

The underlying physiological mechanisms whereby anticholinergics alleviate asthma

Morgan Gazzola, Samuel Mailhot-Larouche, Clémentine Beucher, and Ynuk Bossé

Abstract: The mechanisms whereby anticholinergics improve asthma outcomes, such as lung function, symptoms, and rate of exacerbation, can be numerous. The most obvious is by affecting the contraction of airway smooth muscle (ASM). The acetylcholine released from the cholinergic nerves is the most important bronchoconstrictor that sets the baseline degree of contractile activation of ASM in healthy individuals. Although the degree of ASM's contractile activation can also be fine-tuned by a plethora of other bronchoconstrictors and bronchodilators in asthma, blocking the ceaseless effect of acetylcholine on ASM by anticholinergics reduces, at any given moment, the overall degree of contractile activation. Because the relationships that exist between the degree of contractile activation, ASM force, ASM shortening, airway narrowing, airflow resistance, and respiratory resistance are not linear, small decreases in the contractile activation of ASM can be greatly amplified and thus translate into important benefits to a patient's well-being. Plus, many inflammatory and remodeling features that are often found in asthmatic lungs synergize with the contractile activation of ASM to increase respiratory resistance. This review recalls that the proven effectiveness of anticholinergics in the treatment of asthma could be merely attributed to a small reduction in the contractile activation of ASM.

Key words: long-acting muscarinic antagonists, airway smooth muscle, airway narrowing, airway hyperresponsiveness, respiratory resistance.

Résumé : Les modes d'action par lesquels les anticholinergiques améliorent l'asthme — sur le plan de la fonction pulmonaire, des symptômes et de la fréquence des exacerbations — peuvent être nombreux. Le plus évident constitue les effets sur la contraction du muscle lisse des voies aériennes (MLVA). L'acétylcholine libérée des nerfs cholinergiques représente le plus important bronchoconstricteur dans l'établissement du niveau de base de l'activation contractile du MLVA chez des personnes en bonne santé. Même si le degré d'activation contractile du MLVA peut aussi être réglé finement par une pléthore d'autres bronchoconstricteurs et bronchodilatateurs dans l'asthme, l'inhibition des effets incessants de l'acétylcholine sur le MLVA à l'aide d'anticholinergiques permet de réduire, en tout temps, le degré total d'activation contractile. Puisque les relations qui existent entre le degré d'activation contractile, la force du MLVA, le raccourcissement des cellules du MLVA, le rétrécissement des voies aériennes, la résistance au débit d'air et la résistance pulmonaire ne sont pas linéaires, de petites diminutions de l'activation contractile du MLVA peuvent être amplifiées de façon très marquée et donc se traduire par des avantages importants sur le plan du bien-être du patient. En plus, de nombreuses caractéristiques de l'inflammation et du remodelage, que l'on retrouve souvent dans les poumons de patients atteints d'asthme, entrent en synergie avec l'activation contractile du MLVA afin d'augmenter la résistance pulmonaire. Cet article de synthèse rappelle que l'efficacité avérée des anticholinergiques dans le traitement de l'asthme pourrait être simplement attribuable à une faible réduction de l'activation contractile du MLVA. [Traduit par la Rédaction]

Mots-clés : antagonistes muscariniques à longue durée d'action, muscle lisse des voies aériennes, rétrécissement des voies aériennes, hyperréactivité des voies aériennes, résistance pulmonaire.

Introduction

The symptoms of asthma are either persistent or episodic and generally coincide with perennial or time-varying exposure to environmental factor(s). The diagnosis of asthma is usually straightforward as it is established based on common symptoms such as respiratory distress, cough, wheezing, and thoracic oppression. However, the molecular pathogenesis that drives asthma is characterized by inter-individual variability, as well as within-individual complexity and instability (Carr and Bleecker 2016). The inter-individual variability mainly stems from the fact that the offending environmental factors in asthma vary greatly be-

tween afflicted individuals. In turn, this largely depends on a number of host factors, such as genetic and epigenetic susceptibilities, history of environmental exposures, age, hormones, smoking status, and co-morbidities. The within-individual instability mainly stems from the fact that the severity of asthma at any given moment is determined by the total number of triggering and aggravating environmental factors involved, as well as by their respective concentration and sequence of exposure and co-exposure. In turn, this distinct but intricate and ever-changing offending environmental exposure elicits symptoms of varying sort and varying intensity by regulating several endogenous inflammatory molecules. Again, the assortment of endogenous in-

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M. Gazzola,* S. Mailhot-Larouche,* C. Beucher, and Y. Bossé. Quebec Heart and Lung Institute, affiliated with Université Laval, Quebec City, Quebec G1V 4G5, Canada.

Corresponding author: Ynuk Bossé (email: ynuk.bosse@criucpq.ulaval.ca).

*These authors contributed equally to this work.

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Table 1. Asthma medication.

Type of drugs	Class of drugs	Generic name	Dosing regimen*
Bronchodilators	SABAs	Albuterol	100 or 200 µg, inhaled, prn
		Terbutaline	0.5 mg, inhaled, prn
	LAMAs	Acclidinium†	400 µg, inhaled, bid
		Glycopyrronium†	50 µg, inhaled qd
		Tiotropium	18 µg, inhaled, qd
Anti-inflammatory	Inhaled corticosteroids	Umeclidinium†	62.5 µg, inhaled, qd
		Beclomethasone	Variable dose (≤250 to >500 µg/day), inhaled, bid
		Budesonide	Variable dose (≤400 to >800 µg/day), inhaled, bid
		Ciclesonide	Variable dose (≤200 to >400 µg/day), inhaled, bid
		Fluticasone	Variable dose (100 to >500 µg/day), inhaled, bid
		Mometasone	Variable dose (≤200 to >800 µg/day), inhaled, bid
		Prednisone	Variable dose (5–60 mg), p.o., qd
Anti-leukotrienes	Oral corticosteroids	Montelukast	10 mg, p.o., qd
Combination therapies	LTRAs	Budesonide + formoterol	Variable doses (200/12 to 800/24 µg), inhaled, bid
	Inhaled corticosteroids + LABAs	Fluticasone + salmeterol	Variable doses (200/100 to 1000/100 µg), inhaled, bid
		Mometasone + formoterol	Variable doses (400/20 or 800/20 µg), inhaled, bid
		Fluticasone + vilanterol	Variable doses (100/25 or 200/25 µg), inhaled, qd
Biologics	Anti-IgE	Omalizumab	150–375 mg s.c. every 2 or 4 weeks‡
	Anti-IL-5	Mepolizumab	100 mg s.c. every 4 weeks
		Reslizumab	3 mg/kg i.v. every 4 weeks

Note: bid, twice a day; IgE, immunoglobulin E; IL-5, interleukin-5; i.v., intravenous; LABAs, long-acting β_2 -agonists; LAMAs, long-acting muscarinic antagonists; LTRAs, leukotriene-receptor antagonists; p.o., oral; prn, as needed; qd, once a day; SABAs, short-acting β_2 -agonists; s.c., subcutaneous.

*Dosing regimens are for adults.

†Not approved for asthma.

‡The dose depends on body mass and the serum concentration of total IgE.

flammatory molecules involved is different between patients and depends on host factors and the pattern of exposure to environmental factor(s).

Given that the molecular pathogenesis of asthma is variable between patients, as well as complex and unstable in each patient, it may not be surprising that targeting a single molecular defect at a given point in time benefits only a few, and only slightly (Chang and Bossé 2016). Indeed, many attempts were made with new drugs to block individually the endogenous molecules that are thought to be transiently or persistently dysregulated in asthma. The outcomes of these trials thus far are trivial, even when the patients were enrolled based on well-defined clinical, phenotypic, and endotypic characteristics (Chang and Bossé 2016). Based on recent large clinical trials, the only exceptions to this rule seem to be the anticholinergics. Indeed, the anticholinergics provide significant improvements in symptoms and lung function in broad populations of asthmatic individuals (Busse et al. 2016).

Although vagal activity has always been thought to be more important in chronic obstructive pulmonary disease than in asthma, the anticholinergics are increasingly used to treat asthma today. The most successful in asthma are the ones that antagonize the function of the muscarinic receptors for an extended period of time. They are generally active throughout the day and are thus prescribed in a once-daily dosing regimen. They are commonly called the long-acting muscarinic antagonists (LAMAs). A list of LAMAs, along with other drugs that are currently approved in Canada to treat asthma, is presented in Table 1.

The LAMAs listed in Table 1 are all approved to treat chronic obstructive pulmonary disease. However, tiotropium is the only LAMA that is currently approved in Canada to treat asthma. It was approved in May 2015. Tiotropium is indicated as add-on maintenance bronchodilator treatment in adult patients who remain symptomatic despite the combination of an inhaled corticosteroid and a long-acting β_2 -agonist (LABA) and who experienced at least 1 severe exacerbation in the previous year.

The efficacy of LAMAs in asthma was shown to be comparable with the LABAs in recent large clinical trials (Bateman et al. 2011; Kerstjens et al. 2015). The LABAs actively relax airway smooth muscle (ASM) and thereby bronchodilate the airways no matter the bronchoconstrictor(s) involved in ASM contraction. It may

thus sound surprising that the LAMAs, which can merely block 1 bronchoconstrictor, namely acetylcholine, are sometimes as effective as the LABAs. Plus, evidence pointing toward derangements in cholinergic regulation in asthma is scarce, albeit existent, and discussed later in this chapter (Fryer et al. 2006; Minette et al. 1989; Mitchell et al. 1991; Molfino et al. 1993). However, it is important to remember that cholinergic input originating from the vagal nerves is omnipresent in human lungs (Canning 2006). In fact, an elaborated network of nerves interweaves the ASM bundles (West et al. 2015). Although a better description of the anatomy and the function of these nerves throughout the airway tree is eagerly needed, physiological studies clearly demonstrated that the release of acetylcholine by the nerve endings constantly contributes to raise the degree of ASM's contractile activation (Canning 2006). The non-neuronal cholinergic system may also contribute (Pieper 2012). The LAMAs are thus expected to reduce, at any given moment, the contractile activation of ASM that is attributed to cholinergic tone. For that very reason, the therapeutic benefits of LAMAs may not be surprising after all. This is because the contractile activation of ASM is at the very base of a chain of events that raises respiratory resistance. This chain of events is described in the first part of this review. Importantly, this chain of events is further amplified by diverse arrays of aberrations seen in asthmatic lungs. This latter topic is specifically covered in the second part of this review. Overall, this review expatiates on the many ways by which a small additive reduction in ASM's contractile activation, such as the one induced by LAMAs, may culminate into significant improvement in a patient's well-being.

A chain of events amplifying the effect of ASM's contractile activation

ASM stress

The stress, which is exclusively defined herein as the force per cross-sectional area generated by ASM, depends on the degree of ASM's contractile activation. In turn, the degree of contractile activation depends on the potency, the efficacy, and the concentration of the bronchoconstrictor involved. The relationship that exists between the degree of contractile activation and ASM stress

is described by a sigmoidal curve (Fig. 1A). This implies that the degree of activation needs to reach a certain threshold to instigate the generation of stress. Above this threshold, further increases in activation then translate into important changes in stress. This is true until the rate of gain in stress per unit increase in activation tapers down to attain a plateau, beyond which point additional increases in contractile activation cause no additional gain in stress. Taken alone, this sigmoidal relationship between the degree of ASM's contractile activation and the stress can explain why small changes in bronchoconstrictor concentration can sometimes exert large changes in stress, while large changes in bronchoconstrictor concentration can sometimes exert no effect at all.

It is important to emphasize that the degree of ASM's contractile activation in vivo is not solely determined by the concentration of 1 bronchoconstrictor. It is rather determined by the collective effect of all the bronchoconstrictors and all the bronchodilators that are acting simultaneously on ASM. Owing to the sigmoidal activation–stress curve described above, an additive effect of 2 bronchoconstrictors in terms of ASM activation can sometimes be perceived as a synergistic effect in terms of stress generation. For example, the use of 2 bronchoconstrictors, each at concentration under the threshold required to generate stress when used alone, can have a measurable effect when combined. Similarly, if the concentration of a bronchoconstrictor raises the degree of activation just above the threshold needed to instigate the generation of stress, the subsequent addition of a different bronchoconstrictor is expected to cause a greater gain in stress simply because it is now acting on a steeper part of the activation–stress curve. For the same reason, blocking the contractile activation of ASM induced by 1 bronchoconstrictor can also decrease the stress generated by other bronchoconstrictors.

By blocking the bronchoconstricting effect of acetylcholine, the LAMAs move the degree of ASM's contractile activation leftward on the sigmoidal activation–stress curve. Depending of the position on the activation–stress curve at which this overall decrease in ASM activation occurs, and depending on the magnitude of the cholinergic tone, this can substantially decrease ASM stress. As alluded to above, this can also reduce the stress generated by other bronchoconstrictors. In fact, it can eliminate the effect of other bronchoconstrictors completely if the overall degree of ASM's contractile activation is brought sufficiently below the threshold needed to instigate the generation of stress. Importantly, this effect of LAMAs is expected to occur regardless of the molecular defects leading to excessive bronchoconstriction in asthma.

ASM shortening

Once activated to contract, ASM strives to shorten. Shortening is a major contractile property of ASM, as it ultimately determines the amount of airway narrowing. The amount of ASM shortening within an airway depends on the total tension (stress per unit of airway length) generated by ASM, which is not only determined by the stress generated by ASM, but also by the orientation of the ASM bundles, the fraction of the total airway length containing ASM bundles, and the thickness of the ASM bundles. The amount of ASM shortening is also greatly influenced by the load opposing airway constriction. The load impeding ASM shortening is auxotonic; i.e., it increases non-linearly as the muscle shortens (Lambert et al. 1993). A typical ASM length–load curve is depicted in Fig. 1B. The load impeding ASM shortening in vivo mainly stems from its surrounding structures. It takes into account 3 components. The first component is the transmural distending pressure afforded by the transpulmonary pressure, which also dictates the initial (pre-constricted) length of ASM at the far right of the length–load curve. The 2 other components are only manifested during and after ASM shortening. They include (i) the progressive transfer of the load that is initially carried by the passive compo-

nents of the airway wall prior to the constriction to the ASM; and (ii) the stress required to deform the parenchyma surrounding the airway wall during constriction (Lambert et al. 1993). More specifically, the load increases approximately hyperbolically upon ASM shortening until it reaches a critical point, where the load stabilizes or even slightly descends with further ASM shortening. Beyond that critical load, ASM shortening can go uninterrupted for a while, as the load is relatively constant over a large range of airway narrowing before it rises again. The steep rise at the far left of the length–load curve occurs due to closure, when constriction-induced epithelium apposition completely obstructs the lumen with the mucus and the airway-lining fluid. At such point, further ASM shortening would need to compress the material internal to ASM.

The shortening can also be limited by the differing capacity of ASM to generate stress at different lengths. Indeed, the stress-generating capacity of ASM instantaneously diminishes as its operating length decreases (Herrera et al. 2005). It is also clear that shorter ASM generate less stress for any given bronchoconstrictor concentration (Gazzola et al. 2016; Lee-Gosselin et al. 2013). Therefore, shortening can sometimes be limited because the stress generated at higher lengths suddenly reaches a shortened length that can no longer exert a stress exceeding the load. This occurs even more often in the portion of the length–load curve where the load is stable or slightly descending, simply because of the large length reduction that ASM undergoes once the stress it generates surpasses the critical load.

It is also important to mention that the auxotonic load that impedes ASM shortening is always fluctuating due to breathing maneuvers. The fluctuating load and the consequent strain that ASM undergoes during breathing maneuvers significantly impact the contractile capacity of ASM (Fredberg et al. 1997). The dynamic environment within which ASM operates also implies that other contractile properties than the stress-generating capacity may contribute to set the caliber of the airways. These include shortening velocity, as well as the ability to tolerate and recover from the fluctuating load inflicted by breathing maneuvers. These topics were reviewed elsewhere and will not be described in detail herein (Bossé and Paré 2013; Ijpma et al. 2012). Altogether, the shortening of ASM in response to any given contractile stimulus proceeds until the stress generated by ASM within a range of oscillating lengths equals the dynamic load impeding ASM shortening (Oliver et al. 2007).

Regardless of the magnitude by which breathing maneuvers strain and weaken ASM, the shape of the ASM length–load curve clearly illustrates how a change in ASM stress impacts the amount of shortening (Fig. 1B). By decreasing the stress generated by ASM in response to acetylcholine, the LAMAs thus reduce the load that can be overcome by ASM and thus move ASM length rightward on the length–load curve. Depending on the position on the length–load curve at which this overall decline in stress occurs, this can substantially improve airway patency. The major impact occurs when the decline is just enough to bring ASM stress below the critical load.

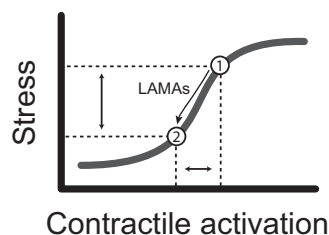
Airway constriction

The constriction of the airway induced by ASM shortening is amplified for geometrical reasons. The relationship between airway wall perimeter and the cross-sectional area of the airway is not linear. Assuming a cylindrical geometry for the airway, the luminal cross-sectional area changes according to the square of changes in the perimeter of the airway wall (Fig. 1C). Because the ASM bundles are not oriented perfectly perpendicular to the length axis of the airway, the changes of luminal area induced by ASM shortening is slightly less than that. More specifically, the ASM bundles are on average oriented 75 degrees off the length axis of the airway (Ijpma et al. 2017; Smiley-Jewell et al. 2002). This number is highly variable though and largely depends on the

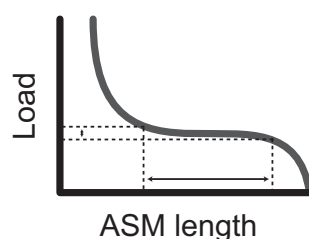
Fig. 1. Sequential amplifying mechanisms whereby small changes in the contractile activation of airway smooth muscle (ASM) can translate into large changes in a patient's well-being. (A) The stress generated by the contractile activation of ASM can be amplified depending on the position on the activation–stress curve at which it occurs. The dashed lines illustrate how a small change in bronchoconstrictor activation translates into a large increase in stress when it occurs on a steep portion of the activation–stress curve. By reducing the degree of ASM activation by a small extent, for example from the position labeled 1 on the curve to the position labeled 2, the long-acting muscarinic antagonists (LAMAs) can dramatically change the amount of stress generated by ASM. (B) The amount of ASM shortening elicited by ASM stress can be amplified depending on the position on the ASM length–load curve at which it occurs. The dashed lines illustrate how a small increase in ASM stress that surpasses a critical load translates into a substantial amount of ASM shortening. (C) The constriction of the airway is amplified by ASM shortening due to geometrical reasons. This is because the cross-sectional area of the airway changes according to the square of changes in the perimeter of the airway wall ($\sim$ ASM length). (D) The resistance to airflow is also amplified during luminal narrowing. This is because the resistance to airflow is inversely related to the cross-sectional area of the airway lumen at the second power. (E) Finally, respiratory resistance can be amplified depending on the pattern of constriction throughout the airway tree. In particular, a heterogeneous pattern of constriction increases respiratory resistance by distributing airflow into the more patent airways and thereby fostering ventilation defects by decreasing parenchymal tethering in the parts of the lung subtended by very constricted airways. A, area; l, length; P, perimeter; R, airway resistance to airflow; μ , fluid viscosity.

Contractile Activation

A. ASM Stress

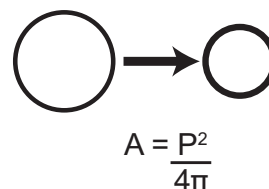


B. Load as a Function of ASM Shortening

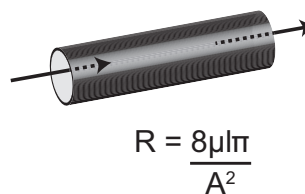


Sequential Amplifying Effects

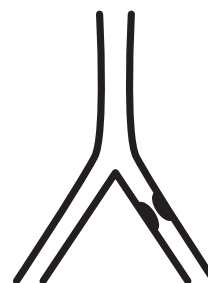
C. Airway Constriction



D. Airflow Resistance



E. Respiratory Resistance (Heterogeneity)



localization of the airway within the lung. The angle is on average 90 degrees in the trachea and the main bronchi, where the ASM bundles occupy approximately 1/3 and 2/3 of the total airway circumference, respectively. The angle then tends to decrease progressively while moving deeper along the airway tree, where the ASM bundles spiral along the entire circumference of the airway wall. The orientation of the ASM bundles is also different at airway bifurcations, on average 25 degrees off the length axis of the airways (Smiley-Jewell et al. 2002). The effect of ASM shortening on airway wall perimeter is thus highly variable, depending on the orientation of the ASM bundles and the proportion of the total airway wall circumference containing ASM bundles. Nevertheless, assuming an average orientation of 75 degrees in an airway in which the ASM bundles are present along the entire circumference, the changes in wall perimeter amount to 96.6% of the changes in ASM length. The average in luminal area thus changes according to the square of 0.966 times the changes in ASM length. This also assumes that the orientation of the ASM bundles remains constant during shortening.

The exponential relationship that exists between ASM shortening and the cross-sectional area of the airway also indicates that a given percentage of ASM lengthening translates into a larger percentage of airway dilation. By reducing ASM shortening caused by acetylcholine, LAMAs can thus exponentially decrease airway constriction. In absolute terms, the major impact of this geometrical effect occurs when the cross-sectional area of the airway is small; viz., when ASM lengthening occurs in either a small-sized airway or a very constricted airway.

Resistance to airflow in a single airway

The resistance to airflow within an airway, hereinafter called airflow resistance (R), is calculated by measuring the pressure drop between 2 points (ΔP) within an airway and by then dividing by airflow ($\dot{V}$) ($R = \Delta P / \dot{V}$). Airflow resistance is not simply proportional to the cross-sectional area of the lumen. Assuming a laminar flow and a luminal volume with a cylindrical geometry, Poiseuille's equation predicts that the change in pressure between 2 points within an airway is proportional to the length of the airway (l) and the viscosity of the fluid (μ) and inversely proportional to the radius (r) at the fourth power ($\Delta P = 8l\mu\dot{V}/\pi r^4$). Airflow resistance during a laminar flow can thus be estimated by $R = 8l\mu/\pi r^4$. Because radius at the denominator is raised to the fourth power, airflow resistance increases dramatically during luminal narrowing. In fact, halving the radius increases airflow resistance by 16-fold. When related to the cross-sectional area of the airway lumen, airflow resistance changes inversely according to the square of changes in luminal area (Fig. 1D).

As ventilation increases, the propensity for flow to transit from a laminar to a turbulent flow increases. In fact, this transition occurs when the Reynolds number is between 2000 and 3000. The Reynolds number is dimensionless and calculated as follows: (gas density $\times$ gas velocity $\times$ airway diameter) / gas viscosity. During turbulent flow, airflow resistance becomes also dependent on flow and affected by air density (ρ) rather than viscosity ($R_{aw} = 8l\rho\dot{V}/\pi r^4$). Any structural deviation from a perfectly cylindrical geometry of the airway lumen, as well as the many bifurcations within the tracheobronchial tree, also increase the propensity of turbulence in flow. Nevertheless, the inverse relationship that exists between airflow resistance and the radius to the fourth power remains. This suggests that, regardless of the flow regime, small decreases in airway luminal radius always translate into disproportionally larger increases in airflow resistance.

The exponential change in airflow resistance induced by constriction of the airway lumen also indicates that a given percentage of airway dilation translates into a larger percentage decrease in airflow resistance. By reducing airway constriction caused by acetylcholine, LAMAs can thus exponentially decrease airway resistance. Once again, the impact of this effect is greater in airways

with a smaller cross-sectional luminal area, although it only applies to convective airways (i.e., airways within which air is transported by convection).

Respiratory resistance

Respiratory resistance refers to the resistance of the entire respiratory system, including the resistance required to displace the chest wall and the lung tissue, as well as the resistance to accommodate airflow within the entire tracheobronchial tree. Because of the geometrical considerations mentioned above, mere heterogeneity in airway patency for any given average in airway caliber increases the resistance to airflow within the tracheobronchial tree (Bates 1993). This is because the increase in resistance caused by narrowing of some airways is not compensated by the decrease in resistance caused by dilation of other airways. Obviously, this is only true when the flow is not redistributed into the more patent airways.

Yet, compelling empirical data demonstrated that airway caliber heterogeneity, at baseline or induced by bronchoconstriction, is associated with increased respiratory resistance (Chen and Johnson 2004; Dame Carroll et al. 2015; King et al. 2004) (Fig. 1E). This is now attributed to closure or near closure of peripheral airways (King et al. 1998; Lutchen and Gillis 1997) and the consequent appearance of sizable ventilation defects (Campana et al. 2009; de Lange et al. 2007; Venegas et al. 2005). A mathematical model, which takes into account flow redistribution towards the more patent airways, has also provided a tentative explanation for the mechanisms underlying this phenomenon (Venegas et al. 2005). According to this model, initial heterogeneity between 2 daughter airways can suddenly foster a dichotomic response to a rising degree of ASM activation, where 1 daughter airway constricts excessively while the other dilates. This is because airflow is redistributed into the more conductant daughter airway, which raises the insufflation pressure in the regional lung subtended by this airway and thereby increases the load impeding ASM shortening. Contrastingly, diminution of flow in the other daughter airway causes regional lung deflation and a consequent decline in parenchymal tethering recoil. The load impeding ASM shortening in this latter thus decreases, which fosters more narrowing for any degree of ASM's contractile activation. Because of the interdependence of airways in series, all the smaller airways downstream of both the excessively closed daughter airway and the paradoxically dilated daughter airway are also affected. In fact, the size of the ventilation defects depends on the first airway generation afflicted by this dichotomic response. Overall, the model demonstrates how a degree of heterogeneity at baseline can trigger a self-perpetuating feedback loop of flow redistribution that renders downstream pathways unstable because of changes in parenchymal elastic recoil. This ultimately leads to cluster of peripheral airway constriction, the emergence of sizable ventilation defects and a consequent increase in respiratory resistance. In support of the results of this model, the degree of ventilation heterogeneity correlates with the increased respiratory resistance elicited by challenging the lung with inhaled methacholine (Downie et al. 2007; Hardaker et al. 2011).

The heterogeneity of airway caliber at baseline and provoked by bronchoconstriction are examples showing that the behaviour of the whole system is not merely the sum of its parts. Indeed, closure or near closure of a few airways markedly increases the resistance of the respiratory system. By reducing the overall constriction of the airways caused by acetylcholine and thereby the propensity for airway closure, LAMAs can thus decrease respiratory resistance substantially.

To reiterate, this part of the review describes how (i) a small decrease in ASM's contractile activation can greatly reduce stress; (ii) a small decrease in stress can greatly reduce ASM shortening; (iii) a small decrease in shortening can greatly reduce airway constriction; (iv) a small decrease in airway luminal constriction can

greatly reduce resistance to airflow; and (v) a small decrease in closure of a few airways throughout the lung can greatly reduce respiratory resistance. At the very base of this chain of events is the degree of contractile activation of ASM. Thus, the net impact of a small decrease in the overall degree of contractile activation caused by a LAMA should not be underestimated.

Additional amplifying factors in asthma

The chain of events described above is effective with or without asthma. However, many steps within this chain of events can be further amplified in the presence of lung aberrations that are commonly found in asthma. This topic was previously reviewed in detail and is only briefly examined herein (Bossé et al. 2010).

At the level of ASM activation, many bronchoconstrictors are dysregulated in asthma, such as histamine (White and Eiser 1983), leukotrienes (Lam et al. 1988), endothelin-1 (Mattoli et al. 1991), prostaglandin D₂ (Murray et al. 1986), thromboxane A₂ (Wenzel et al. 1991), adenosine (Driver et al. 1993), bradykinin (Liu et al. 1991), anaphylatoxin C3a and C5a (Krug et al. 2001), substance P (Tomaki et al. 1995), sphingosine-1-phosphate (Ammit et al. 2001), and platelet-activating factor (Grandel et al. 1985). As explained above, some bronchoconstrictors act additively on ASM's contractile activation and may thus be perceived as synergistic in terms of stress generation. This occurs especially when the concentrations of bronchoconstrictors used are low. Indeed, many of the previously documented examples of synergism between 2 bronchoconstrictors in studies conducted either *in vivo* (Jones et al. 1992; Kanazawa et al. 1992; Leff and Munoz 1981; Mitchell and Bouhuys 1976; Watanabe et al. 2004) or *in vitro* (Catalli and Janssen 2004; Gerthoffer 1996; Gunst et al. 1987; Liu et al. 2006) were involving low concentrations of bronchoconstrictors. Concordantly, some of the reported combinations of bronchoconstrictors were not synergistic at higher concentrations (Gerthoffer 1996; Gunst et al. 1987; Leff and Munoz 1981). However, other interactions between bronchoconstrictors, including bronchoconstrictors acting through muscarinic receptors, truly act synergistically at the level of ASM's contractile activation (Catalli and Janssen 2004; Creese and Bach 1983; Liu et al. 2006; Millar et al. 1995; Oguma et al. 2007). In these synergisms, the degree of contractile activation that is achieved by stimulating ASM with 2 bronchoconstrictors is beyond the one predicted by the additive effect of each of them. The convergence of diverse intracellular pathways, which transduce the contractile signal downstream from each of the 2 distinct G protein-coupled receptors that are being activated by the combined bronchoconstrictors, seems to be at the heart of these synergisms. By blocking the cholinergic tone, LAMAs are thus expected to abrogate the synergistic enhancement of ASM's contractile activation caused by the co-activation of the muscarinic receptors with some of the other bronchoconstrictor receptors.

Still at the level of ASM activation, other environmental triggers and endogenous inflammatory molecules act through the nerves, causing a bronchoconstrictive reflex and (or) an increased efficiency of cholinergic neurotransmission (Allen et al. 2006; Canning et al. 2001; Dixon et al. 1979; Gold et al. 1972; Mills and Widdicombe 1970). Either way, the result is a net increase in the release of acetylcholine. Many cells or mediators in asthma also decrease neuronal acetylcholine reuptake by altering the function of the M₂ receptor (Fryer et al. 2006; Minette et al. 1989; Rynko et al. 2014). Some observations also suggested that acetylcholine half-life is increased in asthma because of a decreased expression of acetylcholinesterase (Mitchell et al. 1991). All of the above factors may contribute to the elevated cholinergic tone observed in asthma (Molfinio et al. 1993) and should be directly inhibited by LAMAs.

At the level of ASM stress, many molecular inflammatory mediators increase the stress-generating capacity of ASM. This rather quick lability of ASM's contractile capacity was recently described

in detail (Auger et al. 2016). The potentiating effect of all these mediators on the stress generated in response to acetylcholine is expected to be blocked by LAMAs. Interestingly, ASM tone (i.e., the sustained contraction of ASM with bronchoconstrictors) itself can also increase the contractile capacity of ASM through a phenomenon dubbed "force adaptation" (Gazzola et al. 2017). Therefore, beyond blocking directly the constriction induced by acetylcholine, LAMAs should also prevent the gain in ASM's contractile capacity induced by cholinergic tone. Similarly, LAMAs should also block the gain in ASM stress acquired through a process dubbed "length adaptation", at least when length adaptation arises following ASM shortening induced by acetylcholine (McParland et al. 2005).

At the level of ASM shortening, many alterations in asthmatic lung may affect the force of interdependence between the airway wall and the parenchyma (Paré and Mitzner 2012). A reduced force of interdependence decreases the load impeding ASM shortening and thereby triggers more airway narrowing for the same amount of ASM stress. Loss of lung recoil (Gelb et al. 2002), physical decoupling caused by breakages of the parenchymal attachments on the abluminal side of the airway wall (Mauad et al. 2004) and geometrical decoupling caused by thickening of the adventitia (Paré and Bai 1995), are aberrations sometimes found in asthma that reduce the force of interdependence and should thus accentuate ASM shortening. Other aberrations in the mechanical properties of the airway wall can also modify the load impeding ASM shortening and thereby affect the degree of ASM shortening caused by any given degree of cholinergic activation (Paré 2003). Inflammation within the lumen, as well as altered mucus content and composition, can also alter the surfactant and thereby increase surface tension. In turn, this enhances shortening because the surface tension conspires with ASM to overcome the load (Wagers et al. 2004b). The amplifying effects of all these aberrations on acetylcholine-induced ASM shortening are expected to be blocked by LAMAs.

At the level of airway constriction, thickening of the airway wall enhances luminal narrowing induced by ASM shortening. This occurs purely because of geometrical reasons. The space occupied by air (i.e., the lumen) is easier to compress than the space occupied by the rest of the materials enclosed by ASM. Therefore, the changes in the luminal space during ASM shortening are exaggerated when a greater fraction of the total space internal to ASM is occupied by less compressible materials (i.e., the space containing everything but air). All the layers that constitute the airway wall are thicker in asthma, including the epithelium, the basal lamina, the lamina propria, and the ASM itself (Bossé et al. 2008). This geometrical effect is thus likely to contribute to airway hyperresponsiveness in asthma (Paré et al. 2007). Various materials inside the airway lumen, such as mucus, inflammatory cells, plasma exudates, desquamated epithelial cells, and creola bodies exert the same detrimental effect; i.e., increase constriction of the airway lumen for any given degree of ASM shortening (Kuyper et al. 2003; Van de Graaf et al. 1991). Altogether, many inflammatory and remodeling features can aggravate the obstruction of the airway lumen elicited by ASM shortening. At the level of airway resistance to airflow, these effects are further potentiated because, as explained above, the changes in airflow resistance are inversely related to the square of changes in the cross-sectional area of the airway lumen. All of these potentiating effects are expected to be blocked by LAMAs when airway luminal narrowing and the subsequent increased resistance to airflow are elicited by acetylcholine-induced ASM shortening.

At the level of respiratory resistance, the patchy nature of the ventilation defects observed in asthma implies that an important degree of spatial heterogeneity exists (Campana et al. 2009; de Lange et al. 2007; King et al. 1998; Venegas et al. 2005). Indeed, asthma is characterized by focal zones showing a wide spectrum of disease severity, encompassing zones of very sick bronchi to zones of virtually normal bronchi (Richmond et al. 1996). This

heterogeneity can stem from a diversity of factors, such as focal variations in the degree of inflammation, ASM's contractile activation, ASM stress, ASM shortening, structural remodeling, edema and (or) mucus. Beyond the effect of each of these factors on the luminal narrowing of individual airways, the heterogeneity that they collectively provoke is, by itself, a major determinant of increased respiratory resistance and lung dysfunction in asthma. The materials inside the lumen of asthmatic airways is particularly alarming as they seriously augment the propensity of plugging and thus the risk of complete airway closure (Kuyper et al. 2003). Any means by which the cholinergic activation of ASM aggravates heterogeneity might be relevant to the demonstrated benefits of LAMAs in asthma.

To reiterate, this part of the review describes how diverse arrays of aberrations in asthma serially interact with an amplifying chain of events to adversely potentiate the effect of abnormal (or perhaps normal (Wagers et al. 2004a)) contractile activation of ASM on respiratory resistance. This further reinforces the aforementioned notion stating that the net impact of a small decrease in the overall degree of ASM's contractile activation caused by a LAMA should not be underestimated.

Conclusion

This review recalls that the proven effectiveness of anticholinergics in the treatment of asthma could be merely attributed to a small reduction in the contractile activation of ASM. The molecular signature of asthma pathogenesis is highly variable between patients and is constantly changing and complex within every patient. Treating a group of asthmatic individuals with a drug that specifically targets 1 inflammation-derived molecule at a given point in time is not tenable (Chang and Bossé 2016). This is because a single molecular defect that is accountable for a significant part of symptoms in all, or a subgroup of, asthmatic patients may simply not exist. In contradistinction, the cholinergic tone is omnipresent, even in an individual devoid of respiratory disorders (Canning 2006). This implies that, although it is the concerted action of all the bronchoconstrictors and bronchodilators in asthma that dictates the contractile degree of ASM's activation, the binding of acetylcholine on the muscarinic receptors always contributes to raise this degree of contractile activation. Anticholinergics, such as the LAMAs, are thus expected to decrease the overall contractile activation of ASM irrespective of their timing of administration and irrespective of the disparities in the molecular defects leading to asthma between patients. This may concurrently explain why a broad population of asthmatic individuals benefits from LABAs (Busse et al. 2016).

We have seen that the effect of small changes at the level of ASM's contractile activation should not be underestimated. The nonlinear relationship that exists between the degree of contractile activation and ASM stress implies that small changes in the contractile activation can greatly affect the stress generated by ASM. Plus, the stress is nonlinearly related to ASM shortening and the changes in both airway constriction and resistance to airflow are greatly amplified during ASM shortening due to geometrical reasons. Moreover, other mechanisms not necessarily apparent in individual airways are operational at higher biological scales and can exaggerate the responses of the respiratory system to changes in the contractile activation of ASM. Finally, many aberrations found in asthma further potentiate the gain in respiratory resistance induced by the contractile activation of ASM by acting at one or several levels within this amplifying chain of events.

It is also important to underline that a decrease in ASM's contractile activation can only be favourable in asthma, irrespective of the degree of contractile activation at which it arises. Further relaxation is even beneficial on ASM that is already at a degree of contractile activation below the threshold needed to instigate stress, as this elevates the bronchoconstrictor burden that is re-

quired on a subsequent asthma attack to elicit a response. The degree of ASM's contractile activation is also likely to be variable between airways within the same individual. Therefore, supplemental relaxation may exert very little effect in some airways but may be dilating other airways substantially. In fact, it is more likely that only the more affected airways may benefit from additional relaxation conferred by LAMAs. Yet, the importance of relieving a few airways should not be undervalued, as this simultaneously re-establishes homogeneity in airway patency and thereby decreases respiratory resistance substantially.

Of course, the benefits of LAMAs in asthma may be ascribed to alternative mechanisms. Notwithstanding these other possibilities, we surmise that merely moving the contractile activation of ASM leftward on the activation-stress curve by blocking the cholinergic tone, alone or in combination with a supplemental leftward displacement by a LABA, may account for a major part of the reported benefits of LAMAs in asthma.

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