Chapter 10

MECHANISMS AND BIOMARKERS OF EXERCISE-INDUCED BRONCHOCONSTRICTION

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KEY WORDS

Hyperpnea; water loss; osmolarity; epithelium; mast cells; eosinophils; eicosanoids; sensory nerves

KEY POINTS

- The conditioning of inhaled air during exercise-hyperpnea initiates osmotic and vascular events that lead, in susceptible individuals, to airway narrowing.
- A loss of physical barrier intergrity and impairment in signaling and secretory functions of the airway epithelium increases the susceptibility to EIB.
- Airway smooth muscle contraction and mucin release in individuals with EIB are mediated predominantly by release of inflammatory mediators with associated activation of neural pathways.
- Cysteinyl leukotrienes and prostaglandin D_2 are the primary inflammatory mediators released into the airways from mast cells and eosinophils during EIB.

SYNOPSIS

Exercise is a common trigger of bronchoconstriction. In recent years, there has been increased understanding of the pathophysiology of exercise-induced bronchoconstriction (EIB). While evaporative water loss and thermal changes have been recognized stimuli for EIB for almost 50 years, accumulating evidence points toward a pivotal role for the airway epithelium in orchestrating the inflammatory response linked to EIB. Overproduction of inflammatory mediators, relative underproduction of protective lipid mediators, and infiltration of the airways with eosinophils and mast cells are all established contributors to EIB. Sensory nerve activation and regional airway closure maybe important in EIB, but further research is warranted.

(100 words)

INTRODUCTION

The underlying basis for exercise-induced bronchoconstriction (EIB) is becoming increasingly understood. Initial work starting in the 1970s revealed the major determinants of EIB in susceptible individuals. The aim of this review is: to examine the respective roles of evaporative water loss and thermal changes as stimuli to EIB; to provide evidence that loss of physical barrier functions of the epithelium during exercise-induced hyperpnea is associated with the development of bronchoconstriction in susceptible individuals; to discuss the central role of leukocyte activation and the associated generation of lipid mediators and release of neuropetides that sustain bronchoconstriction during EIB; and to consider the role that regional airway closure may play in the development of EIB.

CONDITIONING THE AIR INSPIRED DURING EXERCISE

Under most conditions of exercise the air inspired needs to be heated and humidified to body conditions [37^{o}C, 100% relative humidity (RH), or 44 mg H_2O/L] before it enters the alveoli. As a result, heat and water are lost from the airway surface during inspiration. The number of generations involved in conditioning depends on the level of ventilation reached and sustained during exercise, and the temperature and water content of the inspired air.(1)

Heat loss as a stimulus to airway narrowing

Cooling of the airways, from heat lost through vaporisation of water and from heating the inspired air, was initially identified as a potential stimulus for EIB.(2) The proposal was subsequently extended to include a rewarming of the airways after exercise.(3) (Figure 1) This hypothesis suggested that cooling initiated

vasoconstriction during exercise, followed by a reactive (or rebound) hyperaemia at the end of exercise.(3) These vascular events are most significant when air of sub zero temperature is inspired during intense exercise of 4 minutes or more (4) and when the smaller airways are recruited into the conditioning process, but are unlikely to occur in temperate or hot environments. These vascular events may be relevant to anyone performing vigorous exercise in cold conditions – although people with asthma may have a more rapid and exaggerated vascular response (5) –, and may serve to amplify airway narrowing in individuals with asthma with EIB.(6)

Water loss as a stimulus to airway narrowing

It is now well recognised that cooling and rewarming of the airways are not prerequisites for EIB. In individuals with asthma, EIB occurs when hot dry air is inhaled during exercise (7-9) and when the airways are not cooled below their temperature at rest.(10)

The concentration of water in the inspired air is an important determinant of EIB (11), with the rate of water loss during exercise relating directly to the severity of the airway response in an individual.(12) Importantly, the stimulus to EIB acts at the surface of the airways, because EIB is prevented simply by inhaling fully humidified air at body temperature.(11, 13) A loss of water by evaporation, in humidifying large volumes of air in a short time, is thought to cause a transient increase in concentration of ions (e.g., Na^+, Cl^-, Ca^{++}) in the airway surface liquid (ASL).(14) (Figure 1) This osmotic stimulus occurs after intense exercise when dry air of any temperature (cold or hot) is inspired, providing the duration of exercise is sufficient.

Because the surface area of the first 10 generations of airways is 742 cm^2 and contains less than 0.7 ml of ASL (15, 16), only a small loss of water would increase osmolarity above the normal value of ~320-340 mosm. A mathematical model, based on *in vivo* observations (17), predicted that breathing air of 26^oC and 35% RH at 60 L/min would result in a cumulative loss of 0.44 mL *per* minute from the first 12 generations of airways.(18, 19) It is likely that this rate of water loss exceeds the rate of replacement across the epithelium, leading to a transient increase in osmolarity of the ASL.

Although methodological limitations render the accurate measurement of changes in ASL osmolarity difficult (20), there is direct evidence for evaporative water loss from the airways involved in conditioning the air. Mucociliary clearance rate was shown to be reduced during hyperpnea with dry air (but not humid air).(21) This reduction, however, occurred more markedly in people with asthma compared with healthy individuals, suggesting that the rate of water movement across the epithelium towards the lumen is slower in populations prone to EIB.(21)

An increase in ion concentration of the ASL during exercise would act as an osmotic stimulus for transport of water to the airway surface from the epithelial cells and other cells on, or near the airway surface. Because the basolateral membrane is less permeable to water than the apical membrane, it was proposed that epithelial cells, as well as other cells, would have a reduction in volume. (22) It is the regulatory volume increase following cell volume decrease that is thought to provide the signal for mediator release. The source of water to replace the epithelial cell volume is the submucosa and small changes in its osmolarity could be the signal to the increase in

bronchial blood flow that occurs when breathing dry air.(23-25) The osmotic stimulus to EIB is further supported by evidence that airway narrowing can be provoked in asthmatics by inhalation of hyperosmolar saline.(26) Further, the airway response to hypertonic saline is related to mast cell number (assessed by brush biopsy).(27) Mediators released in response to osmotic challenge with dry powder mannitol (28) are the same as exercise (29) and blocked by the same drugs.(30, 31)

The transient increase in osmolarity of ASL during exercise creates an environment known to be favourable for release of mediators from cells in, or near the airway surface (e.g., epithelial cells, sensory nerves, mast cells and eosinophils). While some mediators [prostaglandin E_2 (PGE_2)] may induce bronchodilation during exercise, other mediators [neurokinins, PGD_2, cysteinyl leukotrienes (cyst-LTs)] provoke airway narrowing by acting, either directly on bronchial smooth muscle or, *via* sensory nerves. (Figure 2) (29, 31)

Temperature as a modulator of time course and severity of EIB

The severity of EIB is similar in the same individual over a wide range of inspired air temperatures, from cool (9^oC) to hot (36^oC), (Figure 3) providing that the inspired water content is low and remains the same. (7-9) This is consistent with the relatively constant value measured for water content of expired air (29-33 mg/L), when breathing air at 22-40°C that contains <13 mg H_2O /L.(24) The maximum airway response is thought to occur when generations 10 to 12 are recruited, and this may take 8 minutes when the inspired air is warm. It is here, in the non-cartilaginous airways, that the density of mast cells is high in individuals with asthma.(32) This may explain why warm dry air can still cause a similar fall in expiratory flow to cool

air.(8) The cooler the inspired air, the faster the required generations will be recruited, and the earlier the response will occur.

EPITHELIAL DYSFUNCTION IN EIB

The airway epithelium is the first structural barrier to the inhaled environment in the airway mucosa. Under normal circumstances, the epithelium forms a highly regulated and tight barrier that limits penetration of inhaled allergens, pathogens, pollutants and other toxic compounds into the internal lung environment. Exercise hyperpnea can cause acute disruption of the airway epithelium (33-35). Shedding of epithelial cells into the airway lumen after exercise challenge has been shown in individuals with asthma with EIB (36). A (partial) loss of physical integrity of the epithelial barrier is likely to disrupt airway homeostasis at a number of levels. Disruption of ASL balance (resulting in malfunction of the mucociliary escalator), plasma exudation (resulting in edema), and attraction and activation of inflammatory cells in response to injury can all compromise airway homeostasis. Instrinsic dysfunction of the airway epithelium – as found in individuals with asthma (37) – and/or extreme levels of ventilation – as developed by endurance athletes (38) – are likely to increase the susceptibility to /extent of airway epithelial injury and, thereby, explain the high prevalence of EIB reported in both these populations (39, 40).

Breaking through the epithelial barrier

As minute ventilation increases during exercise, so does the load of foreign substances entering the airways. Disruption of epithelial tight junctions and/or gaps into the epithelium facilitates the penetration of foreign substances, enhancing the potential for toxic, immune, and inflammatory responses, with ensuing

bronchoconstriction in susceptible individuals. During the pollen season, the severity of airway narrowing after outdoor exercise is increased in individuals with asthma with birch pollen allergy (41). The cellular infiltration and activation of mast cells and T cells (and possibly eosinophils) observed during natural allergen exposure in atopic individuals with asthma (42) would render the airway smooth muscle more responsive to the stress of exercise. Further, common air pollutants and irritants (such as ozone or chlorine derivatives) have the potential *per se* to acutely disrupt the lung epithelial barrier (43, 44). Inhalation of noxious airborne agents during exercise hyperpnea could therefore aggravate the damage to the epithelium, and increase the risk/severity of EIB. In support of this concept, a high prevalence of EIB has consistently been reported in athletes who train and compete in polluted environments. (1)

Disturbance of ASL

Apart from being a barrier, the airway epithelium plays other roles, including maintenance of ASL levels (45). Intact epithelial cells sense and autoregulate ASL height by active ion transport (46). A damaged airway epithelium would loose its ability to tightly regulate water movements, potentiating the osmotic effects of exercise hyperpnea and, potentially increasing the severity of EIB. In individuals with asthma with EIB, the extent of airway injury (as assessed by the percentage of columnar epithelial cells in sputum) at baseline has been shown to correlate with the severity of the airway narrowing after exercise (29).

Mucus dysfunction

Abnormal water movement, as a consequence of airway epithelial injury, also leads to alterations in rheological properties of mucus, with subsequent impairement of

mucociliary clearance and plug formation. A thicker and more elastic mucus is harder to clear from the airways and provides an environment for microbial growth, leading to infection and inflammation (47). In children with asthma (a population prone to EIB), viral infections of the airways are frequent and commonly associated with disease exacerbations (48). Further, in elite athletes with asthma and/or EIB, an increase respiratory infection susceptibility has recently been reported (49).
Mucus plugs could also directly contribute to lumenal obstruction in EIB. In individuals with asthma with EIB, exercise was found to initiate the release of MUC5AC (i.e., the predominant gel-forming mucin of goblet cells) (50). Moreover, an hyper-activity of the mucus-secreting goblets has been reported in both, individuals with asthma with EIB (50) and elite athletes (51). Therefore, alterations in mucosal rheology secondary to epithelial dysfunction are likely to contribute to airflow obstruction and post-exercise symptoms (such as cough and mucus hypersecretion).

Epithelial damage-restitution

Restitution of the epithelium upon injury is associated with many tissue responses including, amongst others, plasma exudation (52). Plasma exudate in the airway lumen could not only contribute to EIB through mucosal/submucosal edema, but may also exacerbate some of the events described previoulsy (i.e., shedding of epithelium, impairment of mucociliary transport and mucus plug formation) (53). Further, plasma exudate may cause airway inflammation and constriction due to its content of powerful mediators (52). In the context of elite sport, repeated plasma exudation has been proposed, *via* release of a wide range of chemoattractant factors and plasma proteins (such as cytokines and growth factors), to contribute to the development of EIB (54). In elite athletes, late onset (>25 yr) of EIB is common (55) and a change of

the contractile properties of the airway smooth muscle is likely to result from combined effects of inflammatory and physical environmental stimuli. In asthma, due to pre-existing inflamed airways, the potent plasma-derived proteins-peptides released in response to hyperpnea-induced epithelial injury could activate an inflammatory cascade, with ensuing bronchoconstriction.

LEUCOCYTE ACTIVATION AS A FUNDAMENTAL MECHANISM OF EIB

The acute response

There is strong evidence that the sustained bronchoconstriction following exercise and dry air hyperpnea challenges is due to the release of leukocyte-derived inflammatory mediators in the airways. Similar evidence indicates that hyperosmolar aerosols lead to the release of leukocyte-derived mediators. (28) Specifically, leukocyte-derived eicosanoids including the leukotrienes (LT) and prostaglandins (PG) play a pivotal role. Following exercise challenge in asthmatics with EIB, there is sustained release of cysteinyl LTs (Cys-LTs, LTs C_4, D_4 and E_4) and PGD_2 into the conducting airways, corresponding with the development of acute bronchoconstriction (29, 31, 56). The release of these mediators is attenuated under conditions that reduce the severity of EIB (29, 31, 56, 57). Pharmacological inhibitors of this pathway unequivocally demonstrate that the release of these mediators play a causative role in the pathogenesis of EIB. (29, 31, 58) Inhibition of the key initial enzyme in LT biosynthesis, 5-lipoxygenase (5-LO) with either a 5-LO inhibitor or through inhibition of the 5-LO activating protein (FLAP)(59), significantly reduces the severity of EIB (60). Since LT receptor antagonists (LTRAs) that block the Cys-LT_1 receptor similarly reduce the severity of EIB (29, 60), LTD_4 is likely to mediate bronchoconstriction (as LTD_4 is the primary agonist for the $CysLT_1$ receptor).

However, the inhibition of EIB by LT modifiers is incomplete, implicating other bronchoconstrictive eicosanoids [such as PGD_2 and 15*S*-Hydroxyeicosatetranoic Acid (15*S*-HETE)], and/or the reduction in bronchoprotective mediators (such as PGE_2). PGD_2 binds to two major receptors; DP1 and DP2, or CRTH2. There has been intense interest in development of CRTH2 inhibitors in chronic asthma because this receptor serves as a key regulator of leukocyte activation, but the results of such inhibitors for treatment of stable asthma have been modest (61). With respect to EIB, the DP1 receptor may serve as the key receptor mediating bronchoconstriction and cough, as the DP1 receptor serves as a key activator of capsaicin sensitive sensory neurons (see below)(62).

Although mediators that initiate bronchoconstriction directly are largely attributed to products derived by leukocytes, the epithelium can directly release mediators, such as 15*S*-HETE, and can serve as a source of the initial products of eicosanoid metabolism, leading to leukocyte-derived mediators by transcellular metabolism. In some cases, these alterations in eicosanoid synthesis can lead to shunting of arachidonic acid (AA) away from the production of PGE_2 and to an imbalance between the pro-inflammatory mediators, such as Cys-LTs relative to PGE_2 (63). In addition to shunting of AA away from PGE_2 production, there are other alterations in the epithelium under the influence of IL-13 that reduce the production of PGE_2 (64).

Mast cells and eosinophils are strongly implicated as the cellular sources of Cys-LTs and other eicosanoids (such as PGD_2) in EIB. Following exercise challenge, histamine and the mast cell protease tryptase are released into the airways, demonstrating mast cell degranulation (29). The eosinophil product eosinophilic

cationic protein (ECP) is released into the airways following challenge, and the amount of ECP release varies with the severity of the EIB under different experimental conditions (56). In an analogous situation, pharmacological inhibitors administered prior to mannitol challenge revealed that histamine and possibly PGD_2 are responsible for the early onset of bronchoconstriction, (30, 31, 65), while the release of CysLTs is responsible for sustained bronchoconstriction (30, 66).

The cellular and molecular phenotype associated with EIB

The susceptibility to develop bronchoconstriction in response to hyperpnea challenge varies widely among subjects with established asthma and among individuals without a prior diagnosis of asthma. Thus, the presence of EIB following a specific challenge tests represents clinically recognizable phenotype of asthma. Recent work to understand the biological basis of this syndrome has revealed a consistent biological endotype related to cellular inflammation, particularly the density or mast cells in the airway epithelium (67, 68). Quantitative morphometry of airway biopsies, using design-based stereology, showed that the density of mast cells localized to the airway epithelium is significantly higher in subjects with EIB relative to individuals without asthma and those with asthma who do not have EIB (Figure 4 A&B) (69). Genome-wide expression studies of airway epithelial samples have also revealed high expression of the mast cell genes tryptase and carboxypeptidase A3 (CPA3), but low expression of chymase (Figure 4 C&D) (69, 70). This unique intraepithelial mast cell phenotype is also a biomarker of the "Th2 high" molecular phenotype of asthma (71, 72) that is IL-13 mediated (73, 74). The association between airway eosinophilia and the severity of EIB also provides evidence of this association with "type-2" inflammation (36, 75). Further, modifications of airway proteins that reflect

eosinophil activation are associated with the severity of EIB (76). Individuals with EIB have higher levels of Cys-LTs in induced sputum (36) and in exhaled breath condensate (EBC) (77). Higher levels of Cys-LTs in this group likely reflects the ability of mast cells and eosinophils to direct the eicosanoid pathway towards the synthesis of Cys-LTs as these cells are the predominant source of LTC_4 synthase (LTC_4S), the key enzyme directing this pathway towards Cys-LT formation in the airways (78). The rate-limiting step in eicosanoid formation occurs at the initial release of AA by the hydrolysis of the sn-2 position of membrane phospholipids by a family of phospholipase A_2 (PLA_2) enzymes. Although the cytosolic PLA_2s are known to serve this regulatory function, recent work has revealed that secreted PLA_2 ($sPLA_2$) activity is increased in asthma and is predominantly derived from $sPLA_2$ groups IIA and X (i.e., $sPLA_2$-IIA and $sPLA_2$-X) (63, 79). Further work has revealed that the level of $sPLA_2$-X is elevated in the airways of subjects with asthma, and tends to be higher in subjects with EIB (Figure 5) (80). In addition, $sPLA_2$-X is post-translationally activated by transglutaminase 2 (TGM2), an enzyme that is overexpressed in the airways of patients with EIB (70). As the key source of $sPLA_2$-X is the epithelium, and the enzyme can serve as an activator of myeloid cells, such as eosinophils, to generate the production of Cys-LTs (81), these results raise the possibility that $sPLA_2$-X serves as a critical initiator of eicosanoid production in response to exercise-hyperpnea.

Other markers of airway inflammation have also been associated with EIB, including an increase in the fraction of exhaled nitric oxide (F_{ENO}) (82), especially in subjects with atopy (83). The levels of 8-isoprostanes, non-enzymatic products of phospholipid oxidation, are increased in EBC of asthmatics with EIB, and correlated with the

severity of EIB (84). A reduction in the level of the protective eicosanoid lipoxin A4 has also been described in EIB (85).

In summary, patients who are susceptible to EIB have epithelial shedding, overproduction of inflammatory mediators, such as Cys-LTs, relative underproduction of protective lipid mediators, and infiltration of the airways with eosinophils and mast cells (Figure 2).

Refractoriness to repeated exercise

A refractory period after EIB for ~2 hours occurs in 50 to 60% of people with asthma. This refractoriness is not due to a reduction in heat or water loss from the airways with repeated exercise. Many theories have been suggested to explain refractoriness.(86) The most compelling involve release of mediators during exercise and their sustained presence after exercise. One theory relates to the bronchodilator effect of PGE_2 and the second relates to development of tolerance to the mediators of bronchoconstriction, particularly to the cyst-LTs. The finding that non-steroidal anti-inflammatory agent indomethacin could prevent refractoriness to EIB was explained on the basis of blocking production of PGE_2 during initial challenge.(87) It is also known that refractoriness to exercise occurs after challenge with LTD_4.(88) Increased levels of PGE_2 has recently been reported during the refractory period following hyperpnea of dry air.(89) It has been proposed that repeated stimulation of the cyst-LT receptors lead to their internalisation on the bronchial smooth muscle that would explain refractoriness. When mediators were measured in response to repeated challenge with inhaled mannitol, the subjects most refractory to the second challenge had significantly higher levels of mediators following challenge.(90) Both

prostaglandins and leukotrienes work through G protein coupled receptors and the events proposed to explain refractoriness are illustrated in Figure 6.(86)

NEUROGENIC FACTORS AND EIB

Sensory nerve activation

The airways are innervated by network of sensory nerves that sense mechanical, nociceptive and inflammatory signals through afferent C and A fibers. These sensory neurons send signals centrally, leading to a coordinated response with the efferent parasympathetic nervous system (see below), as well as through a local reflex called retrograde axonal transmission. It is becoming increasingly clear that airway hyperresponsiveness (AHR) is mediated through alterations in both the sensory and autonomic nervous system pathways in the airways (91). This neural plasticity in asthma is the result of changes in the composition of the different types of sensory neurons, the production of neurokinins, such as neurokinin A (NKA) and substance P (SP), and the density of transient receptor potential (TRP) channels, such as TRP vanilloid receptor 1 (TRPV1) which serves as the receptor for capsaicin. These changes are the consequence of chronic inflammation and the generation of neurotropins, such as nerve growth factor (92). In murine models, the sensory neurons are required for the development of AHR (93). The precise anatomical changes in the sensory nervous system of the airways in human asthma are not known in detail; however, the threshold for activation of the sensory nerves by the TRPV1 agonist capsaicin is lowered in asthma (94).

In animal models of EIB, it is well established that the sensory nerves mediate bronchoconstriction in response to a period of dry air hyperpnea, and that the

generation of leukocyte-derived eicosanoids plays the dominant role in activating this neural pathway. In a guinea pig model of hyperpnea-induced bronchoconstriction (HIB), inhibition of Cys-LT signaling, with either a 5-LO inhibitor or a LTRA, inhibited HIB and the release of neurokinins, while a neurokinin 2 receptor antagonist inhibited HIB, but not the release of leukotrienes, suggesting that leukotrienes cause bronchoconstriction *via* sensory nerves during HIB (95). In a dog model, a combination neurokinin 1 and 2 receptor antagonist inhibited HIB and the generation of LTs that are known in this model to cause HIB (96). Sensory nerves are activated directly by osmotic stimuli, but several eicosanoids can either directly activate or alter the activation threshold of sensory nerves (97). A recent study demonstrated that PGD_2, which is predominantly derived from mast cells, initiates sensory nerve activation through the DP1 receptor, regulating airway tone (62). Although the function of neurokinins during EIB has not been fully tested, there is evidence that the levels of NKA and Cys-LTs in the airways are correlated with the amount of bronchoconstriction following exercise challenge and with the release of MUC5AC (50). Overall, these findings suggest that in humans, bronchoconstriction and mucus release following exercise challenge is the consequence of sensory nerve activation that is initiated by the release of eicosanoids from airway leukocytes, such as mast cells.

THE ROLE OF REGIONAL ALTERATIONS IN THE AIRWAYS AND THE DEVELOPMENT OF EIB

In model systems, particularly in animal models with either airway injury or inflamed airways, the mechanism leading to AHR includes regional altertions in airway diameter that are heterogenous, leading to regional airway closure that contributes to

the development of airflow obstruction (98, 99). With the use of hyperpolarized helium imaging to assess regional ventilation, it is apparent that the development of airflow obstruction after exercise represents heterogeneous effects on airway caliber, ranging from conducting airway narrowing to frank airway closure (100). Treatment to reduce the severity of EIB reduces these heterogeneous regional defects in ventilation (101). These findings suggest that the stimulus for EIB causes bronchoconstriction in regions of the airways that are specifically susceptible to this stimulus, most likely because of alterations in the airway wall related to aiway inflammation. Such regional alterations in ventilation are also known to be present at baseline in subjects with asthma, and are associated with the severity of asthma (102). There is evidence that areas of regional heterogeneity in ventilation tend to persist over time, further suggesting that these regions of the airways have heightened susceptibility to airway narrowing (103). Additional studies are needed to fully understand the nature of these regional alterations in airway structure and airway dysfunction in humans.

SUMMARY/FUTURE CONSIDERATIONS

Water evaporation from the airways and thermal changes during exercise-hyperpnea are well established stimuli to EIB. In recents years, it has become increasingly clear that a dysfunctional airway epithelium – characterised by a loss of physical barrier integrity and impairment in signaling and secretory functions – renders individuals with asthma, as well as elite athletes susceptible to EIB. In these two populations, lipid mediator release (cys-LTs and PGD_2) has repeatedly been observed in response to exercise challenge (or its surrogates), highlighting the inflammatory nature of the condition. Additional work is warranted to define the exact role of sensory nerves and

of regional alterations in ventilation in the pathogenesis of EIB. As EIB represents a precise asthma phenotype that can be characterized clinically, and is associated with an increasingly well defined biological endotype, these findings should prove useful to target therapy for this important aspect of asthma.

(4,132 words)

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FIGURE CAPTIONS

Figure 1. Both airway cooling and mucosal dehydration occur in response to evaporative water loss from the airway surface. These events lead to exercise-induced bronchoconstriction. The figure is taken from Rundell et al. (1), and modified, with permission, from the original by Anderson and Daviskas (24).

Figure 2. Cells involved in exercise-induced bronchoconstriction. The epithelial cell layer is the source of prostaglandin E_2, a bronchodilating prostaglandin. The levels are lower in asthmatics. The mast cells are the source of prostaglandin D_2 and cysteinyl leukotrienes and eosinophils are also a source of cysteinyl leukotrienes. These bronchoconstricting mediators likely act via sensory nerves to cause smooth muscle contraction and airway narrowing. This has been demonstrated indirectly by the increase in leukotrienes being related to the increase in mucin (MUC5A) released from goblet cells and the release of neurokin A. The bronchial smooth muscle may also be stimulated directly by some of these same mediators. The figure is taken from Rundell et al. (1), and modified, with permission, from the original by Hallstrand et al. (104).

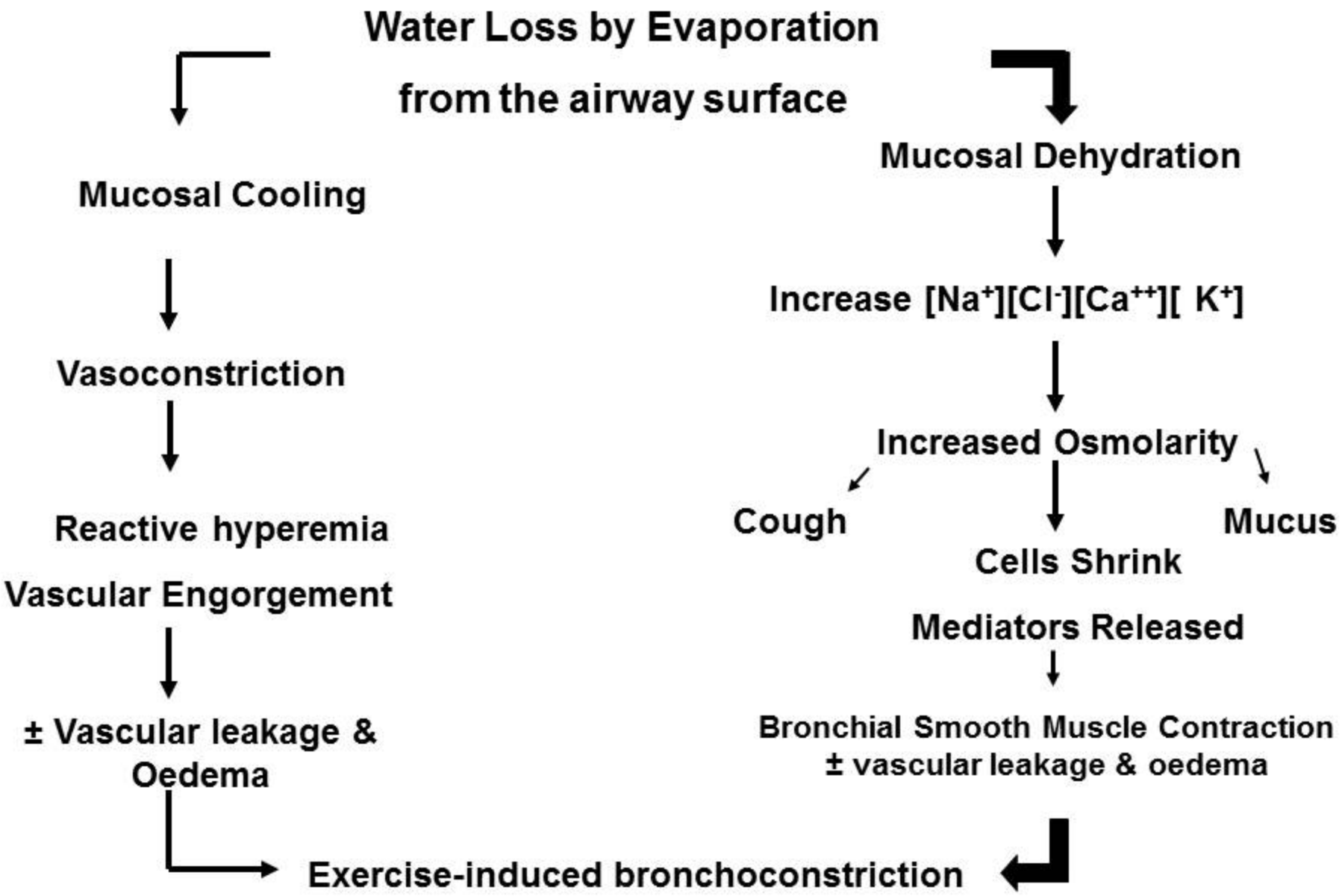
Figure 3. The severity of the bronchoconstriction after exercise is similar in the same individual over a wide range of inspired air temperatures. Panel A drawn from Deal et al., (2), with the reduction in forced expiratory volume in 1 sec (FEV_1) after exercise expressed as percentage of the pre-exercise level; Panel B drawn from Hahn et al. (8), with the reduction in peak expiratory flow rate (PEFR) expressed as percentage of the pre-exercise level.

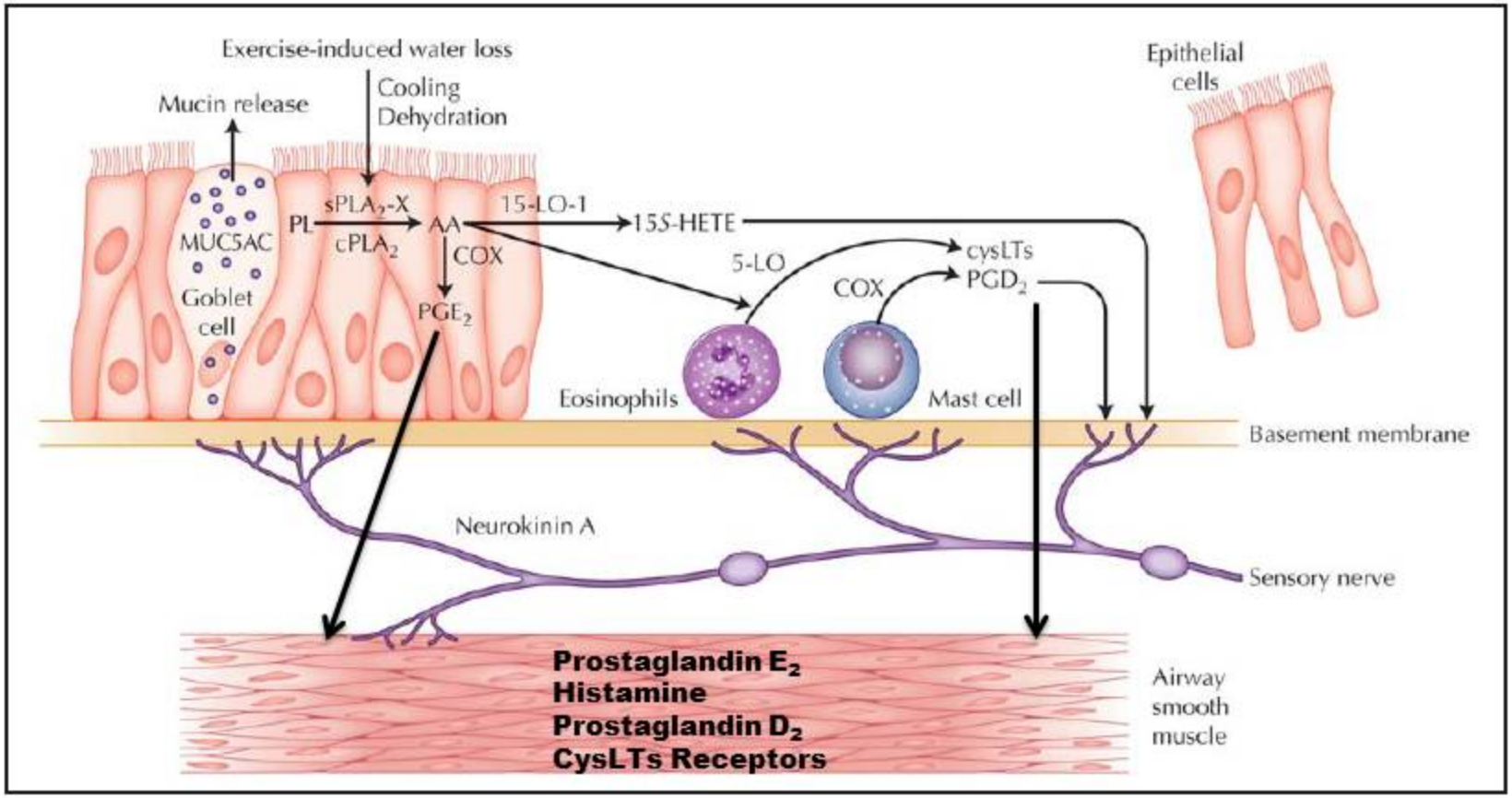
Figure 4. Mast cell infiltration of the airway epithelium in asthma. Quantitative morphometry of endobronchial biopsy samples and epithelial brush gene expression from individuals without asthma (Ctrl) compared with individuals with asthma and EIB (EIB+) and individuals with asthma without EIB (EIB-). Quantitative morphometry reveals that the number of mast cells per volume of airway epithelium (A) and the number of mast cells per area of the basal lamina (B) are substantially increased in individuals with EIB compared to asthmatics without EIB and normal controls. Epithelial brushings show a pattern of mast cell gene expression of tryptase (C) and CPA3 (D) with high expression of these genes in EIB, but lower expression of Chymase (data not shown). Adapted from Lai et al. (69), with permission.

Figure 5. Secreted phospholipase A_2 (sPLA$_2$-X) protein levels in the airways in asthma. Quantitative assessment of sPLA$_2$-X protein levels in induced sputum supernatant and lysates of epithelial brushings from the lower airways in individuals without asthma (Ctrl) compared with individuals with asthma and EIB (EIB+) and individuals with asthma without EIB (EIB-). Induced sputum levels of sPLA$_2$-X were elevated in asthma, particularly in individuals with EIB (A). The levels of sPLA$_2$-X in the epithelium were elevated only in the asthma group with EIB (B). Adapted from Hallstrand et al. (80), with permission.

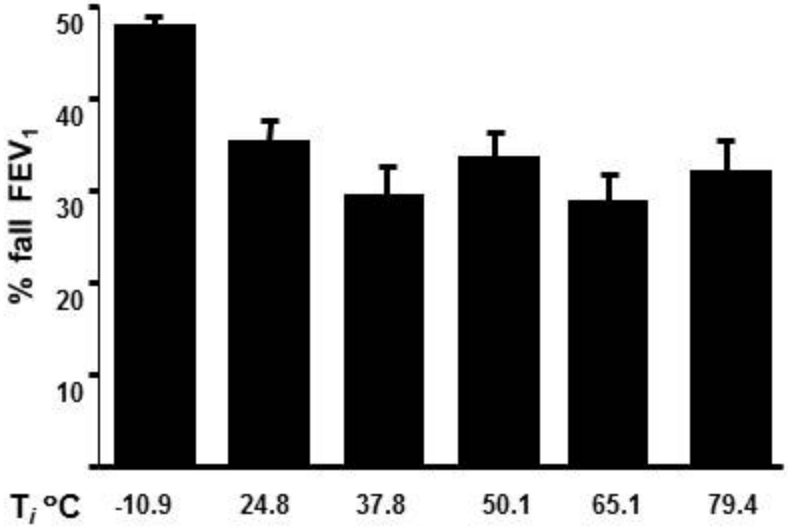
Figure 6. Desensitization of Cys-LT1 as a mechanism of refractoriness. With exercise, there is release of cysteinyl leukotrienes from mast cells that stimulate the GPCR Cys-LT1 causing contraction of the muscle and exercise-induced bronchoconstriction. The PGs generated during the same exercise could, via stimulation of the GPCRs DP1/EP1-4/TP, lead to crosstalk so that the Cys-LT1 is

desensitized through phosphorylation and internalization. Refractoriness to a second exercise challenge within a short period occurs as a result. When the levels of mediators return to baseline values, the Cys-LT1 receptors are recycled back to the cell surface and the muscle becomes sensitive to further stimulation. When generation of PGs is inhibited by agents, such as indomethacin and flurbiprofen, then there is limited desensitization of the Cys-LT1 and, therefore, limited refractoriness. Cys-LT1, cysteinyl leukotriene receptor1; DP1, prostaglandin D receptor1; EP1-4, prostaglandin E receptor1-4; GPCR, G-protein coupled receptor; TP, thromboxane receptor. From Larsson et al. (86), with permission.

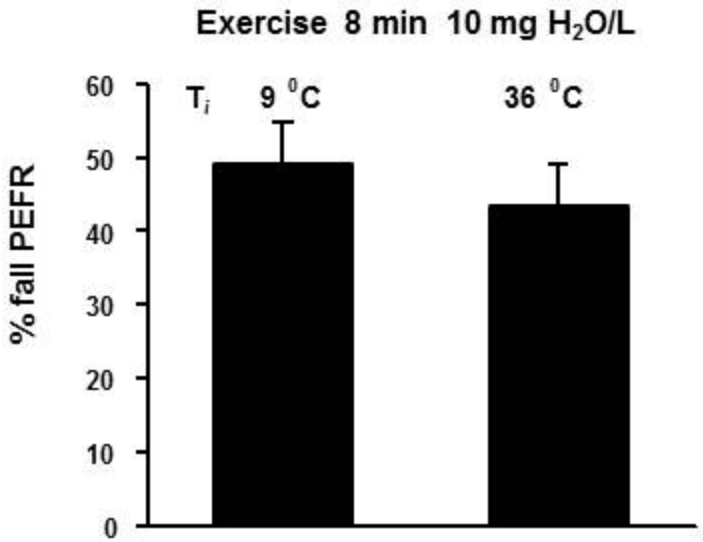

Water Loss by Evaporation
from the airway surface
Mucosal Cooling
Vasoconstriction
Reactive hyperemia
Vascular Engorgement
± Vascular leakage & Oedema
Mucosal Dehydration
Increase [Na+][Cl-][Ca++][K+]
Increased Osmolarity
Cough
Mucus
Cells Shrink
Mediators Released
Bronchial Smooth Muscle Contraction
± vascular leakage & oedema
Exercise-induced bronchoconstriction

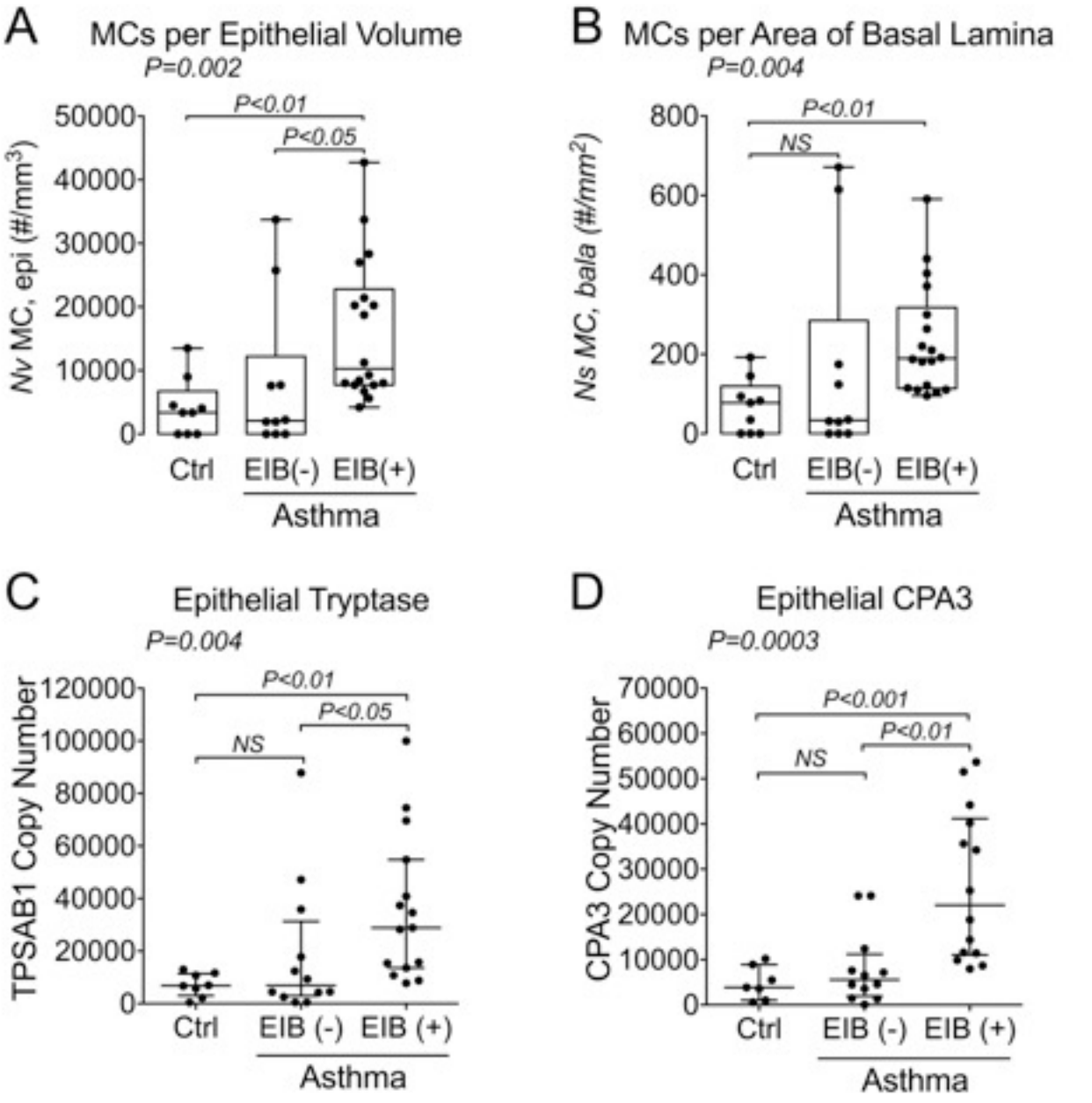

Exercise-induced water loss
Cooling
Dehydration
Mucin release
Epithelial cells
MUC5AC
Goblet cell
PL
sPLA2-X
cPLA2
AA
COX
PGE2
15-LO-1
15S-HETE
5-LO
cysLTs
PGD2
COX
Eosinophils
Mast cell
Basement membrane
Neurokinin A
Sensory nerve
Prostaglandin E2
Histamine
Prostaglandin D2
CysLTs Receptors
Airway smooth muscle

A


Exercise 4 min Dry Air
% fall FEV1
50
40
30
20
10
Ti °C
-10.9
24.8
37.8
50.1
65.1
79.4

B


Exercise 8 min 10 mg H2O/L
% fall PEFR
60
50
40
30
20
10
0
Ti 9 °C
36 °C


A
MCs per Epithelial Volume
P=0.002
P<0.01
P<0.05
Nv MC, epi (#/mm3)
50000
40000
30000
20000
10000
0
Ctrl
EIB(-)
EIB(+)
Asthma
B
MCs per Area of Basal Lamina
P=0.004
P<0.01
NS
Ns MC, bala (#/mm2)
800
600
400
200
0
Ctrl
EIB(-)
EIB(+)
Asthma
C
Epithelial Tryptase
P=0.004
P<0.01
P<0.05
NS
TPSAB1 Copy Number
120000
100000
80000
60000
40000
20000
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Ctrl
EIB (-)
EIB (+)
Asthma
D
Epithelial CPA3
P=0.0003
P<0.001
P<0.01
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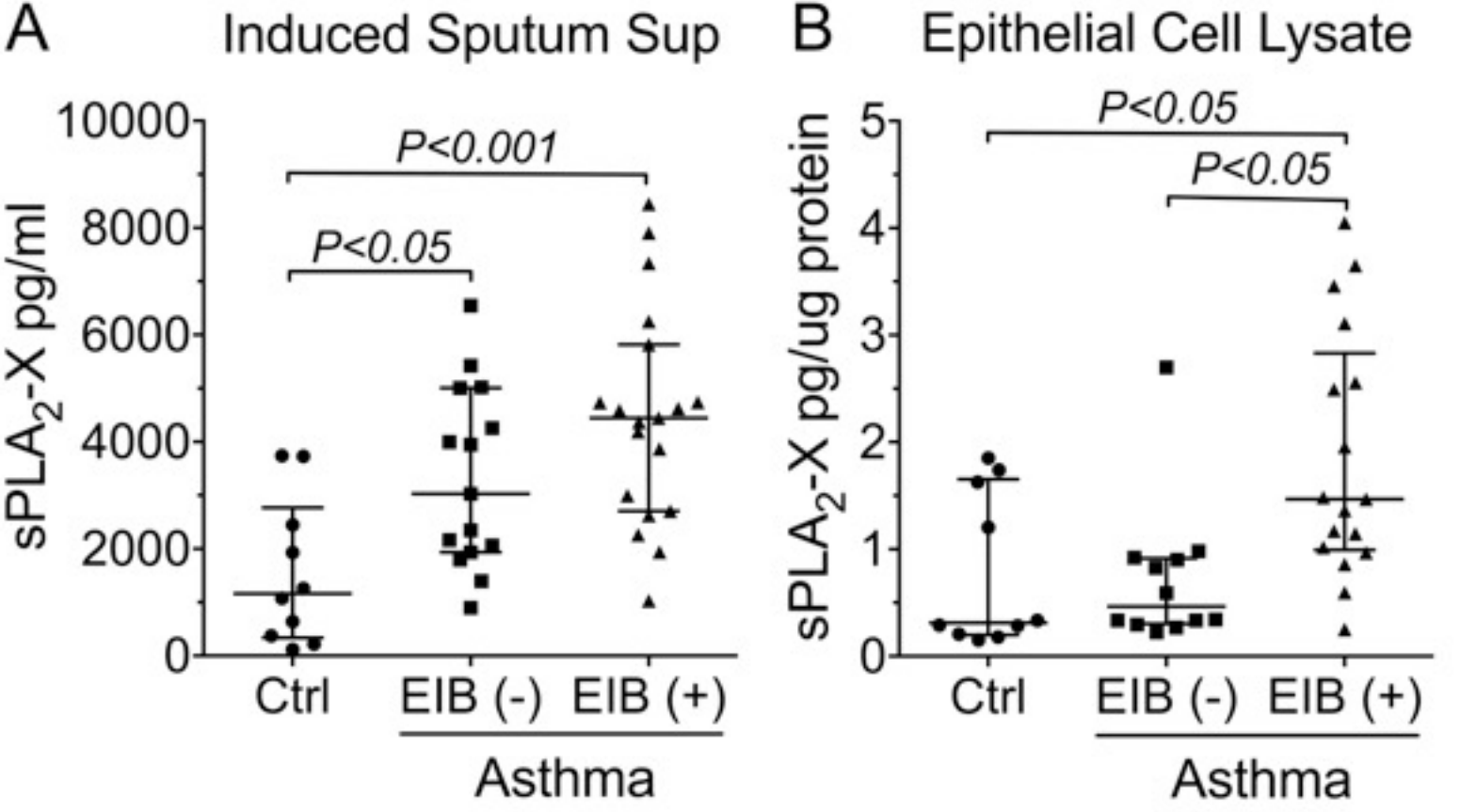
Asthma


A
Induced Sputum Sup
sPLA2-X pg/ml
10000
8000
6000
4000
2000
0
P<0.001
P<0.05
Ctrl
EIB (-)
EIB (+)
Asthma
B
Epithelial Cell Lysate
sPLA2-X pg/ug protein
5
4
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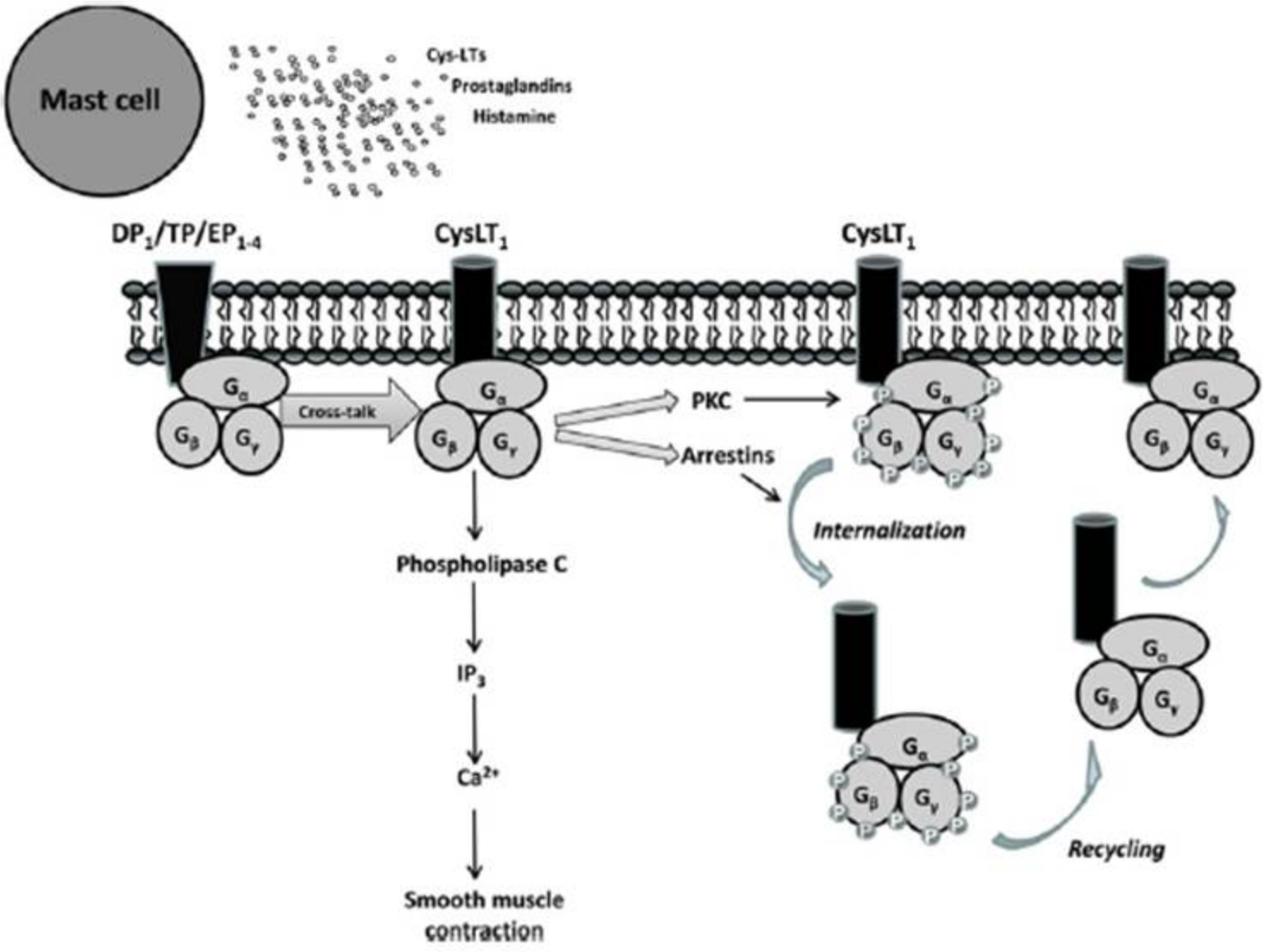
Asthma


Mast cell
Cys-LTs
Prostaglandins
Histamine
DP1/TP/EP1-4
CysLT1
CysLT1
Gα
Gβ
Gγ
Cross-talk
PKC
Arrestins
Internalization
Phospholipase C
IP3
Ca2+
Smooth muscle contraction
Recycling