Endocrine regulation of airway contractility is overlooked

Ynuk Bossé

Institut Universitaire de Cardiologie et de Pneumologie de Québec, Université Laval, Québec, Québec, Canada G1V 4G5

Correspondence should be addressed to Y Bossé
Email
ynuk.bosse@criucpq.ulaval.ca

Abstract

Asthma is a prevalent respiratory disorder triggered by a variety of inhaled environmental factors, such as allergens, viruses, and pollutants. Asthma is characterized by an elevated activation of the smooth muscle surrounding the airways, as well as a propensity of the airways to narrow excessively in response to a spasmogen (i.e. contractile agonist), a feature called airway hyperresponsiveness. The level of airway smooth muscle (ASM) activation is putatively controlled by mediators released in its vicinity. In asthma, many mediators that affect ASM contractility originate from inflammatory cells that are mobilized into the airways, such as eosinophils. However, mounting evidence indicates that mediators released by remote organs can also influence the level of activation of ASM, as well as its level of responsiveness to spasmogens and relaxant agonists. These remote mediators are transported through circulating blood to act either directly on ASM or indirectly via the nervous system by tuning the level of cholinergic activation of ASM. Indeed, mediators generated from diverse organs, including the adrenals, pancreas, adipose tissue, gonads, heart, intestines, and stomach, affect the contractility of ASM. Together, these results suggest that, apart from a paracrine mode of regulation, ASM is subjected to an endocrine mode of regulation. The results also imply that defects in organs other than the lungs can contribute to asthma symptoms and severity. In this review, I suggest that the endocrine mode of regulation of ASM contractility is overlooked.

Introduction

Asthma is a prevalent and debilitating lung disorder (Masoli *et al.* 2004, Haselkorn *et al.* 2010). The symptoms of asthma are driven by multifarious inflammations that are triggered in susceptible individuals by exposure to a diverse variety of environmental factors, such as allergens, viruses, and pollutants. The most distinctive feature of asthma is intermittent airflow obstruction. The waxing and waning nature of asthma symptoms is due to recurrent inflammatory flares. These flares occur upon inhalation of the liable environmental factors and each leads to transient constriction of the airways. The smooth muscle embedded within the airway wall, hereafter called airway smooth muscle

(ASM), is the motor driving airway constriction. Indeed, ASM is arranged nearly circumferentially within the airway wall and strives to shorten once stimulated to contract. Consequently, ASM shortening converges the airway wall within the lumen and, by doing so, provokes respiratory distress by impeding the flow of air into the lung.

ASM activation and airway responsiveness in asthma

The amount of shortening that ASM undergoes and the concomitant degree of airway constriction achieved

depend on the force generated by ASM. In turn, the force is mainly dictated by the level of activation of ASM, which is determined by a balance between endogenous spasmogens (i.e. contractile agonists) and bronchodilators (Fig. 1). The level of ASM activation is difficult to appraise *in vivo*. One strategy is to measure the change in lung function evoked by the inhalation of drugs that relax ASM (i.e. exogenous bronchodilators). In asthma, exogenous bronchodilators substantially improve lung function. In fact, a >12% reversal of airway obstruction pre- vs post-bronchodilators is the most objective criterion used to diagnose asthma according to current guidelines (National Heart Lung and Blood Institute National Asthma Education and Prevention Program 2007,

Bateman *et al.* 2008). The greater responsiveness to bronchodilators represents a solid evidence that ASM activation is elevated in asthma.

Apart from being putatively overactivated, the airways of asthmatic individuals also respond excessively to a bronchoprovocative challenge with a spasmogen (Crapo *et al.* 2000). This typical feature of asthma is called airway hyperresponsiveness (AHR). Representative curves that describe the level of airway responsiveness in asthmatic and non-asthmatic individuals are illustrated in Fig. 2. However, it is important to understand that AHR does not necessarily imply that ASM is hypercontractile, as many other factors found *in vivo* can act additively and synergistically with ASM contraction to increase the
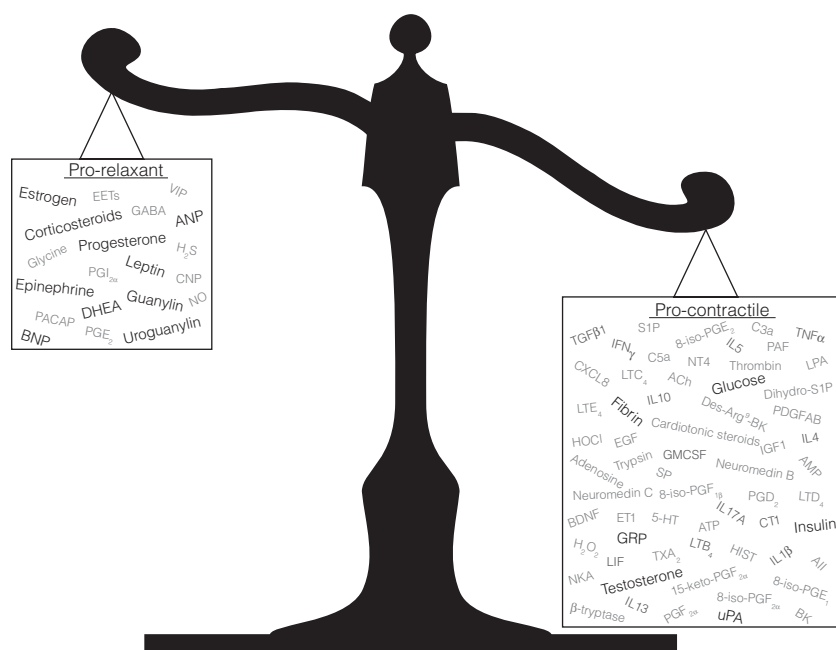
Figure 1

The level of activation of airway smooth muscle (ASM) is controlled by a balance between spasmogens and bronchodilators. Airway contractility is further affected by other types of mediators that influence the responsiveness of ASM to either spasmogens or bronchodilators. Excessive airway constriction in asthma can thus be a consequence of the following: i) a greater expression of spasmogens; ii) a decreased expression of bronchodilators; iii) an altered expression of mediators affecting ASM responsiveness to spasmogens and bronchodilators; or iv) expression of a mixture of any of these elements. Owing to the variable nature of airway obstruction and the well-recognized heterogeneity of asthma pathogenesis, the mediators involved are likely to differ in time and between afflicted individuals. The paracrine mediators that affect ASM contractility directly and indirectly are given in light and dark gray respectively.
The endocrine mediators affecting ASM contractility are given in bold. AII, angiotensin II; ACh, acetylcholine; ANP, A-type natriuretic peptide; BDNF, brain-derived neurotrophic factor; BK, bradykinin; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; CT1, cardiotrophin 1; CXCL8, ligand 8 of the CXC subfamily of chemokines (also called IL8); DHEA, dehydroepiandrosterone; EETs, epoxyeicosatrienoic acids; EGF, epidermal growth factor; ET1, endothelin 1; GABA, gamma-aminobutyric acid; GM–CSF, granulocyte–macrophage colony-stimulating factor; GRP, gastrin-releasing peptide; H_2S, hydrogen sulfide; HIST, histamine; 5-HT, 5-hydroxytryptamine (also called serotonin); HOCl, hypochlorous acid; IGF1, insulin-like growth factor 1; IFNγ, interferon gamma; IL, interleukin; LPA, lysophosphatidic acid; NKA, neurokinin A; NO, nitric oxide; NT4, neurotrophin 4; LIF, leukemia inhibitory factor; LT, leukotriene; PACAP, pituitary adenylate cyclase-activating peptide; PAF, platelet-activating factor; PDGFAB, platelet-derived growth factor AB; PG, prostaglandin; SP, substance P; S1P, sphingosine-1-phosphate; TGFβ1, transforming growth factor beta 1; TNFα, tumor necrosis factor alpha; TXA$_2$, thromboxane A$_2$; uPA, urokinase-type plasminogen activator; VIP, vasoactive intestinal peptide.
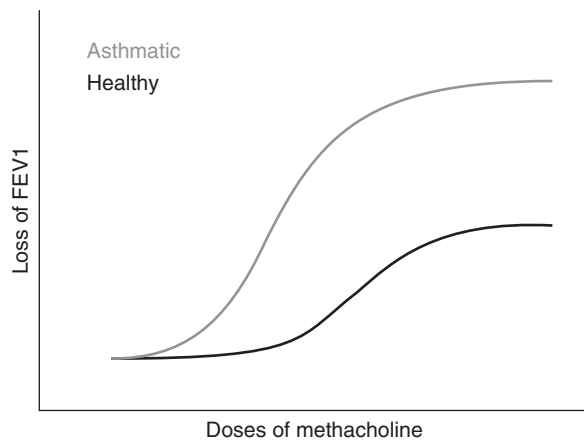
Figure 2

Airway responsiveness is increased in asthmatics. The level of airway responsiveness is assessed by measuring the decline of forced expiratory volume in 1 s (FEV1) before and after each incremental doses of inhaled methacholine. While healthy individuals (black curve) demonstrate normal airway responsiveness, asthmatic individuals (grey curve) demonstrate increased airway responsiveness, called hyperresponsiveness (AHR).

level of airway responsiveness (Bosse *et al.* 2010). The level of airway responsiveness is also variable in time, in great part due to the contractile plasticity of ASM.

ASM plasticity

The response of ASM to a given stimulus is not fixed but rather plastic (i.e. adaptable). A diverse array of mediators influences the contractile capacity of ASM. Inflammation-derived spasmogens, such as leukotrienes, histamine, prostaglandin D_2, and many others, stimulate ASM to contract. The expression and activity of spasmogens are often dysregulated in asthmatic lungs. In addition to triggering ASM contraction, sustained exposure to spasmogens increases ASM contractile capacity over time (Bosse *et al.* 2009, Pascoe *et al.* 2012). Many other mediators, although not spasmogenic themselves, can potentiate the contractile response of ASM to spasmogens. This is the case for many environmental factors that trigger asthma, such as viruses, allergens, and pollutants, as well as endogenous mediators that are produced in response to environmental factors, such as tumor necrosis factor alpha (TNFα), interleukin 13 (IL13), and IL1β (reviewed in Bosse (2012)). Alternatively, other mediators attenuate the contractile effect of spasmogens. This is the case for many proteases that catabolize spasmogens, such as acetylcholinesterases (Mitchell *et al.* 1991), which degrade acetylcholine, neutral endopeptidases (Ratti *et al.* 2001), which degrade spasmogenic

tachykinins such as substance P and neurokinin A, and acetylhydrolase (Stafforini *et al.* 1999), which degrades platelet-activating factor.

Conversely, a diverse array of mediators influence the relaxing capacity of ASM. Many endogenous bronchodilators, such as nitric oxide, prostaglandin E_2, prostacyclin, and many others, stimulate ASM to relax. The expression and activity of bronchodilators are often dysregulated in asthmatic lungs. Other mediators, although not bronchodilators themselves, can potentiate the relaxing effect of bronchodilators. Among other examples cited below, this is the case for glycine (Yim *et al.* 2011) and dopamine (Mizuta *et al.* 2012). Alternatively, other mediators attenuate the relaxing effect of bronchodilators. This is the case for many inflammatory insults, such as lipopolysaccharides, allergens, and viruses, as well as many endogenous mediators, such as TNFα, IL13, and sphingosine-1-phosphate (Bosse 2012).

Mechanical stress can also alter ASM contractility (Fredberg *et al.* 1997, Lavoie *et al.* 2012, Noble *et al.* 2013, Pascoe *et al.* 2013). For example, the change in length that ASM undergoes during breathing maneuvers, such as during a deep inspiration, decreases the contractile capacity of ASM.

The kinetics of change in ASM response to a given stimulus can thus occur at different rates. The kinetics of change fluctuates to such an extent that it can be measured using the following timescales: i) timescales of hours and days, in the case of inflammatory mediators; ii) a timescale of minutes, in the case of the gain of contractile capacity induced by a sustained exposure to spasmogens; or iii) timescales of seconds and fraction of seconds, in the case of the decline of force induced by changes in the ASM length caused by breathing maneuvers. Together, these results indicate that the magnitude of the response of ASM to a given stimulus (e.g. a chosen dose of methacholine) depends on its current state of activation, the external cues to which it has been previously exposed, and its length history. The plasticity of ASM contractility is of high significance, as it is likely to underlie the time-dependent variability of airway responsiveness.

Asthma treatments

Owing to the prominent role played by ASM contraction in the manifestation of symptoms, it is not surprising that exogenous bronchodilators are the most prescribed drugs to treat asthma (von Mutius & Drazen 2012). Among bronchodilators, the β2-agonists are the most widely used.

For the more severe forms of asthma, a proper treatment regimen also includes inhaled glucocorticoids. Gluco-corticoids act partially by decreasing the level of ASM activation, mainly indirectly, but also directly, by decreasing the expression of inflammation-derived spasmogens (see below). It is fascinating that the two pillar classes of drugs to treat asthma are mimetics of endogenous mediators that are secreted by adrenal glands; β2-agonists are catecholamine mimetics selective for the adrenergic β2 receptor and glucocorticoids are mimetics of endogenous corticosteroids. Despite that, the level of ASM activation is mainly regarded as being under the control of a paracrine mode of regulation. Indeed, dysregulated releases of spasmogens and bronchodilators by epithelial cells, nerves, and, most importantly, inflammatory cells that are mobilized in vicinity of ASM are considered at the origin of the elevated level of ASM activation in asthma. It is also not intuitively appealing to study organs other than the lungs, such as the adrenals, to elucidate the underlying mechanisms responsible for the excessive airway narrowing in asthma. However, this may just be a misconception. Mounting evidence indicates that ASM contractility is influenced by endocrine factors. In this review article,

I suggest that the regulation of ASM contractility by mediators secreted by remote organs is overlooked.

Mediators released by remote organs on ASM contraction

The regulation of ASM contractility by remote organs can provide important clues to further our understanding of asthma pathogenesis and to elucidate the origin of asthma heterogeneity (Holgate 2011, Wenzel 2013). It may also lead to the development of alternative treatments, which would be important to fulfill an unmet need in patients who are either refractory or not adequately controlled by current asthma treatments (Barnes & Adcock 2009, Bousquet et al. 2009). The following section highlights seminal discoveries that have demonstrated regulatory functions of mediators generally assigned to other organs on airway contractility (Fig. 3). Although this field of research adds a supplemental layer of complexity to the control of ASM contractility, the evidences suggest that it can no longer be ignored. It is also important to point out that several hormones can modify the immune response and the ensuing inflammatory response to inhaled
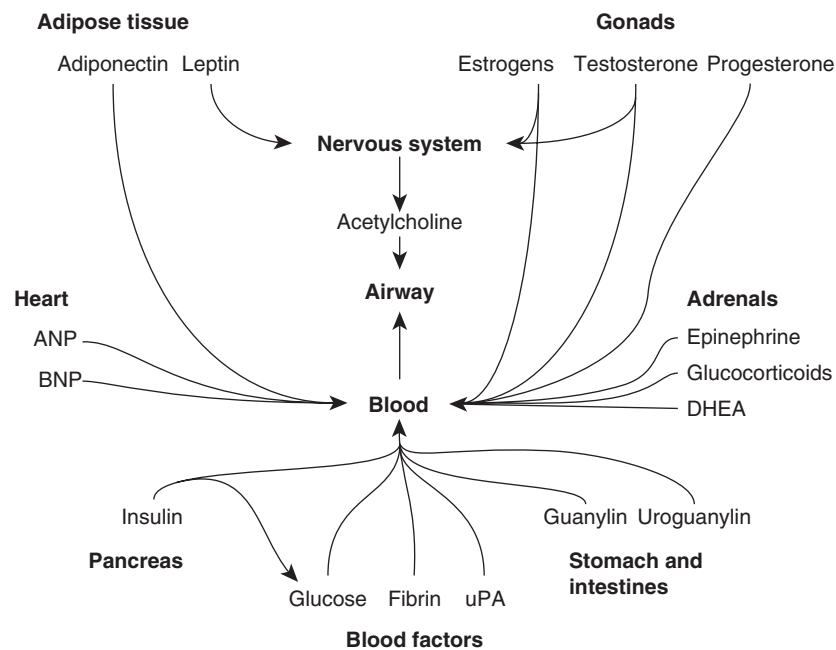
Figure 3

Mediators secreted by remote organs influence airway contractility. The mediators can affect airway contractility directly, by relaxing or contracting airway smooth muscle (ASM), or indirectly, by increasing or decreasing the responsiveness to spasmogens and bronchodilators. Their effect can also require supplementary intermediates. For example, estrogen, testosterone, and leptin act, at least partially, via the nervous system to influence the vagal parasympathetic input that controls the level of cholinergic activation of ASM. Another example is insulin, which affects blood glucose concentration that, in turn, affects the contractile capacity of ASM. More details are provided in the text. Abbreviations are provided in the legend to Fig. 1.

environmental factors that trigger asthma. In turn, this can increase the level of airway responsiveness by altering, or not, the contractile capacity of ASM. For the purpose of clarity, the endocrine regulation of the lung immune system is not addressed in detail in the present review. Readers are referred to recent reviews that are covering this particular topic (Townsend *et al.* 2012*a*, Wright 2012, Sood & Shore 2013).

Adrenals

Epinephrine Epinephrine has long been recognized for its potent bronchodilator effect (von Mutius & Drazen 2012). Epinephrine acts on ASM by binding on the same receptors as the β₂-agonists. However, whether or not the endocrine regulation of airway contractility by endogenous epinephrine is impaired in asthma is yet to be elucidated. The fact that β-blockers induce bronchoconstriction certainly indicates that the bronchodilator effect of endogenous catecholamines, such as epinephrine, is omnipresent (Ind *et al.* 1985*a*, 1989). Severe asthmatics also fail to raise their plasma level of epinephrine during an acute episode of asthma, and this failure can play a role in the manifestation of symptoms (Ind *et al.* 1985*b*). β-blockers are also contraindicated for asthma patients, which also testifies that circulating catecholamines are still suspected to contribute to airway patency. Interestingly, certain asthma triggers, such as respiratory syncytial virus, may alter bronchoconstriction in an animal model of asthma by altering the level of endogenous catecholamines (Li *et al.* 2012).

Glucocorticoids Glucocorticoids are the mainstay therapy for the treatment of mild to severe forms of asthma. In addition to their well-recognized anti-inflammatory virtue, glucocorticoids attenuate ASM contractility. Part of it is mediated by direct and indirect genomic (i.e. transcriptional) effects (Yick *et al.* 2013), and part of it is exerted by a rapid non-genomic effect (Sun *et al.* 2006). Indeed, acute or prolonged exposure to glucocorticoids reduces the contraction elicited by spasmogens and potentiates the relaxing response to β₂-adrenergic receptor activation. The mechanisms involved are several and have been reviewed previously (Hirst & Lee 1998).

DHEA Dehydroepiandrosterone (DHEA), a metabolic intermediate in the synthesis of androgens and estrogens, is the most abundant steroid produced by the adrenals and the one found at the highest concentration in the circulation. DHEA inhibits the contraction of ASM elicited by either spasmogens or an allergen (Espinoza *et al.* 2013). DHEA also suppresses the *in vivo* allergic response (Espinoza *et al.* 2013) and the development of AHR (Cui *et al.* 2008) induced by allergic airway inflammation in animals. Most importantly in humans, nebulized DHEA is also successful to improve asthma control (Wenzel *et al.* 2010).

Pancreas

Insulin The mutual exclusion of type 1 diabetes, a disease in which insulin is deficient, and asthma led to the suspicion that insulin may contribute to asthma (Stene & Nafstad 2001). Association studies that report relationships between insulin and asthma abound. Insulin resistance, a complication in which insulin is over-expressed, is frequent in children with asthma (Arshi *et al.* 2010, Cottrell *et al.* 2011) and is associated with asthma-like symptoms in adults (Thuesen *et al.* 2009). Insulin resistance is actually a potential risk factor for asthma (Al-Shawwa *et al.* 2007) and is suggested to drive the widely discussed association between obesity and asthma (Husemoen *et al.* 2008).

The mechanisms by which insulin altered the development/severity of asthma can certainly be several. However, many reports indicate that insulin adversely influences airway contractility. *In vivo*, inverse association exists between blood levels of insulin and lung function (Huang *et al.* 2012). Supplementation of insulin also causes mild decrease in pulmonary function (Ceglia *et al.* 2006) and increases airway responsiveness to methacholine (Terzano *et al.* 2009). The absence of insulin in diabetic rats also down-regulates airway responsiveness (Cavalher-Machado *et al.* 2004). *Ex vivo*, insulin is actually capable of causing a sustained contraction of ASM (Schaafsma *et al.* 2007). Insulin also increases the capacity of ASM to generate force in response to spasmogens (Gosens *et al.* 2003). The potentiation of insulin on the contractile effect of spasmogens is mediated by the transformation of ASM into a hypercontractile phenotype (Gosens *et al.* 2003, Schaafsma *et al.* 2007, Dekkers *et al.* 2009) and a decreased function of the inhibitory muscarinic M₂ receptors (Belmonte *et al.* 1997, 1998, Coulson *et al.* 2002). These results have recently been confirmed in rats (Nie *et al.* 2014).

Insulin may also affect ASM contractility by altering blood glucose concentration. Glucose concentration in airway lining fluid of non-diseased subjects was estimated to be 12.5 times lower than blood glucose concentration (Baker *et al.* 2007). Glucose concentration in airway secretion increases when blood glucose concentration

reaches 6.7–9.7 mmol/l (Wood *et al.* 2004). This is of considerable interest, especially for diabetics, because glucose increases the contractile capacity of ASM (Cazzola *et al.* 2012). Together, these results offer a potential explanation for the association between blood glucose concentration and impaired lung function (Walter *et al.* 2003, Davis *et al.* 2004).

Adipose tissue

Leptin Leptin is a satiety hormone predominantly expressed in adipocytes, especially in subcutaneous adipose tissue. OBR (LEPR), the leptin receptor, is expressed on ASM (Nair *et al.* 2008). However, leptin has no direct effect on ASM contraction (Nair *et al.* 2008, Arteaga-Solis *et al.* 2013). Leptin rather acts through the central nervous system to attenuate the constitutive muscarinic activation of ASM that emanates from the parasympathetic inputs of the vagi (Arteaga-Solis *et al.* 2013). The fact that leptin-deficient (*ob/ob*) and leptin receptor-deficient (*db/db*) mice are hyperresponsive is also supportive of the bronchodilator effect of leptin (Shore *et al.* 2003, Lu *et al.* 2006, Johnston *et al.* 2007, Arteaga-Solis *et al.* 2013). Leptin and leptin receptor deficiencies also worsen AHR in a murine model of asthma elicited by ozone exposure (Shore *et al.* 2003, Lu *et al.* 2006). In addition, infusion of leptin intracerebroventricularly successfully inhibits AHR observed in high-fat diet-induced obese mice, *ob/ob* but not *db/db* mice, as well as in mice with allergic airway inflammation (Arteaga-Solis *et al.* 2013).

However, the effect of leptin in the context of asthma is not clear. This subject has been reviewed recently (Sood & Shore 2013). The level of leptin in the blood and adipose tissue is generally elevated in asthmatics. The blood level further increases during exacerbation (Tsaroucha *et al.* 2013) and following allergen challenge (Shore *et al.* 2005). Circulating level of leptin also correlates inversely with lung function (Tsaroucha *et al.* 2013) and the expression of leptin in adipose tissue positively correlates with the level of airway responsiveness (Sideleva *et al.* 2012). In agreement with a potentially deleterious effect of leptin in asthma, continuous infusion of leptin by subcutaneously implanted micro-osmotic pumps aggravates AHR in mice sensitized and challenged with an allergen (Shore *et al.* 2005). This later result may seem to be counterintuitive to the protective effect on airway tone mentioned above (Arteaga-Solis *et al.* 2013). However, the method of leptin delivery was different between these studies; i.e. i.c.v. (Arteaga-Solis *et al.* 2013) vs implanted micro-osmotic pumps (Shore *et al.* 2005). Together, these results indicate that the route of leptin administration can greatly affect the outcome. Systemic delivery by micro-osmotic pumps may decrease lung function in the context of allergic inflammation by enhancing the immunization process (Shore *et al.* 2005, Johnston *et al.* 2007), while i.c.v. administration may ameliorate lung function with or without inflammation by mitigating the contractile effect of the parasympathetic nervous system on the airways (Arteaga-Solis *et al.* 2013).

Adiponectin The role of adiponectin in asthma has been reviewed recently (Sood & Shore 2013). Adiponectin is an adipokine that is mainly produced by visceral fat. It is an insulin-sensitizing hormone and generally has anti-inflammatory effects attributed. Several studies have demonstrated the relationships between adiponectin expression and asthma (Sood & Shore 2013). Increased serum levels of adiponectin seem to confer protection against asthma (Sood *et al.* 2008) and low serum levels predict future risk of asthma (Sood *et al.* 2012). The protective association of adiponectin with asthma is clearer in women than in men (Sood & Shore 2013). Adiponectin level is also decreased during asthma exacerbation in humans (Tsaroucha *et al.* 2013) and mouse models (Shore *et al.* 2006).

The potential mechanistic links between adiponectin and AHR were inferred from animals and *in vitro* studies (Sood & Shore 2013). Both adiponectin receptors, ADIPOR1 and ADIPOR2, are expressed in human ASM cells (Shin *et al.* 2008). A direct effect of adiponectin on ASM contractility is suspected because AHR is attenuated in allergic mouse models of asthma treated with adiponectin (Shore *et al.* 2006, Ionescu *et al.* 2012). However, the direct effect of adiponectin on ASM contractility remains unknown.

Blood-derived factors

Fibrin Fibrin is formed from the cleavage of fibrinogen by thrombin. It has an important role in blood clotting. However, fibrin is also detected along the luminal surface of distal airways in patients dying from asthma (Wagers *et al.* 2004). Inverse association exists between lung function and blood levels of fibrinogen (Thyagarajan *et al.* 2006, Huang *et al.* 2012). Although the effect of fibrin on ASM is undefined, mice lacking fibrinogen demonstrate an attenuated AHR in the context of allergic airway inflammation (Riesenfeld *et al.* 2012). Additionally, a combination of aerosolized fibrinogen and thrombin increases airway responsiveness in mice

(Wagers *et al.* 2004). However, this effect seems largely independent of a direct action on ASM. It is rather related to a decrease in surface tension, owing to the ability of fibrin to inactivate the surfactant.

Urokinase-type plasminogen activator   Urokinase-type plasminogen activator (uPA) is a serine protease that cleaves plasminogen into plasmin, a thrombolytic enzyme. Despite the fact that uPA may attenuate airway responsiveness by its fibrinolytic activity (Wagers *et al.* 2004), it may also foster ASM contractility. uPA is not capable of triggering ASM contraction directly but increases ASM sensitivity to acetylcholine (Nassar *et al.* 2010). Accordingly, ASM from mice deficient in uPA demonstrates reduced sensitivity to acetylcholine (Nassar *et al.* 2010). The effect is independent of uPA receptor and rather relies on the binding and cleavage of the *N*-methyl-D-aspartate receptor 1 (NMDAR1 (GRIN1)).

Stomach and intestines

Guanylin and uroguanylin   Guanylin and uroguanylin are small peptide hormones mainly studied for their effects on gastrointestinal and renal systems. Uroguanylin and guanylin are mainly produced by the intestinal mucosa in response to salt load. As uroguanylin is found in the blood and urine, the possibility that its release from the intestines can act on distant organs such as the airways cannot be precluded, especially in the context of inflammation where vascular permeability is increased. In fact, even in normal conditions, the effects of guanylin peptides on the functions of other organs, such as the kidneys (Moss *et al.* 2008) and the hypothalamus (Valentino *et al.* 2011), are achieved through endocrine mechanisms.

The presence of mRNA for uroguanylin receptor, the guanylyl cyclase C receptor, in the lung was the foundational discovery that had led to the investigation of the effects of guanylin and uroguanylin on airway functions. In the case of uroguanylin, inhalation or i.v. administration inhibits the increased respiratory system resistance induced by either allergen or leukotriene challenge (Ohbayashi & Yamaki 2000). This *in vivo* effect of uroguanylin is mediated, at least partially, by its ability to relax ASM (Ohbayashi *et al.* 1998). In the case of guanylin, its effects on both *in vivo* bronchodilation and relaxation of isolated ASM are rather marginal (Ohbayashi *et al.* 1998). Together, these results suggest that uroguanylin, and to a lesser extent guanylin, may influence airway contractility.

Gastrin-releasing peptide   Gastrin-releasing peptide (GRP) is a neuropeptide that stimulates the release of gastrin from the G cells of the stomach. Blocking GRP prevents the development of AHR in murine models of asthma induced by either ozone or allergen exposure (Zhou *et al.* 2011). Whether these *in vivo* effects are mediated by a direct effect on ASM contraction is not completely clear as GRP blockage also abrogates the inflammatory response. However, GRP contracts ASM more potently than substance P, a prototypical spasmogen (Lach *et al.* 1993).

Thyroid

Triiodothyronine and thyroxine   The effect of thyroid hormones on airway contractility is not clear. On one hand, rats rendered hypothyroid by thyroidectomy demonstrate an attenuated susceptibility for the development of experimental asthma, and this impairment is reestablished by supplementation with thyroxine (T_4; Manzolli *et al.* 1999). On the other hand, triiodothyronine (T_3) supplementation in euthyroid asthmatic children was proven to be beneficial to relieve symptoms and to taper down the usage of asthma medication (abdel Khalek *et al.* 1991). It may sound counterintuitive that the absence of thyroid hormone is protective in rats and adding T_3 in asthmatic children with already normal levels of thyroid hormones is beneficial. Together, these observations suggest that the relationship between thyroid hormones and asthma risk is complex. Recent evidence has suggested that a polymorphism in the gene encoding the thyroid hormone receptor influences the response to bronchodilators (Duan *et al.* 2013). This suggests that thyroid hormones affect asthma diathesis at least in part by mechanisms altering airway contractility.

It is likely that T_3 and T_4 can be either beneficial or deleterious in asthma. The direction of the effect probably depends on: i) the type of asthma and the mediators involved in excessive airway narrowing (Fig. 1); ii) the facets on which thyroid hormones are mainly intervening in any individual to either protect or exacerbate asthma (sensitization/inflammation, ASM contraction, etc.); and iii) the genetic predispositions to respond to either the beneficial or deleterious effect of thyroid hormones.

Heart

A-type natriuretic peptide   Cardiac regulation of airway contractility sounds improbable. However, A-type natriuretic peptide (ANP), which is mainly secreted by

the cardiac atria, is a potent bronchodilator in asthmatics (Hulks & Thomson 1994, Angus *et al.* 1996). The degree of bronchodilation achieved by infused ANP is very similar to the one obtained with β₂-agonists. The bronchodilator effect is mediated by direct relaxation of ASM (Hamel & Ford-Hutchinson 1986). The effect of ANP is quite transient, though, and more potent when administered by infusion than by inhalation (Angus *et al.* 1996). The bronchodilator efficacy of ANP either by inhalation (Angus *et al.* 1996) or by infusion (Angus *et al.* 1995) is prolonged by pretreatment with the neutral endopeptidase inhibitor thiorphan, suggesting that its transient action in mainly due to rapid degradation.

B-type natriuretic peptide B-type natriuretic peptide (BNP), which is predominantly secreted by the cardiac ventricles (Mukoyama *et al.* 1991), is another cardiac hormone that can potentially affect ASM tone. In fact, recombinant BNP is a potent bronchodilator in asthmatic patients, just as potent as β₂-agonists (Akerman *et al.* 2006). BNP mechanism of action also involved ASM relaxation (Matera *et al.* 2009, Edelson *et al.* 2013). However, the relaxing effect is mediated indirectly through the epithelium (Matera *et al.* 2011).

Gonads

Estrogens Several lines of evidence suggest that estrogens, such as estradiol, are bronchoprotective in asthma. Estrogen relaxes pre-contracted ASM preparations (Pang *et al.* 2002) and potentiates the relaxation induced by catecholamines and β₂-agonists (Foster *et al.* 1983, Townsend *et al.* 2012b). Estrogens also reduce the increased contraction observed in ASM passively sensitized with serum of atopic asthmatics (Dimitropoulou *et al.* 2005), as well as the *in vivo* responsiveness to an aerosolized mixture of spasmogens (Pang *et al.* 2002). Estrogen is also accountable for the sex disparity in the development of AHR to allergen exposure (Matsubara *et al.* 2008). On one hand, females are protected from allergic inflammation-induced AHR, and this is reversed by either ovariectomy or by treatment with an estrogen antagonist. On the other hand, treatment of males with estrogen protected them from AHR induced by allergic airway inflammation (Matsubara *et al.* 2008). Estrogen replacement therapy in ovariectomized mice also attenuates the development of airway dysfunction elicited by allergic airway inflammation (Dimitropoulou *et al.* 2009). Additionally, female estrogen receptor alpha-deficient mice are more sensitive to methacholine aerosol, either in the presence or the absence of allergic airway inflammation (Carey *et al.* 2007). This hypercontractile phenotype in receptor α-deficient mice is associated with decreased CHRM2 expression and function (Carey *et al.* 2007). Finally, low dose, but not high dose, of estrogens decreases airway responsiveness in ovariectomized rats in the absence of airway inflammation (Degano *et al.* 2003). This occurs in conjunction with decreased responsiveness of isolated ASM to acetylcholine, associated with an increased release of acetylcholinesterase from the epithelium (Degano *et al.* 2001). In contradistinction, a high dose of estrogens increases ASM contractility *ex vivo* and decreases lung function *in vivo* (Degano *et al.* 2003). The dose-dependent dichotomy of estrogen on ASM contractility may be accountable for the proposed deleterious effect of estrogen on asthma outcomes in humans (Lieberman *et al.* 1995a, Troisi *et al.* 1995). Despite these red flag studies, estrogen therapy in humans is generally beneficial in asthma, as it decreases airway responsiveness (Villa *et al.* 1990, Lieberman *et al.* 1995b) and improves lung function (Carlson *et al.* 2001) and symptoms (Myers & Sherman 1994, Chandler *et al.* 1997).

Androgens Androgens, such as testosterone, have an acute, non-genomic, and non-estrogenic effect on ASM activation (Kouloumenta *et al.* 2006, Bordallo *et al.* 2008, Montano *et al.* 2014). The direction of the effect depends on the state of ASM activation. On one hand, androgens relax pre-contracted ASM (Kouloumenta *et al.* 2006, Bordallo *et al.* 2008, Montano *et al.* 2014) and potentiate the relaxing effect of β₂-agonists (Foster *et al.* 1983, Bordallo *et al.* 2008), which suggest an overall bronchodilator effect. On the other hand, in the same ASM preparations and at the same concentrations, androgens potentiate the contractile response to a spasmogen, which suggest a constrictor effect (Bordallo *et al.* 2008). The controversy is also observed *in vivo*. On one hand, i.v. administration of androgens almost completely abrogates the increased lung resistance elicited by allergen challenge in sensitized animals (Montano *et al.* 2014). Testosterone therapy is also salutary in the treatment of symptoms of women suffering from premenstrual asthma (Wulfsohn *et al.* 1964). One the other hand, indirect evidences suggest that androgens foster contraction. For example, the spontaneously greater level of airway responsiveness observed in healthy male mice compared with females is eliminated by castration (Card *et al.* 2007). Administration of testosterone to either females or castrated males also increases airway responsiveness to methacholine (Card *et al.* 2007). The sex disparities in airway responsiveness

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and the potentiating effect of testosterone on airway responsiveness are abrogated by bilateral vagotomy, suggesting that testosterone increases airway responsiveness indirectly *in vivo* by acting through the vagal nerves (Card *et al.* 2007).

Progesterone The effect of progesterone on airway contractility has received less attention. Progesterone and one of its derivatives, 5β-pregnenolone, attenuate the contraction of isolated ASM (Perusquia *et al.* 1997). Progesterone also increases the relaxation induced by β₂-agonists (Foster *et al.* 1983). *In vivo*, progesterone does not affect the level of airway responsiveness in healthy mice (Hellings *et al.* 2003) but amplifies the development of AHR in a murine model of asthma (Hellings *et al.* 2003). The deleterious effect of progesterone on AHR seems to be a consequence of aggravated allergic airway inflammation (Hellings *et al.* 2003). This demonstrates that the use of a single mediator that relaxes ASM does not guarantee a beneficial outcome in a lung disorder as complex as asthma. Hormones, in particular, display pleiotropic effects and can clearly affect differently, and sometimes in opposite directions, the many facets of asthma. *In vivo*, some evidence suggests that progesterone can be beneficial in humans. For example, i.m. administration of progesterone is beneficial in a subgroup of asthmatics (Beynon *et al.* 1988).

The effect of sex hormones on asthma, airway responsiveness, and ASM contractility has received considerable interest (Townsend *et al.* 2012a). The results obtained so far are informative and promising, but not yet permissive to conclusive statements due to reported discrepancies. Segregating the patients according to sex and stage of life may help but may not be sufficient to predict the outcomes of hormonal therapy. The underlying mechanisms responsible for the manifestation of asthma symptoms are more likely to dictate whether hormonal therapy would be salutary, useless, or detrimental. Thus, the potential to improve the quality of life of asthmatics by tailored hormonal therapy exists, but there is probably no unique treatment regimen that will be efficacious for all afflicted patients.

Concluding remarks and future perspectives

The symptoms of asthma are largely related to airway narrowing caused by ASM shortening. Even though ASM is the effector tissue causing excessive airway narrowing, the defects that lead to its malfunction can originate from anywhere in the body. However, it is customary in asthma research to focus on lung factors that affect, via a paracrine mode of regulation, the contraction of ASM. The mechanisms by which dysfunctions of distant organs can affect airway contractility and asthma development/severity are also not intuitively appealing and have commensurately received less attention.

Nevertheless, solid evidences now indicate that ASM contractility is under the influence of many blood-derived mediators that originate from diverse organs, including the adrenals, pancreas, adipose tissue, gonads, heart, intestines, and stomach (Fig. 2). These mediators can act directly on the airways by either modifying the level of ASM activation (Fig. 1) or by altering the response to spasmogens and bronchodilators. Additionally, they can act indirectly via the brain or local nerve endings to amplify the parasympathetic influence of the vagi on the cholinergic activation of ASM. Together, these results highlight the importance of studying the whole organism, in addition to isolated cells, tissues, and organs.

The slow progress in asthma therapy (Holgate 2011, von Mutius & Drazen 2012), despite the extensive and long-standing research interest for that lung disorder, suggests that important mediators might have been overlooked. The endocrine regulation of airway contractility and its dysregulation in asthma are key areas of research that clearly deserve further investigation. These avenues of research are not conventionally addressed by current scientific approaches and are conducive to unexpected and breaking discoveries. A better understanding of the endocrine regulation of ASM contractility can actually be a prerequisite to leap forward the implementation of personalized medicine.

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