

Refractoriness to Exercise Challenge

A Review of the Mechanisms Old and New

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KEYWORDS

• Asthma • Exercise • Refractoriness • Leukotriene • Prostaglandin

KEY POINTS

- Exercise-induced bronchoconstriction is defined as a decrease in forced expiratory volume in 1 second of 10% to 15% or more and is seen in most patients with asthma.
- Refractoriness, a decreased responsiveness to repeated exercise challenge, is an inborn protective mechanism with an unknown mechanism of development.
- Refractoriness is not associated with decreased levels of the primary mediators of bronchoconstriction, cysteinyl leukotrienes, or prostaglandin D₂, as previously suggested.
- Desensitization of the cysteinyl leukotriene receptor (CysLT₁) through interplay between leukotrienes and prostaglandins is suggested as a possible mechanism for refractoriness.

INTRODUCTION

“Exercise-induced bronchoconstriction (EIB) and exercise induced asthma are the terms used to describe the transient increase in airways resistance that follows vigorous exercise.”¹ Most untreated patients with asthma will experience bronchoconstriction after exercise,^{2–4} and the prevalence of positive responses in those being

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treated with inhaled corticosteroids is about 50%.^{5,6} EIB is identified by measuring a decrease in forced expiratory volume in the first second of expiration (FEV₁) after exercise of at least 10% to 15% from the pre-exercise value. Recovery of the FEV₁ normally occurs within 30 to 60 minutes but can be aided by inhaling a β_2 -agonist. Most of those with EIB are atopic.^{7,8} Late asthmatic responses are very uncommon, if ever present following EIB.^{9,10}

When the same exercise challenge is repeated within 1 hour, about 50% of patients with asthma will demonstrate less than half the response they had on the initial challenge, as measured by a decrease in FEV₁ or peak expiratory flow (Fig. 1).^{11,12} This decreased response to repeated challenges is called *refractoriness* and its duration is called the *refractory period*. To avoid false identification of refractoriness, it is essential that the lung function returns to close to the baseline value before the challenge is repeated (see Fig. 1). The precise mechanism for refractoriness is not known, although there have been several proposals from studies performed in patients with clinically

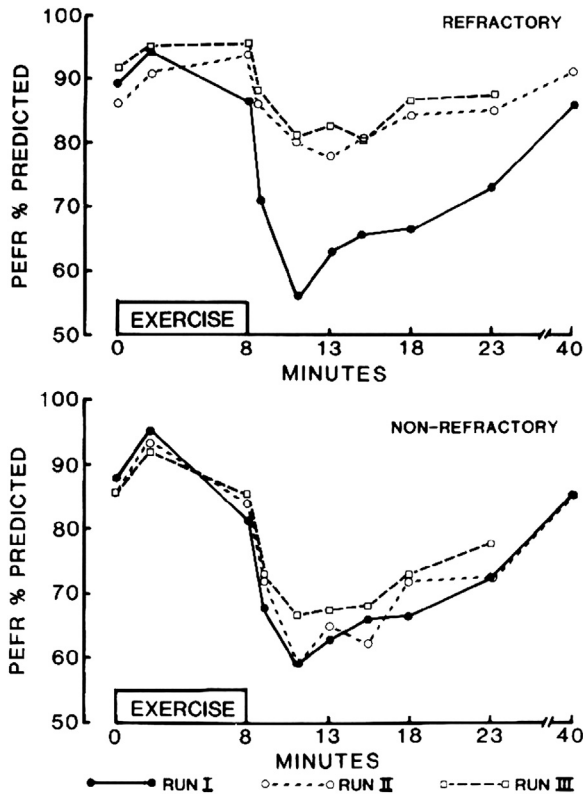


Fig. 1. Bronchoconstrictive response in patients with asthma on repeated exercise challenge. Peak expiratory flow rate (PEFR) expressed as a percentage of the predicted normal value before, during, and after 3 exercise challenges separated by 40 minutes in 16 patients with asthma. PEFR has now been replaced by FEV₁ as the lung function test to measure EIB. The patients included all returned to within 10% of baseline lung function before the start of the subsequent challenge. Refractoriness was seen in 8 patients, whereas no protection was seen in 8 patients. (From Schoeffel RE, Anderson SD, Gillam I, et al. Multiple exercise and histamine challenge in asthmatic patients. *Thorax* 1980;35:164-70; with permission.)

recognized asthma over the last 30 years. Identification of this protective mechanism is advantageous, first, because it has the potential to improve control and resolution of one of the most common causes of an attack of asthma and, second, because elucidation of the mechanism also has the potential to introduce a new target for treatment of asthma in general. The authors have reviewed the current clinical investigations into the stimulus and mechanism of EIB and the previously proposed mechanisms for refractoriness. The mechanism most generally accepted involves the release of a protective prostaglandin (PG), presumably PGE₂, during the first challenge. This mechanism has, however, not been conclusively established. Considering these earlier observations and some recent observations, the authors propose desensitization of the G protein-coupled cysteinyl leukotriene receptor₁ (CysLT₁) as the mechanism of refractoriness and that this desensitization is the effect of interplay between leukotrienes (LTs) and PGs.

WHY DOES EXERCISE PROVOKE AN ATTACK OF ASTHMA?

The most important determinants of EIB are the ventilation reached and sustained during exercise and the water content and temperature of the inspired air. The higher the ventilation reached and the drier the air inspired, the greater the potential to provoke bronchoconstriction. Although the flow rates can start to decrease during exercise, the lowest values usually occur 5 to 12 minutes after 6 to 8 minutes of vigorous exercise.¹³ It is well documented that EIB is inhibited by inhaling fully humidified air at 37°C and the primary stimulus to EIB is most likely water lost by evaporation, in humidifying large volumes of air during exercise.¹³ The evaporation of water is associated with heat loss and dehydration of the airway surface liquid (ASL). The ASL is 5 to 7 µm in depth; over the first 10 generations of airways, its total volume is estimated to be less than 1 mL.¹⁴ There are 2 major theories for the mechanism whereby respiratory water loss causes bronchoconstriction. One theory relates to the thermal effects¹⁵ and the other relates to the osmotic effects of water loss.^{13,14} The thermal theory proposes that the increased ventilation during exercise causes airway cooling and vasoconstriction that is followed by rapid rewarming and a reactive hyperemia of the bronchial mucosal vasculature at the cessation of exercise. These vascular events cause the airways to narrow. The theory does not encompass bronchial smooth muscle or mediator release.¹⁵ The osmotic theory proposes that the water lost by the increased ventilation of exercise transiently increases the osmolarity of the airway surface and possibly the submucosa.^{13,14} This hyperosmolarity, in turn, triggers the release of mediators, such as PGs, LTs; histamine from inflammatory cells, such as mast cells and eosinophils; and possibly neuropeptides from sensory nerves in the airways.^{16–19} These mediators can cause the airways to narrow by contraction of bronchial smooth muscle.¹⁴

The documentation of EIB, under conditions of breathing hot dry air, demonstrates that airway cooling is not a prerequisite for EIB.²⁰ Thus, although the vascular events may play an important role when exercise is performed in low-temperature conditions, these events become less relevant when exercise is performed in moderate- to high-temperature conditions. The importance of increased ventilation, rather than the metabolic effects of exercise, in provoking EIB has been confirmed by studies showing similar results with eucapnic voluntary hyperpnea (EVH).²¹ Moreover, the release of mediators has been demonstrated *in vivo* following inhalation of the osmotically active substance mannitol as well as *in vitro*.^{16,18,22} The protective effect of the mast cell stabilizing agent sodium cromoglycate on challenge by exercise and mannitol supports mediator release from mast cells as an important factor in

provoking EIB.^{23,24} Further, LT antagonists, such as montelukast, significantly attenuate EIB, whereas histamine seems to have a subordinate role with conflicting results using antihistamines.^{25–27}

REFRACTORINESS WITH REPEATED CHALLENGES

McNeill and colleagues²⁸ were the first to describe refractoriness to repeated exercise challenges, and Edmunds and colleagues²⁹ were the first to study its duration. Edmunds and colleagues performed a repeated exercise challenge in 8 patients and looked at the duration of the refractory period and the effect of the workload on the degree of refractoriness. The degree of refractoriness varied between the patients, and the duration of the refractory period varied from 1 to 2 hours (Fig. 2). The more intense the exercise during the first challenge, the more refractory the patients were to the second challenge.

A refractory period occurs not only after exercise challenge but also after other indirect challenges, such as EVH,^{30,31} hypertonic saline,³² adenosine,³³ ultrasonically nebulized distilled water,³⁴ and mannitol.^{35,36} All these indirect challenges are associated with the release of mediators, all are inhibited by premedication with sodium cromoglycate, and all are thought to induce refractoriness via a similar pathway.³⁷ EVH is a good surrogate for an exercise challenge producing a similar reduction in lung function and a similar degree of refractoriness.³⁸ Cross-refractoriness occurs between exercise and EVH,³⁹ between exercise and hypertonic saline,⁴⁰ and between exercise and adenosine 5'-monophosphate.⁴¹ The findings of cross-refractoriness between the different indirect challenges support the concept that they share similar mechanisms. Initial mast cell mediator release is common to all of these challenges.

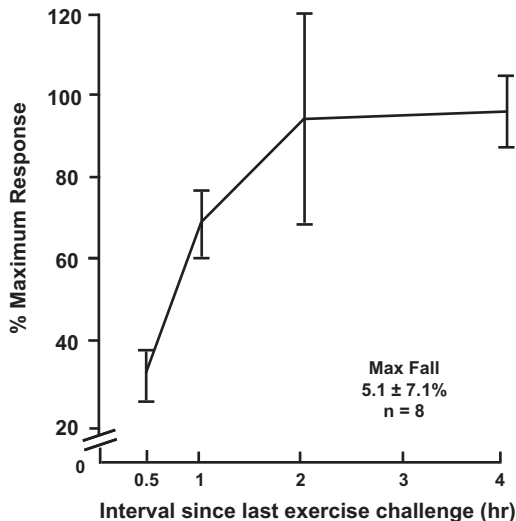


Fig. 2. The duration of refractoriness. Eight patients performed a second running exercise after the specified interval of time. The shorter the time between tests, the less the response on the second test. By 4 hours, the initial response was reestablished. (From Edmunds AT, Tooley M, Godfrey S. The refractory period after exercise-induced asthma: its duration and relation to the severity of exercise. *Am Rev Respir Dis* 1978;117:247–54; with permission.)

SUGGESTED MECHANISMS OF REFRACTORINESS

The mechanisms that have been proposed to account for refractoriness following exercise include high levels of circulating catecholamines, depletion of mast cell mediators, a decrease in release of sensory neuropeptides, a decreased responsiveness of the smooth muscle, release of a protective mediator in response to the initial challenge, and desensitization and/or downregulation of the receptors for the mediators that cause bronchoconstriction after the initial challenge.

Increased Levels of Catecholamines

One of the initial proposals put forward to account for refractoriness was that higher levels of catecholamines released during the first provocation would be protective during the second challenge. This proposal was not supported by studies measuring levels of epinephrine and norepinephrine after exercise and during the refractory period to exercise challenge.^{42,43} Further refractoriness has been reported after EVH and other indirect stimuli that do not involve release of catecholamines.³⁰

Depletion of Mast Cell Mediators

Over the years, the assays to identify inflammatory mediators in the blood, sputum, and urine became more sensitive, and mediator release as the cause of EIB became more widely accepted. The identification of the involvement of mediator release in EIB led some investigators to propose that refractoriness might be caused by a depletion of these mediators by the initial challenge, with insufficient concentration of mediators remaining to cause bronchoconstriction a second time. This theory became accepted, but there are only a few studies that have actually investigated the possibility of the depletion of mediators after repeated challenge. Belcher and colleagues⁴³ studied the release of histamine and catecholamines in plasma and venous blood, respectively, following 2 exercise challenges separated by 60 minutes. No difference in the release of histamine or catecholamines between the first and second challenge was seen even though the subjects were refractory. More recently, the authors reported results on the urinary excretion of LTE₄ and PGD₂ following 2 mannitol challenges, 90 minutes apart.³⁵ The authors found release of both mediators after both challenges, with the subjects who were most refractory having the highest levels of mediators compared with those who were the least refractory. Taken together, these two studies do not support depletion of mediators as a cause of refractoriness, and there are no reports of observations of decreased release during the refractory period.

Decreased Release of Sensory Neuropeptides

Evidence to support EIB being mediated by neuropeptides comes from the inhibitory effect of a neurokinin (NK)₁ receptor antagonist.⁴⁴ There are, however, conflicting data from a study of the effect of a nonselective NK₁/NK₂ receptor antagonist showing no effect on the bronchoconstriction following hypertonic saline.⁴⁵ Further, in addition to their well-known effects on mast cells, sodium cromoglycate and nedocromil sodium also have an inhibitory effect on sensory nerves.⁴⁶ Other evidence comes from the finding that the release of LTs and NKA (an NK₁/NK₂ receptor agonist) were strongly associated, raising the possibility that the cysteinyl leukotrienes (CysLTs) activated the sensory nerves.⁴⁷ This association, however, could also be an effect of the LTs and NKA being released in parallel or that activation of sensory nerves induces the release of mediators from mast cells. The sensory nerves are also activated by increases in osmolarity.¹⁷

The data to support the depletion of sensory neuropeptides in the development of refractoriness come from the finding that there is cross-refractoriness between

sodium metabisulfite, a neurally mediated challenge, and exercise.⁴⁸ However, metabisulfite challenge may also be associated with the release of leukotrienes because the CysLT₁ antagonist zafirlukast has been shown to attenuate the response to sodium metabisulphite.⁴⁹ The involvement of neuropeptides and sensory nerves in the development of refractoriness needs to be further investigated because, to date, only indirect evidence exists and the literature on the effect of neurokinin antagonism is conflicting.⁵⁰

Decreased Responsiveness of the Airway Smooth Muscle

To test if refractoriness is caused by the airway smooth muscle not being able to contract during the second challenge, various pharmacologic agents that act directly on smooth muscle have been administered during the refractory period. The responsiveness of the smooth muscle to inhalation of histamine and methacholine after exercise challenge^{51–53} and methacholine after mannitol challenge has been reported as unchanged in subjects who are refractory.³⁶ A decreased responsiveness to histamine has been documented only after paired, but not single, exercise provocation.⁵⁴ Thus, it seems unlikely that refractoriness to EIB can be accounted for by a general decreased responsiveness of the airway smooth muscle itself. Although the muscle may remain responsive to histamine, this mediator does not play a prominent role in sustaining EIB.²⁷

Tachyphylaxis of the muscle may occur in response to other mediators of EIB. For example, it is well recognized that tachyphylaxis occurs with repeated inhalation of LTD₄ and cross-tachyphylaxis occurs between exercise challenge and LTD₄ challenge (**Fig. 3**).⁵⁵ One of the original observations on tachyphylaxis involved the inhalation of PGF_{2 α} and led to a proposal of a secondary protective mechanism. Fish and colleagues⁵⁶ demonstrated that when PGF_{2 α} was inhaled, it induced bronchoconstriction; but if the inhalation was continued with higher doses, there was a return to baseline lung function. It was not clear if this tachyphylaxis was an expression of decreased responsiveness of the muscle, by downregulation of the receptor, or caused by the release of a protective substance. Yet another possibility is that PGF_{2 α} at the higher concentration, because of its relative lack of receptor specificity, would have also had an effect on other PG receptors, such as the bronchodilating PGE₂ receptors EP₂ and EP₄. It seemed necessary for PGF_{2 α} to be the initial stimulus, but the subsequent response was not specific to PGF_{2 α} because there was also a reduction in responsiveness to histamine (**Fig. 4**).⁵⁶ The investigators suggested that there may be a secondary protective mechanism initiated by PGF_{2 α} . Hyperventilation has been shown to induce the release of bronchodilating PGE₂ and PGI₂, lending support to the idea of a secondary protective mechanism involving PGs.⁵⁷

Release of Protective PGs

The release of a protective PG would seem to be the best argued mechanism for refractoriness to date. The proposal of a protective mediator initially made by Fish and colleagues⁵⁶ was revisited by others who studied the effect of pretreatment with the cyclooxygenase (COX) inhibitors, thereby blocking the production of prostanooids, such as PGs, prostacyclin, and thromboxane. The effect of the COX inhibitor indomethacin has been studied extensively on refractoriness to exercise, EVH, and inhaled water aerosol (**Fig. 5**).^{34,58–60} The findings that indomethacin abolished refractoriness to these 3 challenges, yet had no effect on the response to the initial challenge, strongly support the role of PGs in the development of refractoriness.

The early studies involved an initial exercise breathing hot humid air followed by a second exercise challenge breathing dry air.^{61,62} EIB was inhibited by the hot humid

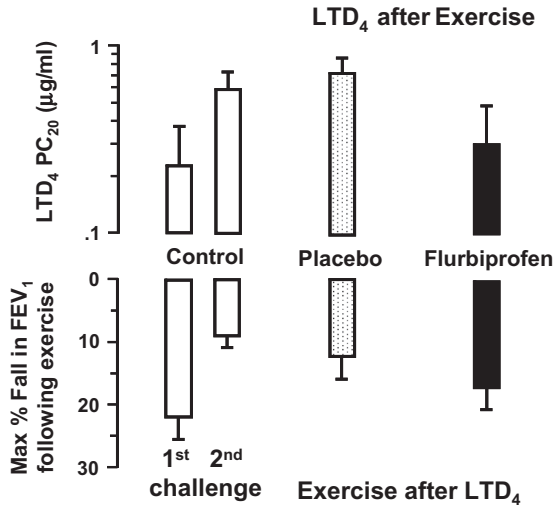


Fig. 3. Cross-refractoriness between exercise and LTD₄. (*Upper panel*) The mean (%SEM) PC₂₀ LTD₄ for two LTD₄ challenges separated by 1 hour and for an LTD₄ challenge 1 hour after exercise during treatment with either placebo or flurbiprofen (50 mg/d for 3 days). LTD₄ tachyphylaxis occurred in control subjects (*open bars*). LTD₄ tachyphylaxis also occurred after exercise during placebo (*dotted bars*), but the effect was significantly attenuated ($P = .027$) during flurbiprofen treatment (*solid bars*). (*Lower panel*) The mean (SEM) percentage decrease in FEV₁ after 2 exercise challenges separated by 1 hour and for exercise 1 hour after LTD₄ challenge during treatment with either placebo or flurbiprofen. Exercise refractoriness occurred after LTD₄ challenge during placebo treatment (*dotted bars*), but the effect was significantly attenuated ($P = .026$) during flurbiprofen treatment (*solid bars*). (From Manning PJ, Watson RM, O'Byrne PM. Exercise-induced refractoriness in asthmatic subjects involves leukotriene and prostaglandin interdependent mechanisms. *Am Rev Respir Dis* 1993;148:950–4; with permission.)

air, but tolerance, to a similar degree as refractoriness, was observed to the second exercise with dry air.^{61,62} These studies were important because they demonstrated that EIB per se was not required for the development of tolerance to the second exercise. Further, the tolerance to the second exercise was abolished by pretreatment with indomethacin.⁶⁰ This finding suggests that protective PGs were induced by the initial exercise challenge, even in the absence of EIB (**Fig. 6**).⁶⁰ It also suggests that PGs play no role in the inhibition of EIB by hot humid air because no bronchoconstriction occurred during indomethacin treatment. The inhibitory effect of indomethacin on refractoriness, however, makes the release of a protective PG following initial challenge seem a likely mechanism. There is one major caveat, however, in that severe EIB has been reported when breathing dry air 20 minutes after an initial challenge with hot humid air when EIB was prevented.^{63,64} Although these two studies were not designed to include the effect of indomethacin, the findings are markedly at odds with those described earlier.

Further support for a protective PG came from a later study in which the effect of repeated exercise and LTD₄ challenge were investigated as well as the effect of LTD₄ preceding or following exercise challenge.⁵⁵ The investigators used the COX inhibitor flurbiprofen to study its effect on the refractory period (see **Fig. 3**). Their findings suggest that LTs induce release of a protective PG. By contrast, there is currently no evidence for a role of histamine in the release of protective mediators.

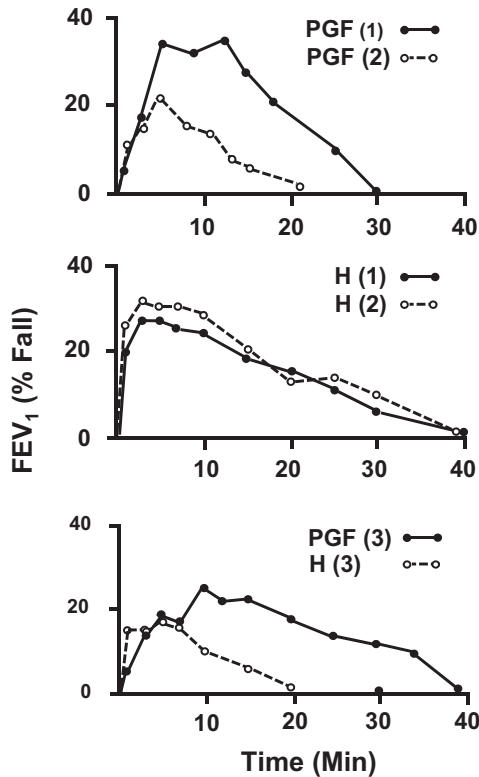


Fig. 4. The effect of repeated inhalation of PGF_{2α} and histamine in patients with asthma. Percent change in FEV₁ after administration of different agonist combinations in a representative subject. Panels, from top to bottom, illustrate the response to first (PGF1) and second (PGF2) challenge with PGF_{2α}, response to first (H1) and second (H2) challenge with histamine, and response to challenge with PGF_{2α} (PGF3) followed by histamine (H3). Tachyphylaxis to the second challenge with PGF_{2α} was seen. Also a decreased response to histamine was seen following challenge with PGF_{2α}. (From Fish JE, Jameson LS, Albright A, et al. Modulation of the bronchomotor effects of chemical mediators by prostaglandin F₂ alpha in asthmatic subjects. *Am Rev Respir Dis* 1984;130:571–4; with permission.)

For example, histamine H₂-receptor antagonists neither affect the airway response to exercise nor the refractoriness induced with repeated challenges.^{65,66} However, the possible role of the more recently discovered H₃ and H₄ receptors remains to be studied.

This protective PG was considered likely to be PGE₂, which had been shown to reduce both the duration and severity of bronchoconstriction following exercise challenge.⁶⁷ Inhalation of PGE₂ does not alter the airway response to methacholine, suggesting it does not act as a functional antagonist.^{67,68} PGE₂ may work through mast cells, neural pathways, or via modulation of receptors or downstream intracellular signaling molecules. PGE₂ is thought to be produced by the airway smooth muscle cells⁶⁹ and the epithelium.⁷⁰ An increase in osmolarity is also a stimulus for the release of PGE₂.⁷¹ PGE₂ also reduces the airway response to metabisulfite. This finding, together with the data supporting a role for leukotrienes in metabisulfite-induced bronchoconstriction, is in keeping with the concept of interdependence between LTs and PGs in the development of refractoriness.

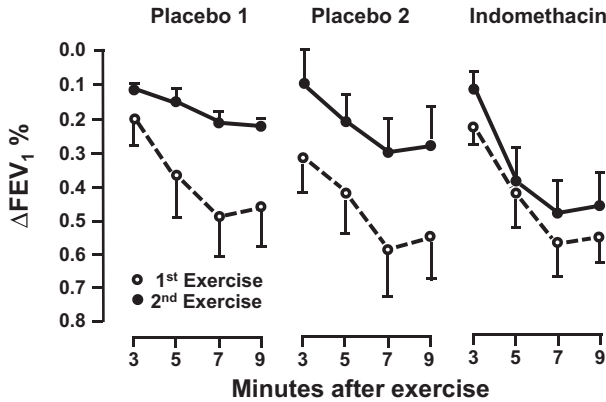


Fig. 5. The effect of indomethacin on the refractory period Change in FEV₁ after exercise on the 2 placebo days. The EIB was similar on the 1st exercise challenge on each test day (*open circles*). Refractoriness occurred after exercise on the 2 placebo days but not after indomethacin. (From O'Byrne PM, Jones GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 1986;134:69–72; with permission.)

Many studies have suggested this protective PG to be PGE₂.^{55,58–60} Although this is the most studied idea, much remains unclear; is there release of PGE₂? If there is release, how does the PGE₂ act to prevent bronchoconstriction? How long lasting is the effect? The reason for this protective PG not affecting the bronchoconstrictor response to the initial challenge, to any significant extent, may be that the release is

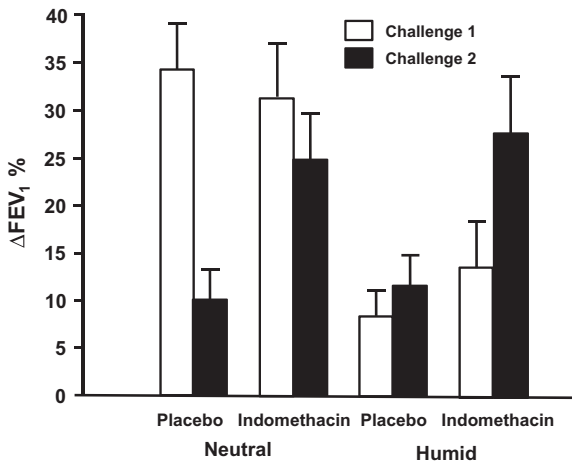


Fig. 6. The effect of breathing conditions on the response to a subsequent challenge performed in thermo neutral conditions. Percentage decrease in FEV₁ (mean $\pm$ standard error of the mean) after exercise compared with pre-exercise values. Placebo and indomethacin pretreatments were compared under thermoneutral and warm-humid breathing conditions. Refractoriness was seen during challenge 2 (*closed bars*) independent of the breathing conditions during challenge 1 (*open bars*). The refractoriness was abolished by indomethacin in both situations. (From Wilson BA, Bar-Or O, O'Byrne PM. The effects of indomethacin on refractoriness following exercise both with and without a bronchoconstrictor response. *Eur Respir J* 1994;7:2174–8; with permission.)

delayed compared with the other mediators. It may also be that it needs to be present before the other mediators are released, as is the case in the study by Melillo and colleagues⁶⁷ whereby the patient inhaled PGE₂ 30 minutes before the challenge. Yet another possibility is that the effectiveness is dependent on interplay with the other released mediators.

WHERE ARE WE NOW?

Since the idea of a protective PG was put forward, 4 different receptors for PGE₂ have been identified, EP₁₋₄. The activation of EP₁ and EP₃ has been shown to induce bronchoconstriction, whereas the activation of EP₂ and EP₄ induces relaxation of the bronchial smooth muscle.^{72,73}

Manning and colleagues⁵⁵ proposed interdependence between LTs and PGs in the development of refractoriness. This idea, however, has never been proven; and how PGE₂ would exert its effect has not been discussed. Also, that levels of PGE₂ are elevated during the refractory period requires confirmation. Considering the recent findings³⁵ of a relation between high levels of mediators and a higher degree of refractoriness, the authors propose a new interpretation of how the interplay between LTs and PGs (PGE₂ and PGD₂) takes place: via downregulation of the CysLT₁.

From studies investigating G protein-coupled receptors (GPCRs), a common finding is agonist-promoted homologous desensitization.⁷⁴ This topic has been extensively investigated with the β_2 -adrenergic receptor agonists (β_2 agonist). In relation to EIB, this desensitization to a β_2 agonist manifests as a reduction in the duration of protection against EIB within days of regular use,⁷⁵⁻⁷⁷ a more prolonged recovery from EIB in response to a β_2 agonist when breakthrough EIB has occurred,⁷⁸ and an increase in the severity of EIB at the end of a dosing period.⁷⁹ With acute stimulation, this desensitization of the β_2 -receptor is associated with phosphorylation of the receptor by GPCR kinases and the second messenger-dependent protein kinase A.⁸⁰ The receptors are then internalized through the effects of arrestins.⁷⁴ With a more chronic stimulation, the agonist exposure leads to receptor downregulation with decreased expression and synthesis.⁸⁰ There is also a correlation between the strength of the stimulus and the desensitization that an agonist can induce.⁸¹

The various GPCRs do not work in isolation but rather they have been shown to exhibit extensive crosstalk between them.⁸² An example of this crosstalk is the activation of the PGE₂ receptor EP₁ leading to a reduction of the bronchodilating function of the β_2 -adrenergic receptor.⁸³ The same has been observed for LTD₄.⁸⁴ Release of PGD₂ and CysLTs has been established with EIB^{19,85,86} and there are also reports on release of PGE₂.⁸⁷ These eicosanoids exert their actions in humans via their respective GPCRs.⁸⁸ For example, LTD₄ has been shown to induce phosphorylation and internalization of the CysLT₁ receptor, a process involving protein kinase C and arrestins.⁸⁹ This process of phosphorylation and internalization of the CysLT₁ has been shown to be initiated rapidly on stimulation and to be recycled back to the cell surface in 15 to 20 minutes after the removal of the stimulation.⁹⁰

The early study of EIB showing a relationship between the workload and refractoriness²⁹ and the more recent study showing higher mediator levels in those who are most refractory³⁵ is in keeping with receptor desensitization as the mechanism of refractoriness. Also in keeping with receptor desensitization is the prolonged increase (60–120 minutes) in the urinary excretion of LTE₄ and 11 β -PGF_{2 α} after challenge with exercise, EVH, or mannitol.^{19,35,85,86} (The nomenclature of the metabolites of different PGs has recently been revised, and 11 β -PGF_{2 α} is the new term for the metabolite previously called 9 α ,11 β -PGF₂ by the authors and others.) Further, the timing of induction

and cessation of receptor internalization would fit with the duration of the refractory period being up to 2 hours. From the authors' experience,^{35,85,86} they have seen that the dynamics of release and the level of mediators vary to a great degree between subjects. These differences between subjects not only help to explain why some subjects are rendered refractory while others are not, but they may also account for the varying duration of the refractory period.²⁹ When the authors compared the most and least refractory subjects following repeated inhalation of mannitol, they found that the most refractory subjects had significantly higher levels of both LTE₄ and the PGD₂ metabolite 11 β -PGF_{2 α} .³⁵ The most refractory subjects also had sustained higher levels and did not return to baseline mediator values before the second challenge.

The consistent finding of increased levels of the PGD₂ metabolite 11 β -PGF_{2 α} during EIB may be relevant in that it may contribute to crosstalk via the thromboxane receptor (TP) receptor leading to desensitization of the CysLT₁ (Fig. 7). In keeping with this, the effect of indomethacin on refractoriness could be explained by PGs having an important role in the crosstalk between GPCRs to induce increased desensitization of CysLT₁ in response to elevated levels of LTs following exercise challenge. When the levels of PGs are decreased, as would be the case with indomethacin pretreatment,

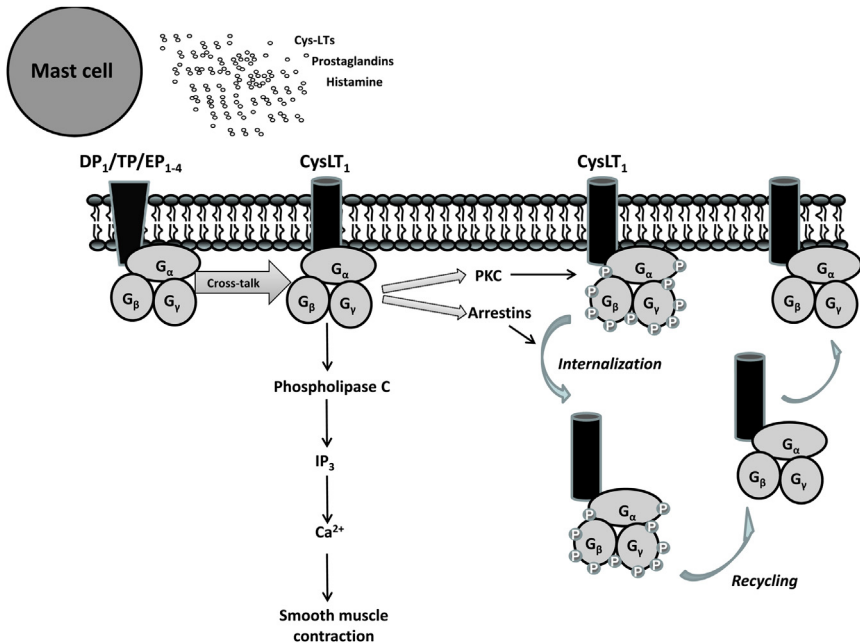


Fig. 7. Desensitization of CysLT₁ as a mechanism of refractoriness. With exercise, there is release of cysteinyl leukotrienes from mast cells that stimulate the GPCR CysLT₁ causing contraction of the muscle and exercise-induced bronchoconstriction. The PGs generated during the same exercise could, via stimulation of the GPCRs DP₁/EP₁₋₄/TP, lead to crosstalk so that the CysLT₁ 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 CysLT₁ 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 CysLT₁ and, therefore, limited refractoriness. CysLT₁, cysteinyl leukotriene receptor₁; DP₁, prostaglandin D receptor₁; EP₁₋₄, prostaglandin E receptor₁₋₄; GPCR, G-protein coupled receptor; TP, thromboxane receptor.

LTs alone would not be able to cause enough phosphorylation to induce refractoriness or at least not as effectively.

Further support for the idea of high levels of CysLTs leading to decreased sensitivity of the same receptor comes from a series of studies of patients with aspirin-intolerant asthma by Lee and coworkers.^{91–93} In this peculiar syndrome, following a positive challenge with aspirin causing bronchoconstriction, continued daily administration of aspirin produces a state of refractoriness to aspirin (ie, aspirin desensitization).

If there is a general desensitization of all the receptors of the mediators released during EIB or if there is desensitization only of a specific receptor (eg, the CysLT₁) remains to be determined. Also, what receptors are involved with this process either directly or via crosstalk is somewhat speculative, but the PGE₂ receptors EP_{1–4}, the PGD₂ receptors DP₁ and TP, and the PGI₂ receptor IP are all likely candidates.

There are also experimental data suggesting a possible role for adenosine or ATP. The bronchoconstriction, and the change in volume that may be expected to occur in the ASL during challenge by exercise, EVH, distilled water, hypertonic saline, and mannitol, is likely to lead to the release of ATP.⁹⁴ The suggested involvement of adenosine or ATP makes extracellular nucleotide receptors P2Y and the adenosine receptor A2b other possible candidates. It is of course also possible that other yet-to-be-identified mediators and their receptors have a vital role in this complex interplay.

In this review, the authors revisit the literature on refractoriness and its suggested mechanisms. The proposed mechanism that has the most supportive evidence is the release of a protective PG, presumably PGE₂. The authors extend this thinking and suggest that it is desensitization of the CysLT₁ that is the mechanism of refractoriness to exercise. This hypothesis is based on the evidence to date and may act as a starting point for further research in the field.

REFERENCES

1. Freed AN, Anderson SD. Exercise-induced bronchoconstriction. Human models. In: Kay AB, Kaplan AP, Bousquet J, et al, editors. *Allergy & allergic diseases*. 2nd edition. Oxford: Blackwell Scientific Publications; 2008. p. 806–20.
2. Anderson SD, Silverman M, Konig P, et al. Exercise-induced asthma. *Br J Dis Chest* 1975;69:1–39.
3. Cabral AL, Conceicao GM, Fonseca-Guedes CH, et al. Exercise-induced bronchospasm in children: effects of asthma severity. *Am J Respir Crit Care Med* 1999;159:1819–23.
4. Godfrey S, Springer C, Noviski N, et al. Exercise but not methacholine differentiates asthma from chronic lung disease in children. *Thorax* 1991;46:488–92.
5. Weiler JM, Nathan RA, Rupp NT, et al. Effect of fluticasone/salmeterol administered via a single device on exercise-induced bronchospasm in patients with persistent asthma. *Ann Allergy Asthma Immunol* 2005;94:65–72.
6. Anderson SD, Lambert S, Brannan JD, et al. Laboratory protocol for exercise asthma to evaluate salbutamol given by two devices. *Med Sci Sports Exerc* 2001;33:893.
7. Koh YI, Choi IS, Lim H. Atopy may be related to exercise-induced bronchospasm in asthma. *Clin Exp Allergy* 2002;32:532–6.
8. Helenius IJ, Tikkanen HO, Haahtela T. Occurrence of exercise induced bronchospasm in elite runners: dependence on atopy and exposure to cold air and pollen. *Br J Sports Med* 1998;32:125–9.
9. Karjalainen J. Exercise response in 404 young men with asthma: no evidence for a late asthmatic reaction. *Thorax* 1991;46:100–4.

10. Hofstra WB, Sterk PJ, Neijens HJ, et al. Occurrence of a late response to exercise in asthmatic children: multiple regression approach using time-matched baseline and histamine control days. *Eur Respir J* 1996;9:1348–55.
11. Schoeffel RE, Anderson SD, Gillam I, et al. Multiple exercise and histamine challenge in asthmatic patients. *Thorax* 1980;35:164–70.
12. Anderson SD. Exercise-induced asthma: stimulus, mechanism, and management. In: Barnes PJ, Rodger I, Thomson NC, editors. *Asthma: basic mechanisms and clinical management*. London: Academic Press; 1988. p. 503–22.
13. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is... *J Allergy Clin Immunol* 2000;106:453–9.
14. Anderson SD. Is there a unifying hypothesis for exercise-induced asthma? *J Allergy Clin Immunol* 1984;73:660–5.
15. McFadden ER Jr, Lenner KA, Strohl KP. Postexertional airway rewarming and thermally induced asthma. New insights into pathophysiology and possible pathogenesis. *J Clin Invest* 1986;78:18–25.
16. Brannan JD, Gulliksson M, Anderson SD, et al. Evidence of mast cell activation and leukotriene release after mannitol inhalation. *Eur Respir J* 2003;22:491–6.
17. Pisarri TE, Jonson A, Coleridge HM, et al. Intravenous injection of hypertonic NaCl solutions stimulates pulmonary C-fibres in dogs. *Am J Physiol* 1991;260(5 Pt 2): H1522–30.
18. Gulliksson M, Palmberg L, Nilsson G, et al. Release of prostaglandin D2 and leukotriene C4 in response to hyperosmolar stimulation of mast cells. *Allergy* 2006;61:1473–9.
19. O'Sullivan S, Roquet A, Dahlen B, et al. Evidence for mast cell activation during exercise-induced bronchoconstriction. *Eur Respir J* 1998;12:345–50.
20. Anderson SD, Schoeffel RE, Black JL, et al. Airway cooling as the stimulus to exercise-induced asthma—a re-evaluation. *Eur J Respir Dis* 1985;67:20–30.
21. Rundell KW, Anderson SD, Spiering BA, et al. Field exercise vs laboratory eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite cold weather athletes. *Chest* 2004;125:909–15.
22. Eggleston P, Kagey-Sobotka A, Schleimer R, et al. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 1984;130:86.
23. Tullett WM, Tan KM, Wall RT, et al. Dose-response effect of sodium cromoglycate pressurised aerosol in exercise induced asthma. *Thorax* 1985;40:41–4.
24. Brannan JD, Gulliksson M, Anderson SD, et al. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. *Eur Respir J* 2006; 27:944–50.
25. Kemp JP, Dockhorn RJ, Shapiro GG, et al. Montelukast once daily inhibits exercise-induced bronchoconstriction in 6- to 14-year-old children with asthma. *J Pediatr* 1998;133:424–8.
26. Anderson SD, Brannan JD. Exercise-induced asthma: is there still a case for histamine? *J Allergy Clin Immunol* 2002;109:771–3.
27. Dahlen B, Roquet A, Inman MD, et al. Influence of zafirlukast and loratadine on exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2002;109: 789–93.
28. McNeill RS, Nairn JR, Millar JS, et al. Exercise-induced asthma. *Q J Med* 1966; 35:55–67.
29. Edmunds AT, Tooley M, Godfrey S. The refractory period after exercise-induced asthma: its duration and relation to the severity of exercise. *Am Rev Respir Dis* 1978;117:247–54.

30. Bar-Yishay E, Ben-Dov I, Godfrey S. Refractory period after hyperventilation-induced asthma. *Am Rev Respir Dis* 1983;127:572–4.
31. Argyros GJ, Roach JM, Hurwitz KM, et al. The refractory period after eucapnic voluntary hyperventilation challenge and its effect on challenge technique. *Chest* 1995;108:419–24.
32. Hawksworth RJ, O'Hickey SP, Lee TH. The effects of indomethacin on the refractory period to hypertonic saline-induced bronchoconstriction. *Eur Respir J* 1992; 5:963–6.
33. Daxun Z, Rafferty P, Richards R, et al. Airway refractoriness to adenosine 5'-monophosphate after repeated inhalation. *J Allergy Clin Immunol* 1989;83: 152–8.
34. Mattoli S, Foresi A, Corbo GM, et al. The effect of indomethacin on the refractory period occurring after the inhalation of ultrasonically nebulized distilled water. *J Allergy Clin Immunol* 1987;79:678–83.
35. Larsson J, Perry CP, Anderson SD, et al. The occurrence of refractoriness and mast cell mediator release following mannitol-induced bronchoconstriction. *J Appl Physiol* 2011;110:1029–35.
36. Suh DI, Lee JK, Kim JT, et al. Airway refractoriness to inhaled mannitol after repeated challenge. *Pediatr Pulmonol* 2011;46(10):1007–14.
37. Joos GF, O'Connor B, Anderson SD, et al. Indirect airway challenges. *Eur Respir J* 2003;21:1050–68.
38. Rosenthal RR, Laube BL, Hood DB, et al. Analysis of refractory period after exercise and eucapnic voluntary hyperventilation challenge. *Am Rev Respir Dis* 1990; 141:368–72.
39. Ben-Dov I, Gur I, Bar-Yishay E, et al. Refractory period following induced asthma: contributions of exercise and isocapnic hyperventilation. *Thorax* 1983;38:849–53.
40. Belcher NG, Rees PJ, Clark TJ, et al. A comparison of the refractory periods induced by hypertonic airway challenge and exercise in bronchial asthma. *Am Rev Respir Dis* 1987;135:822–5.
41. Finnerty J, Polosa R, Holgate S. Repeated exposure of asthmatic airways to inhaled adenosine 5'-monophosphate attenuates bronchoconstriction provoked by exercise. *J Allergy Clin Immunol* 1990;86:353–9.
42. Dosani R, Van Loon GR, Burki NK. The relationship between exercise-induced asthma and plasma catecholamines. *Am Rev Respir Dis* 1987;136:973–8.
43. Belcher NG, Murdoch R, Dalton N, et al. Circulating concentrations of histamine, neutrophil chemotactic activity, and catecholamines during the refractory period in exercise-induced asthma. *J Allergy Clin Immunol* 1988;81:100–10.
44. Ichinose M, Miura M, Yamauchi H, et al. A neurokinin 1-receptor antagonist improves exercise-induced airway narrowing in asthmatic patients. *Am J Respir Crit Care Med* 1996;153:936.
45. Fahy JV, Wong HH, Geppetti P, et al. Effect of an NK1 receptor antagonist (CP-99,994) on hypertonic saline-induced bronchoconstriction and cough in male asthmatic subjects. *Am J Respir Crit Care Med* 1995;152:879–84.
46. Dixon CM, Barnes PJ. Bradykinin-induced bronchoconstriction: inhibition by nedocromil sodium and sodium cromoglycate. *Br J Clin Pharmacol* 1989;27: 831–6.
47. Hallstrand TS, Debley JS, Farin FM, et al. Role of MUC5AC in the pathogenesis of exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2007;119: 1092–8.
48. Pavord I, Lazarowicz H, Inchley D, et al. Cross refractoriness between sodium metabisulphite and exercise induced asthma. *Thorax* 1994;49:245–9.

49. Lazarus SC, Wong HH, Watts MJ, et al. The leukotriene receptor antagonist zafirlukast inhibits sulfur dioxide-induced bronchoconstriction in patients with asthma. *Am J Respir Crit Care Med* 1997;156:1725–30.
50. Joos GF, Pauwels RA. Tachykinin receptor antagonists: potential in airways diseases. *Curr Opin Pharmacol* 2001;1:235–41.
51. Hahn AG, Nogrady SG, Tumilty DM, et al. Histamine reactivity during the refractory period after exercise induced asthma. *Thorax* 1984;39:919–23.
52. Boulet LP, Legris C, Turcotte H. Bronchial responsiveness to histamine after repeated exercise-induced bronchospasm. *Respiration* 1987;52:237–45.
53. Magnussen H, Reuss G, Jorres R. Airway response to methacholine during exercise induced refractoriness in asthma. *Thorax* 1986;41:667–70.
54. Carpentiere G, Castello F, Marino S. Airway responsiveness to histamine in patients refractory to repeated exercise. *Chest* 1988;93:933–6.
55. Manning PJ, Watson RM, O'Byrne PM. Exercise-induced refractoriness in asthmatic subjects involves leukotriene and prostaglandin interdependent mechanisms. *Am Rev Respir Dis* 1993;148:950–4.
56. Fish JE, Jameson LS, Albright A, et al. Modulation of the bronchomotor effects of chemical mediators by prostaglandin F₂ alpha in asthmatic subjects. *Am Rev Respir Dis* 1984;130:571–4.
57. Ishii Y, Kitamura S. Hyperventilation stimulates the release of prostaglandin I₂ and E₂ from lung in humans. *Prostaglandins* 1990;39:685–91.
58. O'Byrne PM, Jones GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 1986;134:69–72.
59. Margolskee DJ, Bigby BG, Boushey HA. Indomethacin blocks airway tolerance to repetitive exercise but not to eucapnic hyperpnea in asthmatic subjects. *Am Rev Respir Dis* 1988;137:842–6.
60. Wilson BA, Bar-Or O, O'Byrne PM. The effects of indomethacin on refractoriness following exercise both with and without a bronchoconstrictor response. *Eur Respir J* 1994;7:2174–8.
61. Ben-Dov I, Bar-Yishay E, Godfrey S. Refractory period after exercise-induced asthma unexplained by respiratory heat loss. *Am Rev Respir Dis* 1982;125:530–4.
62. Wilson BA, Bar-Or O, Seed LG. Effects of humid air breathing during arm or treadmill exercise on exercise-induced bronchoconstriction and refractoriness. *Am Rev Respir Dis* 1990;142:349–52.
63. Hahn AG, Nogrady SG, Burton GR, et al. Absence of refractoriness in asthmatic subjects after exercise with warm, humid inspirate. *Thorax* 1985;40:418–21.
64. Anderson SD, Daviskas E, Schoeffel RE, et al. Prevention of severe exercise-induced asthma with hot humid air. *Lancet* 1979;2:629.
65. Nogrady SG, Hahn AG. H₂-receptor blockade and exercise-induced asthma. *Br J Clin Pharmacol* 1984;18:795–7.
66. Manning PJ, Watson R, O'Byrne PM. The effects of H₂-receptor antagonists on exercise refractoriness in asthma. *J Allergy Clin Immunol* 1992;90:125–6.
67. Melillo E, Woolley KL, Manning PJ, et al. Effect of inhaled PGE₂ on exercise-induced bronchoconstriction in asthmatic subjects. *Am J Respir Crit Care Med* 1994;149:1138–41.
68. Pavord ID, Wisniewski A, Mathur R, et al. Effect of inhaled prostaglandin E₂ on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma. *Thorax* 1991;46:633–7.
69. Delamere F, Holland E, Patel S, et al. Production of PGE₂ by bovine cultured airway smooth muscle cells and its inhibition by cyclo-oxygenase inhibitors. *Br J Pharmacol* 1994;111:983–8.

70. Knight DA, Stewart GA, Lai ML, et al. Epithelium-derived inhibitory prostaglandins modulate human bronchial smooth muscle responses to histamine. *Eur J Pharmacol* 1995;272:1–11.
71. Hjoberg J, Folkerts G, van Gessel SB, et al. Hyperosmolarity-induced relaxation and prostaglandin release in guinea pig trachea in vitro. *Eur J Pharmacol* 2000;398:303–7.
72. Woodward DF, Jones RL, Narumiya S. International union of basic and clinical pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. *Pharmacol Rev* 2011;63(3):471–538.
73. Saffholm J, Dahlen SE, Delin I, et al. Prostaglandin E (2) maintains the tone of the guinea pig trachea through a balance between activation of contractile EP (1) receptors and relaxant EP (2) receptors. *Br J Pharmacol* 2013;168(4):794–806.
74. Perry SJ, Lefkowitz RJ. Arresting developments in heptahelical receptor signaling and regulation. *Trends Cell Biol* 2002;12:130–8.
75. Simons FE, Gerstner TV, Cheang MS. Tolerance to the bronchoprotective effect of salmeterol in adolescents with exercise-induced asthma using concurrent inhaled glucocorticoid treatment. *Pediatrics* 1997;99:655–9.
76. Nelson JA, Strauss L, Skowronski M, et al. Effect of long-term salmeterol treatment on exercise-induced asthma. *N Engl J Med* 1998;339:141–6.
77. Ramage L, Lipworth BJ, Ingram CG, et al. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;88:363–8.
78. Haney S, Hancox RJ. Recovery from bronchoconstriction and bronchodilator tolerance. *Clin Rev Allergy Immunol* 2006;31:181–96.
79. Hancox RJ, Subbarao P, Kamada D, et al. Beta2-agonist tolerance and exercise-induced bronchospasm. *Am J Respir Crit Care Med* 2002;165:1068–70.
80. McGraw DW, Almoosa KF, Paul RJ, et al. Antithetic regulation by beta-adrenergic receptors of Gq receptor signaling via phospholipase C underlies the airway beta-agonist paradox. *J Clin Invest* 2003;112:619–26.
81. Kenakin T. Drug efficacy at G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 2002;42:349–79.
82. McGraw DW, Elwing JM, Fogel KM, et al. Crosstalk between Gi and Gq/Gs pathways in airway smooth muscle regulates bronchial contractility and relaxation. *J Clin Invest* 2007;117:1391–8.
83. McGraw DW, Muhlbachler KA, Schwarb MR, et al. Airway smooth muscle prostaglandin-EP1 receptors directly modulate beta2-adrenergic receptors within a unique heterodimeric complex. *J Clin Invest* 2006;116:1400–9.
84. Rovati GE, Baroffio M, Citro S, et al. Cysteinyl-leukotrienes in the regulation of beta2-adrenoceptor function: an in vitro model of asthma. *Respir Res* 2006;7:103.
85. Kippelen P, Larsson J, Anderson SD, et al. Effect of sodium cromoglycate on mast cell mediators during hyperpnea in athletes. *Med Sci Sports Exerc* 2010;42:1853–60.
86. Kippelen P, Larsson J, Anderson SD, et al. Acute effects of beclomethasone on hyperpnea-induced bronchoconstriction. *Med Sci Sports Exerc* 2010;42:273–80.
87. Hallstrand TS, Moody MW, Wurfel MM, et al. Inflammatory basis of exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2005;172:679–86.
88. Boyce JA. Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol Rev* 2007;217:168–85.
89. Naik S, Billington CK, Pascual RM, et al. Regulation of cysteinyl leukotriene type 1 receptor internalization and signaling. *J Biol Chem* 2005;280:8722–32.

90. Parhamifar L, Sime W, Yudina Y, et al. Ligand-induced tyrosine phosphorylation of cysteinyl leukotriene receptor 1 triggers internalization and signaling in intestinal epithelial cells. *PLoS One* 2010;5:e14439.
91. Arm JP, O'Hickey SP, Spur BW, et al. Airway responsiveness to histamine and leukotriene E4 in subjects with aspirin-induced asthma. *Am Rev Respir Dis* 1989;140:148–53.
92. Nasser SM, Patel M, Bell GS, et al. The effect of aspirin desensitization on urinary leukotriene E4 concentrations in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1995;151:1326–30.
93. Sousa AR, Parikh A, Scadding G, et al. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N Engl J Med* 2002; 347:1493–9.
94. Tarran R. Regulation of airway surface liquid volume and mucus transport by active ion transport. *Proc Am Thorac Soc* 2004;1:42–6.