based on recent data that show a role for muscarinic receptors in modulating airway smooth muscle proliferation, contractility and contractile protein expression is discussed. Based on these findings, we postulate that endogenous acetylcholine contributes to airway remodeling in asthma and COPD.

Keywords: Airway remodelling; Asthma; COPD; Acetylcholine; Airway smooth muscle; Anticholinergic

Contents

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1. Introduction

Airway remodelling is a pathological feature observed both in asthma and in chronic obstructive pulmonary disease (COPD). The nature of this airway remodelling is different, however, as is the palette of inflammatory cells that are involved in the pathophysiology of these diseases. Comparative studies have demonstrated a prominent role for $CD8^+$ lymphocytes, neutrophils and macrophages in COPD; asthma on the other hand is best characterised by eosinophilic inflammation and $CD4^+$ lymphocytes (Jeffery, 2000, 2001). Nevertheless, all of the mentioned inflammatory cells are potential sources of growth factors, proteases, cytokines and chemokines that generate structural changes in the airways (Hirst, 2000; 2003). In COPD, these structural changes include destruction of the lung parenchyma (leading to emphysema), fibrosis, epithelial metaplasia, mucus gland hypertrophy and increases in vascular and airway smooth muscle mass (Jeffery, 2001). As for COPD, asthma is characterised by mucus gland hypertrophy, subepithelial fibrosis and increases in airway smooth muscle mass. However, in asthma, the epithelium is fragile, the basement membrane is thickened and there is no emphysema. In addition, the increased airway smooth muscle mass in asthma may be more pronounced in the larger airways, whereas in COPD this smooth muscle thickening occurs more prominently in the small airways (Barnes et al., 1998; Jeffery, 2000, 2001).

Despite of differences in the pattern of airway smooth muscle thickening, the observation that airway smooth muscle mass is increased in both inflammatory diseases is interesting in view of its putative role in airway hyperreactivity and chronic airways obstruction. In addition, recent findings have shown that airway smooth muscle is not only involved in contraction, but is also capable of dynamically interacting with its environment, especially in inflammatory conditions. Thus, airway smooth muscle cells can proliferate, migrate, secrete substances such as chemokines, cytokines, extracellular matrix proteins and growth factors and, importantly, adapt to these functions by changing its phenotype from contractile to proliferative/synthetic or even hypercontractile (Halayko and Amrani, 2003; Halayko and Solway, 2001; Hirst, 2000, 2003; Panettieri, 2003). As such, airway smooth muscle is now considered to play an active role in the regulation of airway remodelling in inflammatory airway diseases. The functions mentioned above are induced by growth factors and inflammatory mediators from the local environment and support the inflammatory response. Interestingly, a vast number of the acute inflammatory mediators (e.g. bradykinin, leukotrienes, histamine) exert their effect through G protein-coupled receptors (GPCRs) present in the airway smooth muscle cell membrane (Billington and Penn, 2003). Since contractile neurotransmitters, including acetylcholine, also activate GPCRs present in airway smooth muscle, their regulatory role in the airways is likely to exceed contraction. Nevertheless, the potential role of increased cholinergic activity in airway remodelling in asthma and COPD has thus far received little attention.

2. Acetylcholine release in airway inflammation

The primary source of acetylcholine in the airways is the vagal nerve. The release of acetylcholine from the vagal nerve is regulated by a variety of prejunctional receptors, including autoinhibitory muscarinic M_2 receptors (Aas and Maclagan, 1990). In animal models of allergic airway inflammation and asthma, muscarinic M_2 autoreceptor dysfunction has been found to contribute to exaggerated acetylcholine release from the vagal nerve both in vivo and ex vivo (Fryer and Wills-Karp, 1991; Larsen et al., 1994; Ten Berge et al., 1995). This muscarinic M_2 receptor dysfunction is thought to be mediated by eosinophils that migrate to cholinergic nerves and release major basic protein, which acts as an allosteric muscarinic M_2 receptor antagonist (Adamko et al., 1999; Costello et al., 1997; Jacoby et al., 1993). Muscarinic M_2 receptor dysfunction may also be relevant in humans. Thus, muscarinic M_2 autoreceptor function has been reported to be impaired in some, but not all patients with asthma (Minette et al., 1989; Okayama et al., 1994). Taken into consideration that muscarinic M_2 autoreceptor function is more prominent in the larger airways (Ten Berge et al., 1996) and that muscarinic M_2 receptor dysfunction is mediated by eosinophils, this mechanism may be more prominent in asthma when compared to COPD. Indeed, muscarinic M_2 autoreceptors have been reported to be still functional in patients with stable COPD (On et al., 2001), although it should be noted that this does not exclude a dysfunction in acute exacerbations.

In addition to effects on autoinhibition, eosinophil-derived polycations like major basic protein are known to cause epithelial shedding, exposing sensory nerve endings to the airway lumen (Gleich et al., 1988). Together with muscarinic M_2 autoreceptor dysfunction, this may lead to increased cholinergic reflex activity in response to inhaled stimuli and contribute to allergen-induced airway hyperreactivity (Santing et al., 1995). Afferent sensory nerve endings are also involved in central reflex bronchoconstriction upon stimulation by inflammatory mediators such as histamine, bradykinin, serotonin, adenosine and endothelin (Coleridge et al., 1989; Riccio et al., 1995; Undem and Myers, 2001). Tachykinins (neurokinin A, substance P) that originate from non-myelinated C-fibres are also involved in peripheral reflex mechanisms by enhancing ganglionic cholinergic transmission (Undem and Myers, 2001). Furthermore, substance P can possibly induce major basic protein release from eosinohils, causing M_2 dysfunction as described above (Evans et al., 2000). In addition to reduced M_2 autoreceptor function, inflammation-derived prostanoids including prostaglandin D_2, prostaglandin $F_{2\alpha}$ and thromboxane A_2 can augment acetylcholine release from chol-

inergic nerve endings by prejunctional facilitation (Undem and Myers, 2001). Interestingly, airway smooth muscle itself also represents a potential source of prostaglandin D_2, prostaglandin $F_{2\alpha}$ and thromboxane A_2 (McKay and Sharma, 2001).

Taken together, the above data indicate that vagal release of acetylcholine during periods of airway inflammation may be increased by various mechanisms. Although the above data suggest an important role for exaggerated acetylcholine release in asthma, anticholinergics are primarily used by patients with COPD, since in contrast to asthma, vagal tone appears to be the only reversible component of airways obstruction in these patients (Gross, 1988; Chapman, 2001). Nevertheless, mechanisms of increased cholinergic activity are thus far unclear, although it could be envisaged that airway inflammation in COPD augments vagal neurotransmission as well.

Acetylcholine, excreted from non-neuronal tissues has been less well explored. Nevertheless, bronchial epithelial cells, T and B lymphocytes, mast cells, monocytes, granulocytes, alveolar macrophages and airway smooth muscle cells all contain acetylcholine and/or express its synthesizing enzyme, choline acetyltransferase (ChAT) (Kawashima and Fujii, 2003; Wessler et al., 2003a; Wessler and Kirkpatrick, 2001). At present, the role of acetylcholine as an autocrine or paracrine hormone in inflammatory airways diseases has not yet been established. However, patients with atopic dermatitis, a condition often associated with bronchial asthma, express increased levels of acetylcholine in non-neuronal cells in the skin, which may suggest a primed role for non-neuronal acetylcholine in allergic inflammation (Wessler et al., 2003a,b).

3. Cholinergic signalling in airway smooth muscle

In order to better understand the established and potential effects of acetylcholine on airway smooth muscle, insight in the signal transduction that underlies muscarinic receptor activation is essential. Airway smooth muscle expresses both G_i-coupled muscarinic M_2 and G_q-coupled muscarinic M_3 receptors, the former being the predominant population, comprising ~80% of the total muscarinic receptor population (Roffel et al., 1988, 2001). G_q-coupled muscarinic M_1 receptors are not present, whereas the presence of G_i-coupled muscarinic M_4 receptors may be species specific. Thus, muscarinic M_4 receptor mRNA and protein have been observed in bronchiolar airway smooth muscle in the rabbit lung, but not in human bronchiolar as well as bronchial smooth muscle (Mak and Barnes, 1990; Mak et al., 1992, 1993). Therefore, a selective focus on signalling induced by muscarinic M_2 and M_3 receptors seems appropriate. These receptors are part of complex intracellular signalling networks that allow cross-talk with a variety of signalling cascades, including those primarily activated by growth factors, such as mitogen-activated protein (MAP) kinase and phosphatidyl inositol 3-kinase (PI3-kinase) pathways, relevant for airway remodeling.

G_q-coupled muscarinic M_3 receptors in airway smooth muscle activate phospholipase C, causing hydrolytic conversion of phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-trisphosphate ($InsP_3$) and *sn*-1,2-diacylglycerol (DAG) (Meurs et al., 2001). $InsP_3$ is involved in the mobilization of Ca^{2+} from intracellular stores, which generates a rapid and transient increase in $[Ca^{2+}]_i$. DAG generated through muscarinic M_3 receptor activation activates protein kinase C (PKC). Both Ca^{2+} and PKC are involved in the regulation of airway smooth muscle contraction. Different PKC isozymes exist, most of which being expressed in airway smooth muscle. The precise functions of these individual isozymes are not fully known, but they may relate to receptor-specific effects (Webb et al., 2000). PKC can activate the p42/p44 MAP kinase signalling cascade through direct phosphorylation of the MAP kinase kinase kinase Raf-1 (Kolch et al., 1993). This PKC-dependent pathway may be involved in muscarinic agonist-induced p42/p44 MAP kinase activation in bovine tracheal smooth muscle, as shown by its sensitivity to the PKC inhibitor 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide (GF109203X; Fig. 1). Nevertheless, methacholine-induced p42/p44 MAPK activation is not fully inhibited in the presence of GF109203X, which indicates that additional signalling pathways induced by the muscarinic receptor agonist activate the MAP kinase cascade independently of PKC. In this regard, activation of the Ca^{2+}-dependent non-receptor protein tyrosine kinase Pyk2 could play a role, presumably by inducing transactivation of growth factor

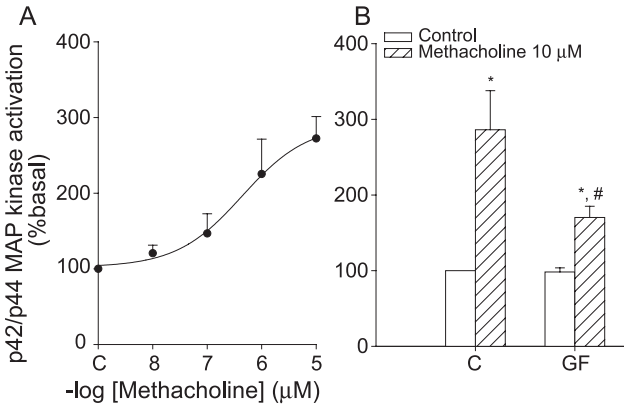

Fig. 1. Methacholine-induced p42/p44 MAPK activation in bovine tracheal smooth muscle is concentration- and PKC-dependent. (A) Intact strips were stimulated with increasing concentrations of methacholine (5 min, 37 °C), homogenised and immunoblotted against phosphorylated p42/p44 MAP kinase. Unstimulated strips were used as a control (C). Shown is the densitometric analysis of four blots. (B) Intact strips were stimulated with methacholine (10 μM) or vehicle for 5 min, after 30 min preincubation with GF109203X (10 μM) or vehicle (C). Subsequently, proteins were separated using electrophoresis and immunoblotted for phosphorylated p42/p44 MAP kinase. Shown is the densitometric analysis of six blots. $^{*}P<0.05$ compared to unstimulated; $^{\#}P<0.05$ compared to the absence of GF109203X.

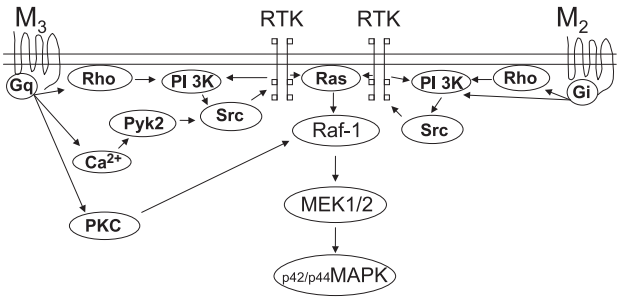

Fig. 2. Putative mechanisms of activation of p42/p44 MAP kinase, Rho and PI3-kinase by muscarinic M_2 and M_3 receptors in airway smooth muscle. These signalling pathways provide potential mechanisms for muscarinic receptors to cross-talk with growth factor-induced signal transduction, relevant for airway remodelling.

receptors (receptor tyrosine kinases) (Della-Rocca et al., 1997; Lev et al., 1995) (Fig. 2).

In addition, p42/p44 MAP kinase activation in response to muscarinic M_2 receptor activation has been reported in canine tracheal smooth muscle (Hedges et al., 2000). Presumably, this occurs via α_i-mediated activation of Ras (Emala et al., 1999) or through $\beta\gamma$-mediated activation of PI3-kinase, which can transactivate receptor tyrosine kinases (Koch et al., 1994; Lopez-Ilasaca et al., 1997; van Biesen et al., 1995) (Fig. 2). PI3-kinase can also modulate transcriptional regulation through activation of protein kinase B (PKB) (Burgering and Coffer, 1995). Activation of PI3-kinase is also achieved by activation of Rho in smooth muscle (Wang and Bitar, 1998). This could imply the involvement of both muscarinic M_2 and M_3 receptors in the activation of PI3-kinase, since both receptor subtypes are known to activate the RhoA/Rho-kinase signalling pathway (Fukata et al., 2001). Therefore, both Rho-dependent, PI3-kinase-dependent and MAP kinase-dependent pathways may be activated in response to muscarinic agonists in airway smooth muscle. As elaborated on below, all of these pathways are involved in effects that could underlie airway remodelling, including the regulation of airway smooth muscle contractility and contractile protein expression, proliferation, secretory function and migration.

4. Cholinergic regulation of airway smooth muscle remodelling

4.1. Phenotype, contractility and contractile protein expression

Accommodating the elements that comprise the contractile machinery, has for a long time been considered the prominent function of airway smooth muscle. This does not imply incapability to self-regulation, however, considering recent findings focusing on plasticity in airway smooth muscle function under pathophysiological conditions (Halayko and Amrani, 2003; Halayko and Stephens, 1994; Halayko and Solway, 2001; Hirst et al., 2000a,b). Airway smooth muscle may be induced to change its phenotype to hypercontractile in response to prolonged growth arrest or in response to insulin (Gosens et al., 2002, 2003b; Halayko et al., 1999; Ma et al., 1998). This hypercontractile phenotype is characterised by more rapid and extensive shortening and by increased expression of contractile and contraction regulatory proteins, such as smooth muscle-specific actin, myosin and myosin light chain kinase (MLCK). In addition, muscarinic M_3 receptor expression is thought to increase under these conditions, since reconstitution of the contractile phenotype in culture also induces functional re-coupling of muscarinic M_3 receptors in canine airway smooth muscle cells (Mitchell et al., 2000). Conversely, airway smooth muscle can also switch to a less contractile phenotype, characterised by decreased contractility, decreased contractile protein expression and decreased muscarinic M_3 receptor expression (Gosens et al., 2002, 2004b; Halayko and Solway, 2001; Hirst et al., 2000a,b). Switching to a less contractile phenotype generally occurs when airway smooth muscle is stimulated to proliferate in response to growth factors or fetal bovine serum and is dependent on p38 and p42/p44 MAP kinase and on PI3-kinase (Gosens et al., 2002, 2004c). Thus, the less contractile phenotype is thought to be associated with an increase in proliferative capacity and could as such contribute to the increase in airway smooth muscle mass, seen in asthma and COPD.

Contractility of airway smooth muscle preparations obtained from patients suffering from asthma and/or COPD has been reported increased in some (Bramley et al., 1994; De Jongste et al., 1987a,b; Saez et al., 2000), but not all patients (Armour et al., 1984; Cerrina et al., 1986, 1989; Taylor et al., 1985). Moreover, isolated cells obtained from asthmatics are hypercontractile (Ma et al., 2002), yet proliferate faster in culture (Johnson et al., 2001). Passive sensitization of human airway smooth muscle in vitro is also known to increase contractility (Schmidt et al., 2000). Furthermore, passively sensitized human airway smooth muscle cells have been found to produce more extracellular matrix proteins when compared to cells obtained from healthy controls and may therefore be considered hypersecretory (Johnson et al., 2000). These seemingly paradoxical results may be explained by the dynamics of phenotype switching, dependent on the inflammatory conditions in the airways, which can be controlled in vitro, but not in lung tissue obtained from patients.

The effects of acetylcholine on airway smooth muscle phenotype are complex as muscarinic receptors may both induce and reduce contractility. As described above, muscarinic receptor stimulation activates RhoA and Rho kinase, which may be involved in induction of contractility. Thus, Rho-kinase has been found to be important in maintaining bovine tracheal smooth muscle contractility (Gosens et al., 2004c) and is known to direct serum response factor to the nucleus, which regulates smooth muscle specific gene expression in airway smooth muscle (Camoretti-Mercado et al., 2000; Liu et al., 2003a).

Indeed, carbachol has been noted to increase smooth muscle specific myosin heavy chain and SM22 protein expression in M_3 transfected cultured canine airway smooth muscle cells through Rho- and Rho-kinase-dependent pathways (Liu et al., 2002). Cholinergic activation of PKC on the other hand has been found to temper carbachol-induced expression of SM22 and myosin in the same cells (Liu et al., 2003b), which implies a role for PKC in reducing contractility, possibly as an autoinhibitory feedback mechanism.

However, prolonged (8-day) exposure of organ cultured bovine tracheal smooth muscle strips to high concentrations of methacholine results in strongly reduced contractility and contractile protein expression (actin, myosin), which is dependent on muscarinic M_3 receptors, but independent of PKC and only partially dependent on p42/p44 MAP kinase and PI3-kinase (Gosens et al., 2004b). This does not represent a phenotypic change comparable to that induced by growth factors, however, since the proliferative capacity of the tissue was not concomitantly increased. Importantly, this also demonstrates that changes in contractility or contractile protein expression do not necessarily have to be interpreted as phenotype 'switching'. The mechanism responsible for this decreased contractility most probably is the prolonged rise of intracellular Ca^{2+} (Gosens et al., 2004b), which is known to negatively regulate contractility in the organ cultured rat tail artery and guinea pig ileum (Gomez and Sward, 1997; Hellstrand, 1998; Lindqvist et al., 1997). It is not clear how the balance of this inhibitory mechanism and the above described Rho/Rho-kinase-dependent stimulatory mechanism relates to cholinergic regulation of contractility in vivo. The phenotypic starting-point may be of critical importance to the outcome, as the highest serum response factor-mediated smooth muscle specific gene transcription is observed in synthetic, not contractile smooth muscle cells (Camoretti-Mercado et al., 2000).

Very recently, we found evidence showing that tracheal smooth muscle contractility and contractile protein expression in lung homogenates has been increased in repeatedly allergen-challenged guinea pigs, which could indicate a role of allergen-induced phenotype-switching in the development of (chronic) airway hyperresponsiveness. Importantly, the increase in contractility and contractile protein expression was reduced by treatment with tiotropium bromide, a long-acting muscarinic receptor antagonist used for the treatment of COPD as well as for asthma (Gosens et al., 2004a). These results for the first time indicate that endogenous acetylcholine may be involved in allergen-induced airway remodelling in vivo. Further experimentation is required to find out whether the muscarinic contribution to allergen-induced airway remodelling is caused by affecting contractility and/or by inducing increased airway smooth muscle mass. Also, the effects of tiotropium bromide on airway remodelling in asthma and COPD warrants investigation.

4.2. Airway smooth muscle proliferation

The increases in airway smooth muscle mass observed in asthma and COPD could in part be mediated by peptide growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1) and basic fibroblast growth factor (bFGF) (Stewart, 2001). These growth factors have all been implicated in airway inflammation as they can be released from inflammatory cells, such as eosinophils and macrophages. In addition, they can be derived from the epithelium, extravasated plasma and the airway smooth muscle itself (Hirst, 2000; McKay and Sharma, 2001). Mechanistically, these growth factors rely on activation of MAP kinases and PI3-kinase (and downstream targets) for their proliferative responses (Karpova et al., 1997; Kelleher et al., 1995; Krymskaya et al., 1999; Walker et al., 1998), which can be activated by muscarinic receptor agonists as well (Fig. 2). Nevertheless, muscarinic receptor stimulation alone is not sufficient to induce an increase in cell proliferation or [^{3}H]thymidine uptake in bovine (Gosens et al., 2003a) and human (Krymskaya et al., 2000) airway smooth muscle cells. This may be explained by the incapability of cholinergic agonists to induce prolonged p42/p44 MAP kinase activation, which is required to induce proliferative responses (Kelleher et al., 1995; Orsini et al., 1999). However, muscarinic receptor stimulation has been described to interact with peptide growth factor signalling, causing synergistic induction of mitogenesis in bovine (Gosens et al., 2003a) and human (Krymskaya et al., 2000) airway smooth muscle cells. This potentiation can be quite effective, as combined administration of *non-mitogenic* concentrations of methacholine and PDGF induce approximately 45% of the maximal control response to PDGF. Despite the complex signalling network that may be activated by muscarinic M_2 and M_3 receptors, this potentiation was found to be mediated solely by muscarinic M_3 receptors in bovine tracheal smooth muscle cells (Gosens et al., 2003a).

Mechanistically, the synergistic induction of mitogenesis by methacholine and PDGF in bovine tracheal smooth muscle could be explained by synergistic activation of p70 S6 kinase but not of p42/p44 MAP kinase, as reported for the combination of carbachol and EGF in human airway smooth muscle cells (Krymskaya et al., 2000). Even though PKC activity has been associated with p42/p44 MAP kinase activation (as described above), PKC may still be functionally involved in the observed synergism, however, by activating other pathways. For instance, we have recently demonstrated that activation of G_q-coupled bradykinin B_2 receptors induces synergistic activation of mitogenesis when combined with EGF, which was dependent on conventional PKC isozymes (Grootte Bromhaar et al., 2004). In addition, the G protein-coupled receptor agonist lysophophatidic acid is synergistic with EGF by activating Rho (Ediger et al., 2003). Since muscarinic M_3 receptors activate both Rho and conventional PKC

isozymes, these pathways may be important in muscarinic receptor-induced synergism with growth factors. Additional research is therefore needed to clarify the role of these pathways.

4.3. Airway smooth muscle secretory function

Airway smooth muscle secretory function has important implications for airway inflammation, as the number of molecules that can be secreted by airway smooth muscle cells is considerable. As a potential source of pro-inflammatory cytokines (e.g. interleukin-5, interleukin-6, interleukin-13) and chemokines (e.g. eotaxin, interleukin-8), airway smooth muscle cells could modulate inflammation in the airways, both directly and indirectly by affecting chemokinesis of inflammatory cells and the mediator production by these cells. In addition, airway smooth muscle cells can produce inflammatory mediators (mainly prostanoids), growth factors (e.g. PDGF, IGF, bFGF), proteases (e.g. matrix metalloprotease I) and extracellular matrix proteins (e.g. pro-collagen, fibronectin, laminin) (Hakonarson and Grunstein, 2003; Hirst, 2003; Johnson and Knox, 1997; McKay and Sharma, 2001). In turn, these secretory components may have effects on airway smooth muscle proliferation and phenotype. Extracellular matrix proteins for instance can affect airway smooth muscle proliferation and contractility. Thus, human airway smooth muscle cells coated on collagen I or fibronectin exhibit a proliferative phenotype, whereas cells coated on laminin switch to a more contractile phenotype (Hirst et al., 2000a,b). Thus, airway smooth muscle may contribute to various aspects of airway remodelling in asthma and COPD by dynamically interacting with its environment through both direct and indirect mechanisms.

Although the majority of studies has focussed on the regulation of airway smooth muscle secretory function by cytokines (e.g. interleukin-4, interleukin-13, tumor necrosis factor α), some have addressed the possibility that these functions can be regulated by GPCR agonists (McKay and Sharma, 2001). Bradykinin for instance is capable of inducing interleukin-6 and interleukin-8 release from human airway smooth muscle (Huang et al., 2003; Pang and Knox, 1998). Importantly, bradykinin-induced interleukin-6 production by these cells is dependent on the short-lived p42/p44 MAPK activation by bradykinin, which could indicate that other GPCR agonists are capable of inducing interleukin-6 release as well. Indeed, histamine and endothelin-1 have been reported to induce interleukin-6 release in human airway smooth muscle cells (McKay et al., 2001). Remarkably, cholinergic regulation of airway smooth muscle secretory function has not been addressed, possibly because the G_q-coupled muscarinic M_3 receptor loses its expression rapidly in culture (Widdop et al., 1993). Nevertheless, cholinergic regulation of airway smooth muscle secretory function may be of great importance and warrants future investigation.

4.4. Airway smooth muscle migration

Recent studies have demonstrated that airway smooth muscle cells in culture have the capacity to migrate. By migrating to a more pro-mitogenic environment, for instance to the collagen-rich matrix in the subepithelial region, airway smooth muscle migration has been postulated to contribute to hyperplasia (Stewart et al., 2004). Indeed, human airway smooth muscle cell migration can be stimulated by pro-mitogenic stimuli, such as PDGF and bFGF (Goncharova et al., 2003). However, the G protein-coupled receptor agonist thrombin was without effect in these cells, even though this agonist is a highly effective mitogen. This would imply that GPCR agonists do not affect migration by themselves. Nonetheless, leukotriene E_4 can augment PDGF-induced migration of human airway smooth muscle cells in which PI3-kinase is the key signalling event (Parameswaran et al., 2002). Likewise, acetylcholine could potentially have effects on airway smooth muscle cell migration, although this has not yet been studied.

5. Concluding remarks

Muscarinic receptor antagonists such as ipratropium bromide and tiotropium bromide are often used for the treatment of COPD and represent an important co-treatment in severe asthmatics (Barnes et al., 1995). They are used as bronchodilators and are generally not considered to have beneficial effects on airway remodelling. Nevertheless, there is evidence that prolonged treatment with these anticholinergics may improve lung function in patients with COPD (Rennard et al., 1996; Tashkin and Kesten, 2003). Although no direct evidence exists to suggest that these effects are due to improve-

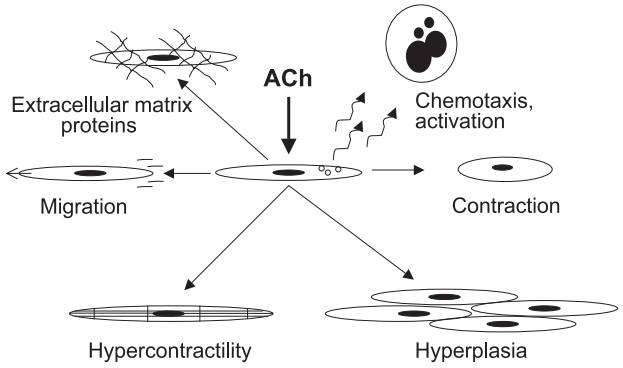

Fig. 3. Proposed mechanisms by which acetylcholine could affect airway smooth muscle remodelling. Acetylcholine has been shown to affect airway smooth muscle contractility, contractile protein expression, pro-mitogenic signalling and proliferation. In addition, like several other G protein-coupled receptor agonists, acetylcholine could also be involved in airway smooth muscle cell migration, extracellular matrix protein production and secretion of cytokines and chemokines. Altogether, these effects could contribute to airway remodelling in asthma and COPD.

ment of airway remodelling, these studies are particularly interesting in view of the recently discovered effects of acetylcholine on airway smooth muscle remodeling. Thus, prolonged stimulation of muscarinic receptors on airway smooth muscle may affect contractility, contractile protein expression, pro-mitogenic signalling and proliferation. In addition, other effects of acetylcholine on airway smooth muscle, including regulation of secretory function and migration, may be envisaged (Fig. 3). Since prolonged neuronal and non-neuronal release of acetylcholine may be induced by several inflammatory processes as observed in asthma and COPD, a role for acetylcholine in airway remodelling could be postulated, a contention confirmed by recent observations using tiotropium bromide inhalations that muscarinic receptor signalling is involved in airway remodelling in allergen challenged guinea pigs.

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