

THE ROLE OF HISTAMINE, LEUKOTRIENE D₄ AND INHIBITORY
PROSTAGLANDINS IN EXERCISE BRONCHOCONSTRICTION
AND REFRACTORINESS IN ASTHMATIC SUBJECTS.

By

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Abstract

Exercise has long been recognized to be an important natural stimulus causing bronchoconstriction in most patients with symptomatic asthma, particularly in children and young adults. In the laboratory, bronchoconstriction caused by exercise is documented by examining a reduction in airway calibre after exercise which is measured as a fall in the forced expired volume in one second (FEV₁). Exercise bronchoconstriction is generally short lived, wearing off within 60 minutes.

More than 50% of asthmatic subjects, experience progressively less and less airway narrowing with repeated exercise challenges on the same day. This progressive decrease in exercise bronchoconstriction is considered to be a potentially important airway inhibitory protective effect and is termed exercise refractoriness. O'Byrne and Jones (O'Byrne and Jones, 1986), first demonstrated the likely involvement of inhibitory prostaglandins in exercise refractoriness, when they demonstrated that indomethacin inhibited the effect in asthmatic subjects.

The precise mechanism underlying exercise bronchoconstriction is unclear. However, the initiating stimulus may involve either alterations in osmolarity of the epithelial-lining fluid or changes in airway temperature resulting from the inhalation of inadequately conditioned air during exercise or both. These changes cause the release of bronchoconstrictor mediators and the development of

bronchoconstriction. The work described in this thesis was undertaken to establish the role of leukotriene (LT) D₄ in the pathogenesis of exercise bronchoconstriction and of histamine-, and LTD₄-induced, inhibitory prostaglandin release in causing exercise refractoriness. The body of research described in this thesis details new findings in relation to LTD₄ and exercise responses in asthmatic subjects. It shows that tachyphylaxis, a potential airway protective mechanism, occurs to repeated challenges with LTD₄ inhalations in asthmatic subjects and that flurbiprofen, a cyclooxygenase inhibitor, blocks this effect implicating inhibitory prostaglandin release in the mechanism. Using a specific LTD₄ receptor antagonist, it shows for the first time, that LTD₄, an important mediator in asthma, is involved in exercise bronchoconstriction in asthmatic subjects. In addition, it demonstrates also that complete cross refractoriness/tachyphylaxis occurs following exercise and LTD₄ stimulation an effect which is also inhibited by flurbiprofen. However, complete cross refractoriness/tachyphylaxis does not occur following exercise and histamine.

The conclusion from the work in this thesis is that LTD₄, rather than histamine, plays the more important role in exercise bronchoconstriction and that LTD₄-induced inhibitory prostaglandin release is the cause of exercise refractoriness in asthmatic subjects.

Acknowledgements

This body of work spans 5 years and now at completion I would like to acknowledge and thank those who have been instrumental in helping me.

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List of abbreviations

FEV ₁	Forced Expired Volume in 1 Second
LTD ₄	Leukotriene D ₄
MMD	Mass Median Diameter
PC ₂₀	Provocation Concentration causing a 20% fall in FEV ₁

Drugs Used

flurbiprofen	Upjohn, Canada.
histamine acid phosphate	ICN Nutritional Biochemicals, Ohio, USA
LTD ₄	Merck-Frosst, Canada
ranitidine	Glaxo, Canada.

Preface

The work described in this thesis has in part, been submitted, and in some cases, published, in peer reviewed journals. The majority of the planning, execution and statistical analysis of the studies in this thesis were carried out by me. The first two studies, outlined in this thesis, were planned and undertaken as a follow-on from work carried out by me on histamine tachyphylaxis and which were published in 1987-1988 (Manning, 1987, 1988). However, Dr. Cindy Hamielec, then a research fellow, collaborated closely with me on these two studies. I carried out the histamine tests and some of the exercise tests, while Cindy performed the remaining exercise tests. While in the other studies, Rick Watson, technologist and research assistant, was invaluable in recruiting patients and helping with the exercise challenges, the majority of the planning, execution and statistical analysis of the studies were performed by me. In study 4, Dr. Margolskee and her colleagues from Merck Sharpe and Dohme (Williams VC, Schwartz JI) are thanked for their financial support and supply of the MK-571 and placebo. Doctor A. Ford-Hutchinson, Merck-Frosst, Canada, is also thanked for supplying the leukotriene D₄ used for inhalation tests in this thesis.

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Am. Rev. Respir. Dis., 148: 950-954, 1993.

2. Manning PJ, Watson RM, O'Byrne PM. The effects of H₂-receptor antagonists on exercise refractoriness in asthma.
J. Allergy Clin. Immunol., 125-126, 1992.

3. Manning PJ, Watson RM, Margolskee DJ, Williams VC, Schwartz JI, O'Byrne PM. Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D₄-receptor antagonist.
N. Engl. J. Med., 323: 1736-1739, 1990.

4. Hamielec CM, Manning PJ, O'Byrne PM. Exercise refractoriness after histamine inhalation in asthmatic subjects.
Am. Rev. Respir. Dis., 138: 794-798, 1988.

Chapter I

Introduction

1.1 Epidemiology of Asthma

Asthma is a common chronic respiratory disease worldwide, particularly in developed countries, such as Canada. However, epidemiological studies examining the incidence, morbidity and mortality of the disease have shown a wide variability in different populations. In Western countries between 1.6 and 20.5% of children have symptoms suggestive of asthma (Sears, 1992). This variability may relate to the fact that criteria for the diagnosis of asthma varies between groups (Sears, 1992). Sears has suggested, in his excellent review on the epidemiology of the disease, that asthma prevalence is increasing worldwide (Sears, 1992). The reason for the increase is unclear and research is continuing in many countries to address this issue.

Asthma morbidity and mortality, particularly in younger asthmatics, has increased in the past 20 years in many of the developed countries including Canada (Mao et al, 1987; Wilkins and Mao, 1993), the United States (Mullally et al, 1984; Evens et al, 1987; Carr et al, 1992), England (Burney, 1986), New Zealand (Beasley et al, 1990), and Australia (Gandevia, 1968) and this increase continues to the present. The economic burden of the disease in these countries is significant. for instance, in the United States the economic cost for asthma in 1990 was estimated

to be over \$6 billion (Weiss et al, 1992). Further increases in the incidence, morbidity and mortality of the disease have the potential for increasing this cost even higher in the future.

Currently, asthma mortality varies between countries but on average, especially in the age group 5 - 34 years, it is usually below 1 per 100,000 of the population (Sears, 1992). This age group is thought to be the most appropriate for use in examining asthma mortality, due to accuracy of diagnosis of the disease and a reduced likelihood of contaminating the diagnosis with another diseases, such as chronic bronchitis and emphysema, diseases which are more likely to occur in the older age group.

While, asthma is a disease recognized since antiquity, the recognition of the importance of the inflammatory process in the mechanism of the disease has only been recognized and investigated, in recent years. Prior to this period, over the years, many different hypotheses were expounded in order to explain the different features of the disease.

1.2 Historical Review of Asthma

The earliest recorded references to asthma are found in the Ebers Papyrus (1550 BC) and the Chinese Neu Ching (1000 BC) and subsequently in ancient Greek

and Hebrew literature (Brewis, 1991, Ellul-Micallef, 1976). However, it was the Greek physicians, Hippocrates (460-357 B.C.), 'the Father of Medicine'; Aretaeus of Cappadocia (81-131 AD); and Galen (130-200 AD), the 'Prince of Physicians', who, for centuries, dominated early medical thinking on the nature of asthma.

Hippocrates, mentioned asthma in his aphorisms suggesting that the disease had its own nature arising from external causes (Brewis, 1991). His ideas were instrumental in guiding medical thought away from the notion that asthma was due to divine intervention. He believed also in a proper balance between the various humours; phlegm; bile (yellow and black); and blood, in order to maintain good health, postulating that disease was due to an imbalance between the humours. Aretaeus subsequently, classified asthma as a disease and was the first to write an accurate description of an acute asthmatic attack (Aretaeus, 1856). Galen, like Hippocrates, accepted a humoral cause for the disease and his ideas formed the basis for medical thought and practice for over a millennium.

During the Medieval period, Moses Maimonides (1130-1204 AD), a Jewish physician, with his Treatise on Asthma (Maimonides, 1963, Rosner, 1981) contributed significantly to the treatment of asthma during that period. Although born in southern Spain, he lived most of his life in Egypt where he practised medicine. While in Egypt, he treated the asthmatic son of Sultan Saladin. In his

Treatise on asthma he documented his advice to the Sultan for management of the disease. His main recommendations included a requirement for maintaining proper daily dietary and excretory habits; inhaling clean air; regular exercise, rest and sleep; and the avoidance of emotional turmoil and to keep an even temper.

The descriptions and judgments of these writers on asthma, especially those of Galen, remained in common use until the Renaissance. An increased interest in the study of the anatomic and physiologic aspects of asthma during the Renaissance enabled the disease to be more clearly defined, leading to new hypotheses concerning the mechanisms of the disease. It is of interest that many of the physicians who contributed to our knowledge of asthma from that period onwards, were asthmatics, including, van Helmont, Floyer, Bostock, Laennec, and Salter.

In 1662, Jean Baptiste van Helmont, the inventor of the term 'gas', compared an asthmatic attack to that of an epileptic fit (Brewis, 1991; Ellul-Micallef, 1976). Subsequently, Thomas Willis (1621-1675 AD), who described the Circle of Willis in the brain, suggested that bronchial asthma had two forms, a convulsive type and a pneumonic form (Brewis, 1991; Ellul-Micallef, 1976). The convulsive type was believed to be due to muscular cramps in the bronchial wall, diaphragm and chest wall. The pneumonic form was believed to be due to obstruction of the

bronchial tree with thick humours, swelling of the bronchial walls and obstruction from without. Subsequently, Sir John Floyer (1649-1734 AD) in his Treatise of the Asthma (Sakula, 1984), gave a classical description of an attack of convulsive asthma. Floyer's teachings on asthma influenced medical thought and practice for over a hundred years. He, like Willis, also differentiated between convulsive asthma due to bronchial muscle contraction and continuous asthma due to some inherent organic pathology. In addition, he recognized the influence of heredity as an important component to asthma.

In the 19th. Century, Franz Reisseneu documented the presence of smooth muscle in the bronchial tree (Brewis, 1991). He noted that with stimulation, the muscle contracted and the airways narrowed. Later, Rene Theophile Hyacinth Laennec (1722-1826 AD), the 'Father of Chest Medicine' and the inventor of the stethoscope, described typical auscultatory features of asthma in his 'L'auscultation mediate' and was convinced that airway constriction was an important feature of asthma (Brewis, 1991, Ellul-Micallef, 1976, Sakula, 1981). An improvement in the diagnosis of asthma along with an increased awareness and understanding of the disease occurred with the increased use of the stethoscope by physicians.

In 1846 John Hutchinson invented the spirometer which has subsequently become an important tool for the investigation and understanding of respiratory pulmonary

function in health and disease even to the present day. A spirometer is used in this thesis to evaluate the pulmonary function in the asthmatic subjects. However, it was not until the development of the more portable dry bellows 'vitalograph' spirometer in the 1950s, that the spirometer became more commonly used to assess and monitor pulmonary function in respiratory clinics and hospitals.

In 1959, Martin Wright, together with McKerrow, introduced the smaller and more portable peak flow meter (Wright and McKerrow, 1959). This device has proved to be easier for patients to use to monitor pulmonary function from day to day, particularly in general clinical practice. Nowadays, various forms of the 'mini' peak flow meter are regularly used by patients as an asthma self-monitoring instrument at home.

Henry Hyde Salter (1823-1871 AD) produced the best book on asthma of the 19th. Century, called 'On Asthma: Its Pathology and Treatment' (Brewis, 1991; Sakula, 1985). Salter reviewed over two hundred cases of asthma from his practice. He noted that hereditary played an important role in about one third of these cases. He was the first to cite various factors including, smoke, animal dander, and nervous excitement and fatigue, as responsible for asthma exacerbations. He also described what are now known as eosinophils, in the sputum of asthmatics.

During that period, John Millar (1733-1805 AD), documented the pathological changes associated with death from asthma in 'Observations on the Asthma and on the Whooping Cough' and noted the presence of hyperinflation in the lungs of asthmatics at post mortem (Brewis, 1991).

At the turn of the Nineteenth Century, William Osler (1849-1919 AD), through his writings, greatly influenced the medical thinking concerning the mechanism and treatment of asthma which lasted until recent years (Osler, 1892). He noted that airway obstruction in acute asthma not only involved muscular contraction but also oedema in the airway mucosa and excessive mucus production in the airways. Osler also recognised the importance of inflammation of the smaller bronchioles in acute asthma. However, he also fostered the opinion that asthma was usually not a fatal disease and is reported to have mentioned that "the asthmatic pants into old age".

In addition to inflammation, allergy was also noted to be important in the development of asthma at that time. John Bostock published a short report in 1819 on the association between asthma and hay fever based on his first hand experience of the conditions (Brewis, 1991). Later, in 1831, John Elliotson (1786-1868) described seasonal attacks of asthma and suggested that pollen was a cause. He also gave a clear description of hayfever associated with rabbit

exposure. Subsequently, in 1872, Morill Wyman of Harvard, described asthma related to ragweed pollen. It is interesting that over 100 years prior to this, Ramazzini (1633-1714 AD), 'the father of Occupational Medicine' had recognized an association between the dusty working environments of millers and bakers and the development of asthma and which are known to have an allergic cause (Brewis, 1991).

The term 'allergy', from the Greek meaning 'other', was first used by Clement von Pirquet in 1906, to describe a reaction to a foreign substance after immunization. Some four years earlier, Portier and Richet used the term anaphylaxis (from the Greek, 'ana' meaning 'backward' and 'phylaxis' meaning 'protection') to describe a paradoxical event that occurred following a immunization procedure to a toxin (allergen) from the sea anemone (Brewis, 1991). Dogs repeatedly immunized with the allergen were subsequently exposed to a small dose of toxin after a number of weeks and died within minutes. Richet was awarded the Nobel Prize, in 1913, for his work on anaphylaxis.

Subsequently, in 1910, Samuel Meltzer postulated that bronchospasm following allergen-induced anaphylactic shock in guinea-pigs might be similar to bronchospasm seen in acute asthma (Meltzer,1910). He suggested that allergic responses may therefore be important in the development of asthma which

subsequently led to further research on an allergic cause for asthma.

Thus by the beginning of the Twentieth Century attention focused on defining the disease in greater detail, due largely to this increased understanding of the importance of allergy and inflammation in the mechanism of asthma and the beginnings of investigation into the pharmacological mechanism involved in the disease.

1.3 Definition of Asthma

The term asthma comes from the Greek word, $\alpha\sigma\theta\mu\alpha$, meaning to breathe or pant (Brewis, 1991). Defining asthma however, has been extremely difficult due to the lack of a clear understanding of the exact pathophysiology of the disease. A simplified definition proposed by Scadding in 1983, now widely accepted, includes the presence of variable airflow obstruction, which can be reversed either spontaneously or following treatment (Scadding, 1983). This is the definition used to describe asthmatic subjects in this thesis. In recent years, a characteristic form of airway inflammation, and the presence of airway hyperresponsiveness are now recognised as important pathophysiological features of asthma (O'Byrne, 1986^a).

1.4 Airway Inflammation

The structural abnormalities present in asthmatic airways; the hyperplasia both of

airway smooth muscle and epithelial goblet cells; collagen deposition below the basement membrane; and epithelial shedding are likely to be due to the chronic inflammatory responses, characteristic of asthma (Dunill, 1960; Lemanske, 1992; Hogg, 1992). These inflammatory changes were particularly noted in the airways of patients dying acutely from the disease (Dunill et al, 1967).

Repeative airway inflammation, with activated mast cells, eosinophils, and lymphocytes is now recognized to play an important role in patients with symptomatic asthma. These inflammatory cells may release mediators such as histamine, leukotrienes, prostaglandins, thromboxane and platelet activating factor which may be involved in the inflammatory response associated with ongoing symptomatic asthma and in acute airway narrowing following exposure to various stimuli such as inhaled allergen, occupational sensitizing agents or following cold air and exercise (O'Byrne, 1986^a; Lemanske, 1992).

1.5 Airway Hyperresponsiveness

Airway hyperresponsiveness describes the ease with which asthmatic bronchoconstrict to various stimuli. Over the past 50 years a marked increase in the understanding of the mechanism of this increased airway bronchoconstrictor response in asthmatics has occurred (Boushey et al, 1980). It has been recognized for many years that histamine (Curry, 1946; Curry, 1947; Curry and Lovell, 1948;

Herxheimer, 1951, 1972; Laitinen, 1976; Cockcroft et al, 1977^a; Hargreave et al, 1981) and the cholinergic agonists acetylcholine, methacholine and carbachol (Tiffeneau and Beauvallet, 1945; Curry 1947; Curry and Lowell, 1948; Parker et al, 1965; Laitinen 1976; Orehek and Gayrard, 1976; Juniper et al, 1978; Hargreave et al, 1981) cause bronchoconstriction in humans and that this response occurs at lower doses of these agonists in asthmatic subjects compared to non-asthmatics.

Airway hyperresponsiveness in asthmatic subjects also occurs to other chemical bronchoconstrictor stimuli including; adenosine (Cushley et al, 1983, 1984); β -adrenergic blockers (McNeill and Ingram, 1966^a; Zaid and Beall, 1966); bradykinin (Varonier and Panzani, 1968, Simonsson et al, 1973; Fuller et al, 1987); leukotrienes (Holroyde et al, 1981; Bisgaard et al, 1985; Adelroth et al, 1986); prostaglandin D₂ (Hardy et al, 1984) and prostaglandin E_{2 α} (Mathé et al, 1973; Mathé and Hedquist, 1975; Thomson et al, 1981; Fish et al, 1984); serotonin (Herxheimer, 1953; Hajos, 1962; Panzani, 1962; Colebatch et al, 1966); as well as to physical stimuli such as, exercise (Chan-Yeung et al, 1971; Anderson et al, 1975; McFadden 1980), cold air (Wells et al, 1960; Deal et al, 1980; O'Byrne et al, 1982; Heaton et al, 1984), distilled water (Anderson et al, 1983^b), and inert aerosols (Sterling, 1969)..

1.5.1 Methods of Measuring Airway Hyperresponsiveness

There are several methods described for measuring the responsiveness of the airways to bronchoconstrictor stimuli. The most commonly used methods involve inhalation of an aerosolized bronchoconstrictor, usually histamine or methacholine, agonist, generated by a nebulizer to provoke bronchoconstriction. Two methods which have been carefully characterized, and compared to each other (Ryan et al, 1981^a, 1981^b) are both employed in this thesis. In one method (Cockcroft et al, 1977^a), which is a modification of a test originally described by de Vries and colleagues (de Vries et al, 1962), aerosol is generated by a Wright nebulizer, delivered either to a face mask or a mouth piece, and inhaled by tidal breathing for 2 min. In the second method (Chai et al, 1975), aerosol is generated by a Devilbiss 646 nebulizer attached to a Rosenthal-French dosimeter, delivered to a mouth piece and inhaled by 5-10 inspiratory capacity breaths. These two methods give very similar results (Ryan et al, 1981^b) and are reproducible from day to day (Juniper et al, 1978), once appropriate standardization of features which influence the response is performed.

1.5.2 Clinical Significance of Airway Hyperresponsiveness

Nowadays, inhalation tests employing histamine and methacholine, are widely used to measure the degree of airway hyperresponsiveness in asthmatic subjects in clinical and research laboratories. Subjects inhale increasing doubling

concentrations of aerosolized histamine or methacholine and the bronchoconstrictor response is measured by a fall in pulmonary function tests, usually the forced expired volume in 1 second (FEV_1). The subsequent dose response curves are analyzed log-linearly (Cockcroft and Berscheid, 1982). It is usual to define the result of the test as the provocative concentration of the agonist that produces a 20% fall in the FEV_1 (PC_{20}) (Cockcroft et al, 1977^a) and this is the method used in this thesis .

Increased sensitivity of the airway to these inhaled bronchoconstrictor agonists is shown by a lowering in the PC_{20} and a leftward shift in the dose response curve. Using the method described by Cockcroft (Cockcroft et al, 1977^a), asthmatic subjects generally have a histamine or methacholine PC_{20} of less than 8 mg/ml, while most nonasthmatics will have a PC_{20} of greater than 16 mg/ml. There is however some overlap, and defining an exact level of airway responsiveness, which would distinguish asthmatic subjects from nonasthmatic subjects, is not possible. This is because there appears to be a continuous distribution of nonspecific airway responsiveness in the general population, with asthmatic subjects in one tail of this distribution (Cockcroft et al, 1983). It is now recognized that asthmatics with lower PC_{20} values, generally have increased asthma symptoms and require more asthma therapy to control these symptoms (Cockcroft et al, 1977^a; Juniper et al, 1981).

For many asthmatic subjects, the responsiveness of the airway to bronchoconstrictor stimuli can be stable over several years (Juniper et al, 1982). However, as has already been described, there are a number of inhaled stimuli such as allergen (Cockcroft et al, 1977^b; Cartier et al, 1982; Boulet et al, 1983), ozone (Golden et al, 1978) and chemical sensitizers, such as toluene diisocyanate (TDI) (Fabbri et al, 1987), or plicatic acid from Western Red Ceder (Chan-Yeung et al, 1982), and upper respiratory viral infections (Empey et al, 1976) which can cause airway hyperresponsiveness in human subjects. This exposure and subsequent development of airway hyperresponsiveness is associated with an increase in both asthma symptoms and in the amount of treatment required to control these symptoms (Juniper et al, 1981). Identifying the significance of these stimuli has been important in the management of individual patients with asthma, so that with removal of the stimulus, particularly with occupational sensitizing agents, normal airway responsiveness is restored (Chan-Yeung and Lam 1986). This means that some patients can have airway hyperresponsiveness and symptomatic asthma at one time but not at another.

Lastly, the degree of bronchoconstriction caused by exercise in asthmatic subjects, is related to the level of airway hyperresponsiveness (Anderton et al, 1979). As this stimulus is considered to act through release of endogenous mediators, it is possible that airway hyperresponsiveness to these endogenous mediators may

play a role in causing symptoms after exercise in asthmatic patients.

1.6 Exercise Bronchoconstriction

Aretaeus of Cappadocia (81-131 AD) was likely the first to record a clear description of exercise bronchoconstriction when he noted that running and gymnastics provoked an asthmatic attack (Brewis, 1991). However, it was not until the Seventeenth and subsequently the Eighteenth centuries that Willis and Floyer, recognized a clear association between exercise and asthma. Floyer described an asthmatic attack following exercise and noted that different forms of exercise induced differing degrees of bronchoconstriction. The mechanism, at that time, was thought to be related to an increased blood volume in the lungs during exercise. In the Nineteenth century, Salter recognized that exercise bronchoconstriction was an important stimulus for asthma in many of the asthmatic subjects that he reported on.

It is now recognized that bronchoconstriction following exercise usually peaks between 5 and 10 minutes and then remits spontaneously, with complete recovery usually by one hour (Lee and Anderson, 1985). Since the late 1960s there has been a growing interest in examining mechanisms underlying exercise bronchoconstriction. Research in this area has suggested that exercise bronchoconstriction may be caused either through alterations in airway

temperature or in the osmolarity of the epithelial lining fluid as a consequence of inhaling inadequately conditioned air during exercise (Chen and Horton, 1977; Anderson et al, 1985^b; Freed et al, 1987; Gilbert et al, 1987).

By the mid-1940s, objective evidence that exercise-induced asthmatic responses were associated with bronchoconstriction began with Herxheimer reporting on the fall in the vital capacity lung volume after running and cycling in asthmatic subjects (Herxheimer, 1946). He, and subsequently others, (Kivity and Souhrada, 1980), suggested that the increased ventilation associated with exercise was the important stimulus for exercise bronchoconstriction. In the 1960s Jones and his colleagues, demonstrated that exercise bronchoconstriction was common in children and young adults with asthma (Jones et al, 1962, 1963).

1.6.1 Mechanism of Exercise Bronchoconstriction in Asthma

In the 1960s and early 1970s, possible stimuli investigated in the mechanism for exercise bronchoconstriction included; metabolic acidosis (Seaton et al, 1969), hypocapnia (McFadden and Lyons, 1968; Rebuck and Read 1968; Fischer et al, 1970), impaired 'oxygen transport' (Katz et al, 1971; McFadden and Lyons, 1968), or hyperventilation-induced bronchoconstriction through enhancement of airway vagal reflexes (Crompton 1968; Simonsson et al, 1972). These effects are now believed to occur as a consequence of exercise rather than playing an important

role as the stimulus for exercise bronchoconstriction (Anderson et al, 1975; O'Byrne et al, 1983).

In recent years, airway cooling due to changes in airway temperature associated with exercise and/or alterations in osmolality of airway lining fluid are now thought to be important stimuli for exercise bronchoconstriction (Anderson et al, 1985^b; Gilbert et al, 1987, 1988; Gilbert and McFadden, 1992). The importance of conditioning of inhaled air and airway cooling during exercise in the mechanism of exercise bronchoconstriction has stemmed from a number of observations. First that swimming, which is associated with breathing of highly humidified air, is associated with less exercise bronchoconstriction than other forms of exercise and secondly that breathing cold dry air with low humidity, induces bronchoconstriction in asthmatics and also that inhaling warm and humid air during exercise inhibits exercise-induced bronchoconstriction (Anderson et al, 1979^a; Bar-Or et al, 1977; Bar-Yishay et al, 1982; Bengtsson et al, 1984). However, the mechanism of how alterations in the conditioning of the inspired air induces bronchoconstriction during exercise is unclear. To date research has focused largely on mechanisms involving either; alterations in osmolality of airway lining fluid; airway cooling; or rapid rewarming of the airway mucosa following exercise (Deal et al, 1979^a, 1979^b, 1979^c; Anderson et al, 1982; Gilbert et al, 1987, 1988; Henriksen et al, 1981; Sheppard and Eschenbacher, 1984; Strauss et al, 1977). The debate continues

in the literature as to which stimulus is the more important for initiating exercise bronchoconstriction (Anderson et al, 1985^b; Ben-Dov et al, 1982; Hahn et al, 1984; Freed et al, 1987; Bianco et al, 1988; Daviskas et al, 1991; Gilbert and McFadden, 1992; Johnston et al, 1992; McFadden et al, 1986; McFadden, 1990; Noviski et al, 1987; Wilson et al, 1990).

Whatever the initiating stimulus, the subsequent bronchoconstrictor response following exercise is believed to involve local release of mediators from mast cells in the airways (Lee et al, 1983^a). The primary focus of the research carried out in this thesis concerns the role of the mediators, histamine and LTD₄, in exercise bronchoconstriction, rather than focussing on the mechanism of the initiating stimulus.

1.6.2 Mediators and Exercise Bronchoconstriction

The evidence for the role of mast cell mediators in exercise bronchoconstriction to date has largely been circumstantial and was first suggested by results from studies using sodium cromoglycate ('Intal') in asthmatic subjects (Davies, 1968). Sodium cromoglycate, a drug derived from the plant extract khellin, has been used since ancient times particularly in Eastern Mediterranean countries for respiratory conditions. Roger Altounyan, an asthmatic physician, and his colleagues developed and subsequently demonstrated the effectiveness of this drug in asthma

therapy in the late 1960s (Altouyan, 1967; Howell and Altouyan, 1967).

This inhibitory effect of 'Intal' on exercise bronchoconstriction, was confirmed in various studies (Silverman et al, 1973; Wallace and Grieco, 1976; Dahl and Henriksen, 1979). Sodium cromoglycate is believed to be an inhibitor of mediator release from mast cells (Cox, 1967, 1971). The fact that treatment with this drug prevents exercise bronchoconstriction when inhaled prior to, but not when given after, exercise (Silverman and Andrea, 1972), supports the hypothesis that mediators released from mast cells are involved in exercise bronchoconstriction.

1.6.3 Mast Cells

In the late 19th. Century, Paul Ehrlich described various tissue metachromatic cells whose the cell cytoplasm inclusion bodies stained differently with aniline dyes as "mastzellen" (Selye,1965) . Twelve years earlier these mast cells were seen, but not described, in frog mesentery by von Recklinghausen (Selye,1965). Unna demonstrated mast cells in cutaneous lesions of urticaria pigmentosa, suggesting a possible clinical role for these cells. The importance of mast cells and their mediators in rat anaphylactic reactions was subsequently demonstrated by Selye in 1935 (Selye,1965). In man, histochemically distinct mast cells exist in the lung compared to other areas of the body, such as the skin (Befus,1987). However it is not known whether a functional distinction also exists between mast cells from

different sites in humans as is the case in the rat where mast cells differ both histochemically and functionally in different areas of the body.

Mast cells are particularly abundant in the lung (Salvato,1961), the majority are located in the airways and the alveolar walls (Hibbs et al, 1960; Salvato, 1961; Brinkman, 1968; Fox et al, 1981; Tomioka et al, 1984; Gibson et al, 1993) usually superficial to the basement membrane (Patterson et al, 1977; Magius et al, 1985) but also between airway epithelial cells or located on the airway surface in asthmatic subjects (Cutz and Orange, 1977). Mast cells are thus ideally situated to be responsible, through bronchoconstrictor mediator release, for exercise bronchoconstriction.

In the 1960s, the mechanism of mast cell degranulation was addressed as a result of the discovery of immunoglobulin E (IgE) (Ishizaka et al, 1966) with the demonstration that mast cell are primed to release various mediators of immediate hypersensitivity following antigen binding to specific cell IgE receptors. IgE antibody in asthmatic serum was simultaneously discovered and reported on by two independent groups of investigators, the Ishizakas and colleagues, and Johansson and Bennich (Ishizakas et al, 1966; Johansson and Bennich, 1967). This discovery led to a better understanding of allergic mechanisms associated with asthma particularly their importance in the binding of allergen to IgE receptors

on mast cells and the release of inflammatory mediators. At this time, Voorhorst and colleagues also demonstrated the importance of house dust mite allergy in the development of asthma (Voorhorst et al, 1967).

Mast cells are now known to possess various mediators of immediate hypersensitivity, including, preformed and newly generated mediators. Preformed mediators include biogenic amines (histamine, serotonin), neutral proteases (tryptase, chymase), acid hydrolases (β -hexosaminidase, β -glucuronidase, β -galactosidase, arylsulfatase), high molecular weight neutrophil chemotactic factors, and proteoglycans (heparin sulfate, chondroitin sulfate). The other mediators, which are synthesized only upon mast cell activation, include products of cyclooxygenase metabolism (prostaglandins, thromboxanes) and lipoxygenase metabolism (hydroxyeicosatetraenoic acids, leukotrienes B₄, C₄, D₄, E₄), and platelet-activating factor (White and Kaliner, 1991). Histamine, a potent bronchoconstrictor, was the first mast cell derived mediator investigated in the mechanism for exercise bronchoconstriction (Anderson et al, 1981; Barnes and Brown, 1981; Hartley et al, 1981; Lee et al, 1982).

1.6.4 Histamine

β -amino-ethylimidazole or histamine, the oldest known mediator of immediate hypersensitivity, was discovered by Dale and Barger in the early 1900s. By 1907

it was synthesized by Windans and Vogt (Windans and Vogt, 1907). Histamine was first noted to occur in various tissues and hence its name which comes from the Greek histos, meaning tissue. Within four years, Dale, Laidlaw and Barger, had described most of the pharmacological actions of histamine, apart from its effect on gastric acid secretion (Dale and Laidlaw, 1910, 1911; Barger and Dale, 1910, 1911). In 1929, Dale and colleagues isolated histamine from fresh tissue, established that histamine was a natural constituent of the body (Best et al, 1929) and reported that lung tissue was one of the richest sources of histamine in humans. The finding that histamine was released from cells in the skin following injury or allergic reactions by Lewis and colleagues led to the description of the "triple response" of redness, flare, and wheal which occurred following an intradermal injection of histamine. These findings led to greater interest and study of histamine as a possible mediator in asthma.

In 1929, Weiss and colleagues investigated the physiological effects of histamine in man reproduced the features of asthma by giving intravenous histamine to asthmatic subjects (Weiss et al, 1929, 1932). Curry confirmed this bronchoconstrictor effect of histamine in 1946 by demonstrating airway obstruction following intravenous and inhaled histamine in asthmatic subjects (Curry 1946). Curry also demonstrated that histamine bronchoconstriction was more severe in asthmatics compared to non-asthmatics suggesting that asthmatics were more

responsive to histamine stimulation than non-asthmatics (Curry, 1946, 1947; Curry and Lovell, 1948). This knowledge led to the development of histamine inhalation tests, widely used now in clinical and research laboratories to document the degree of airway hyperresponsiveness in asthmatic subjects (Cockcroft et al, 1977^a).

By 1951, Herxheimer and colleagues clearly demonstrated that histamine was released from asthmatic lung tissue and thereby suggesting an important role for this mediator in asthma (Herxheimer, 1951). Subsequent research indicated that the different effects following histamine stimulation occurred due to histamine acting through different receptors.

1.6.4.1 Histamine Receptors

The discovery of antihistamines in the late 1940s was preceded by the findings of Bovet and Staub who discovered substances capable of blocking some histamine stimulated effects (Bovet and Straub, 1937). These effects are now known to occur through stimulation of histamine H₁-receptors, a name suggested by Ash and Schild in 1966 (Ash and Schild, 1966). However these anti-histamine drugs did not block all histamine effects particularly gastric acid secretion. Within 6 years, a new drug which blocked these non-H₁-receptor histamine stimulated responses, was described by Black and colleagues and drugs of this type were termed histamine

H₂-receptor blockers (Black et al, 1972). We have demonstrated in recent years that histamine H₂-receptor stimulation is associated with inhibitory prostaglandin release in the airways (Jackson et al, 1988) and the importance of this effect following exercise will be discussed later in the thesis.

The discovery of different histamine receptor blockers opened up further areas of research into histamine function and, provided new drugs for the treatment of allergic disease. Recently, a new class of histamine receptor, the H₃-receptor, had been described (Arrang et al 1987; Ishikawa and Sperelakis, 1987). However, the exact function of these new receptors in the airways is not clear but they may be involved in modulating neuronal mediated bronchoconstrictor responses (Ichinose and Barnes, 1989).

The classical mechanism of histamine release from mast cells occurs when allergen bridges specific IgE antibodies firmly associated with IgE-F_c receptors (Beaven, 1976; Church et al, 1982; Befus 1987) and the subsequent secretory process is activated. Mast cell histamine release may also occur from stimulation by neuropeptides such as substance P; the anaphylatoxins, C_{3A}, C_{4A} and C_{5A}; various therapeutic drugs including, opiates or antibiotics; diagnostic agents, including iodinated contrast media; and to physical stimuli such as hypoxia, light, heat, cold and trauma (Flint et al, 1985; Eggleston et al, 1987; Freidman and

Kaliner, 1987).

The turnover rate of histamine in mast cells is slow and when the cell is depleted of histamine, it can take days or even weeks to replenish normal levels of histamine. While exogenous histamine diffuses quickly into tissues, it is also rapidly metabolized by the tissue. The two major cellular enzymatic pathways for histamine metabolism are the N-methyltransferase/monoamine oxidase pathway and the histaminase (diamine oxidase) pathway. The end products of histamine metabolism are imidazole acetic acid and imidazole acetic acid riboside which are excreted in the urine. Only a small amount of the total histamine (2-3%) is excreted unchanged in the urine (Gilman et al, 1985).

1.6.4.2 Histamine and Exercise Bronchoconstriction

The role of histamine in exercise bronchoconstriction has been suggested by studies documenting increased circulating levels of histamine following exercise and also by demonstrating the ability of histamine H₁-receptor antagonists to inhibit exercise bronchoconstriction. Several studies have demonstrated increases in plasma histamine concentration during exercise bronchoconstriction (Anderson et al, 1981; Barnes and Brown, 1981; Hartley et al, 1981; Lee et al, 1982; Belcher et al, 1988). Anderson has proposed that small increases in plasma histamine concentrations may reflect much larger increases in the lung because the bronchial

circulation is only 1-2% of the cardiac output and there is rapid metabolism of histamine in the peripheral circulation (Anderson, 1983^a). Histamine likely comes from airway mast cells or from 'leaky' circulating basophils, which increase in number after exercise (Barnes and Brown, 1981; Hartley et al, 1981; Morgan et al, 1983; Howarth et al, 1984), and release histamine spontaneously.

Histamine H₁-receptor antagonists only partially inhibit exercise bronchoconstriction (Hartley and Nogrady, 1980; Clee et al, 1984). This result suggests the possibility that other mast cell bronchoconstrictor mediators may be involved in this effect. Possible mediators include SRS-A (slow-reacting substance of anaphylaxis) (McNeill and Ingram, 1966^b; Simonsson et al, 1972), which is now known to contain leukotrienes (LTs). The work to be described later in this thesis, suggests that leukotriene LTD₄, plays an important role in the development of exercise bronchoconstriction.

1.6.5 Leukotrienes

SRS or "slow reacting substance", now called the leukotrienes, was first mentioned by Feldberg and Kellaway in 1938 to describe the release of a substance from guinea pig and cat lung perfused by cobra venom which was associated with a slow onset, sustained contraction of guinea-pig ileum (Feldberg and Kellaway, 1938). This snake venom is now known to contain the enzyme, phospholipase A₂,

an important enzyme in the release of arachidonic acid, the precursor of leukotrienes, from cell membrane phospholipids. Using a similar perfusate preparation to Feldberg and Kellaway, Brocklehurst during his investigation into the mechanism of anaphylactic shock, described the presence of a sustained reaction from the perfusate of sensitized guinea-pig tissue and asthmatic lung tissue following antigen exposure and this led to the addition of anaphylaxis (A) to SRS-A (Brocklehurst, 1955, 1960). It was of interest that Brocklehurst was at that time, an undergraduate Science student with H.O. Schild when he independently rediscovered SRS-A. Within a short period of time the biological constrictor properties of SRS-A on human airways were confirmed in-vivo in normal subjects and in guinea-pigs following inhalation (Herxheimer and Stresemann, 1963).

By the early 1980s, SRS-A was shown to consist of LTC₄, and its metabolites LTD₄ and LTE₄ (Samuelsson et al, 1980, 1983; Lewis et al, 1980). A detailed account of the chemistry, synthesis, metabolism, receptors and pharmacology of leukotrienes is given elsewhere (Lewis and Austen, 1984; Drazen and Austen, 1987; Piper, 1983; Taylor and Morris, 1983; Samuelsson 1987). The term "leukotriene" was used to describe these molecules because of their proposed leucocyte origin (Samuelsson, 1979). The triene referred to three of the 4 double bond occurred alternatively on the carbon atom backbone. The subscript 4 refers to the leukotrienes molecules with four double bonds. Leukotrienes are thus

biologically active substances derived from the metabolism of arachidonic acid by the action of phospholipase A₂ localized in the cell membrane.

On release from the cell membrane, free arachidonic acid can serve as a substrate for the two major enzyme systems, the cyclooxygenase system and the 5-lipoxygenase system (Figure 1).

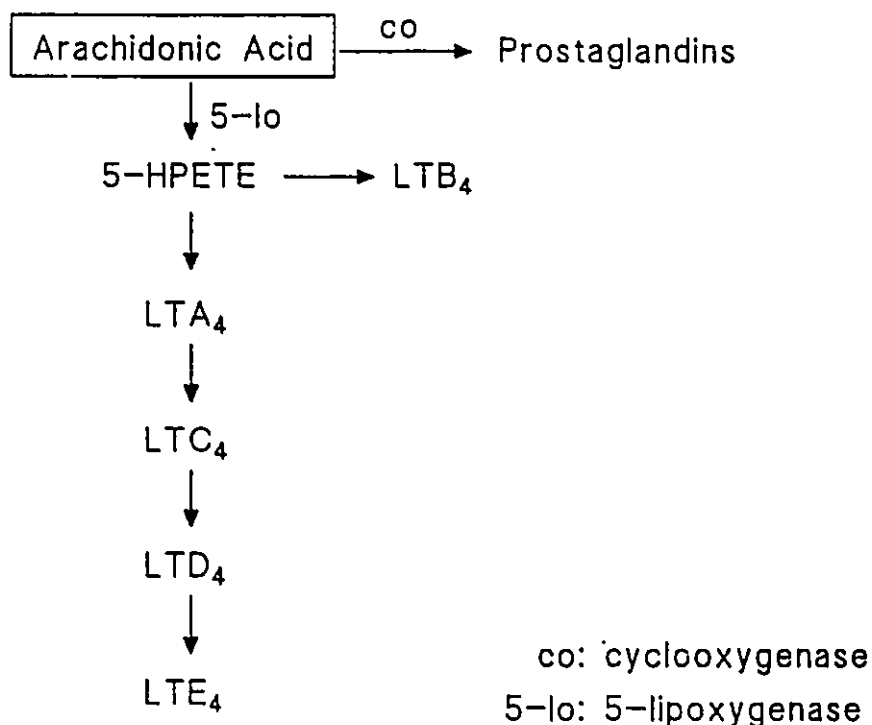


Figure 1.1 The 5-lipoxygenase pathway of arachidonic acid metabolism.

The mechanism involved in arachidonic acid release from cell membranes is as yet poorly defined. Arachidonic acid release can occur following various stimuli which may include, activation of cell receptors, following antigen-antibody

interaction on the cell surface and in response to physical stimuli, such as changes in ionic concentration surround the cell membrane or cold (Drazen and Austen, 1987). These latter stimuli are believed to play a role in the pathophysiology of exercise bronchoconstriction.

Leukotrienes once liberated, are not stored but are metabolized through the 5-lipoxygenase pathway. Arachidonic acid is a 20-carbon polyunsaturated fatty acid which is a natural constituent of most mammalian cells (Samuelsson, 1987).

The leukotrienes were initially divided according to whether or not their molecular structure contained a sulphur linkage and amino acid residues at C-6. These sulphidopeptide leukotrienes included LTC₄, LTD₄, LTE₄ and the while the others included LTA₄ and LTB₄. The sulphidopeptide leukotrienes are now referred to as the cysteinyl leukotrienes because of the presence in the molecule of a cysteine double bond.

The 5-lipoxygenase enzyme catalyses the first two steps in the leukotriene biosynthetic pathway from arachidonic acid. This is an active energy utilization process requires adenosine triphosphate (ATP), calcium and the involvement of 5-lipoxygenase activating protein (FLAP), a specific protein involved in translocation of the 5-lipoxygenase from the cell membrane to the to the cytosol

(Ford-Hutchinson, 1991). Following oxygenation of arachidonic acid the initial metabolites include the unstable intermediate compounds 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE). LTA_4 is then formed from 5-HETE by dehydration, (Lewis and Austen 1984, Taylor and Morris 1983, Piper 1983, Samuelsson 1987). LTA_4 forms either LTB_4 by undergoing hydration, or LTC_4 by the addition of a glutathione molecule. LTC_4 then undergoes further degradation by the removal of a glutamic acid residue to form LTD_4 .

LTE_4 is formed from LTD_4 through the removal of a glycine residue. The conversion of LTC_4 to LTD_4 and LTE_4 occurs in various cells and tissues, particularly in human lung tissue (Aharony et al, 1985) and LTC_4 is the major leukotriene formed in lung mast cells (MacGlashen et al, 1982; Schleimer et al, 1986). LTE_4 appears to be the end product of in the metabolic pathway of the cysteinyl-leukotrienes in the lung and subsequent bioactivation occurs through excretion and/or extrapulmonary metabolism (Parker et al, 1980; Orning et al, 1985; Verhagen et al, 1987; Maltby et al, 1990; Sala et al, 1990).

1.6.5.1 Leukotriene Receptors

Each of the intermediate byproducts of 5-lipoxygenase pathway, LTC_4 , LTD_4 , and LTE_4 are biologically active and may exert their effects through activation of cell

processes following interaction at specific tissue sites. The receptor sites for LTD₄ binding are the best described and appear to be largely confined to the lung (Kuehl et al, 1984; Lewis et al, 1985; Civelli et al, 1987). LTD₄ receptors are composed of a glycoprotein moiety located in the cellular plasma membranes (Drazen and Austen 1987). The cellular response process following LTD₄ receptor binding is complex and involves interaction with cellular G proteins, calcium mobilization and the generation of second messenger systems through phosphoinositide metabolism (Crooke et al, 1990).

In contrast to LTD₄, specific receptor sites for LTC₄ and LTE₄ are not as clearly defined and remain to be elucidated (Drazen and Austen, 1987). However, it is of interest that LTE₄ binds to the LTD₄ receptor in human bronchi. The tissue binding for LTC₄ also occurs in the lung (Lewis and Austen, 1984; Kuehl et al, 1984; Rovati et al, 1985; Drazen and Austen, 1987). Through receptor stimulation, the cysteinyl leukotrienes are potent constrictors of human airway smooth muscle in-vitro (Dahlen et al, 1980; Hanna et al, 1981; Jones et al, 1982), and constrict human airways in-vivo in normal and asthmatic subjects (Weiss et al, 1982, 1983; Biscard et al, 1983; Griffen et al, 1983; Barnes et al, 1984^b; Smith et al, 1985; Adelroth et al, 1986; Kern et al, 1986; Davidson et al, 1987). LTC₄, LTD₄, and LTE₄ have similar airway smooth muscle contractile properties but are 100-1000 times more potent than other constrictor substances such as histamine or

methacholine on a molar basis and have a longer duration of effect (Adelroth et al, 1986, Barnes et al, 1984^b).

LTD₄ is the most potent bronchoconstrictor mediator studied so far in man and as with other constrictor agonists, asthmatics, demonstrate airway hyperresponsiveness to inhaled LTD₄ compared to normal subjects (Adelroth et al, 1986). In addition to their bronchoconstrictor effects, the cysteinyl leukotrienes can increase vascular permeability (Soter et al, 1983) and they are also involved in airway responses to stimulation with allergen (Dahlen et al, 1983; Abraham et al, 1985; Taylor et al, 1989; Manning et al, 1990).

1.6.5.2 LTD₄ Receptor Antagonists

The potential importance of the cysteinyl leukotrienes in the pathogenesis of asthmatic responses has resulted in the pharmaceutical industry launching a major effort in recent years, to modulate the activity of these mediators. This has been achieved either through the development of compounds which either inhibit the leukotriene biosynthetic pathway (5-lipoxygenase inhibitors) or the receptor function (LTD₄ antagonists). A number of LTD₄ receptor antagonists have been developed in recent times. These molecules have been important in detailing the functional activities of LTD₄ on human airway function in the normal state and in disease. Since the discovery of the first LTD₄ antagonist, FPL-55712 (Augstein et al, 1973),

a variety of other receptor antagonists now exist which include; the Smith Klein French compounds, SKF-104,353 and SKF-106,203; Whyeth Laboratories compound, WY 48,252; the Merck compounds, MK-571 and MK-0679; the Imperial Chemical Incorporated (ICI) compounds, ICI-198,615 and ICI-204,219; the ONO Pharmaceuticals compound, ONO-1078; the Searle compounds, SC-41930 and SC-50605; and the Ciba-Geigy compounds CGP-45715 and CGP-44826. Many of these compounds are now undergoing clinical development to determine their clinical usefulness in asthma and other allergic diseases.

The research described in this thesis in examining the role of LTD₄ in exercise bronchoconstriction, utilized the receptor antagonist, MK-571 or [3-(((3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-(dimethyl-amino)-3-oxopropyl)thio)methyl)thio)propanoic acid] (Jones et al, 1989), to inhibit exercise bronchoconstriction in asthmatic subjects, suggesting for the first time the importance of this mediator in exercise bronchoconstriction. In subsequent studies, the role of LTD₄ in exercise refractoriness was also addressed in the thesis.

1.7 Exercise Refractoriness in Asthmatic Subjects

Some asthmatic subjects when exposed to repeated exercise challenges develop progressively less bronchoconstriction with each exercise test even though the degree of exercise achieved is the same and this inhibitory effect may last for up

to 4 hours or more. This reduction in exercise bronchoconstriction is termed exercise refractoriness.

The likely first description of exercise refractoriness was by Thorowgood in 1873, when he noted that an initial bout of mild exercise in some asthmatics could avert a threatened asthmatic attack to a subsequent exercise challenge (Anderson, 1985^a). However, exercise refractoriness was only demonstrated objectively in the mid-1960s (McNeill et al, 1966^b) and this effect was confirmed by other researchers in the 1970s (Anderson et al, 1975; Edmunds et al, 1978).

While the mechanism of exercise refractoriness is unclear, research has demonstrated that exercise refractoriness is not due to a lack of histamine release following exercise implicating some other mechanism in this effect (Belcher et al, 1988^b). O'Byrne and Jones in 1986 suggested that the release of inhibitory prostaglandins are important in the development of exercise refractoriness (O'Byrne and Jones, 1986). These investigators demonstrated that indomethacin, an inhibitor of cyclooxygenase while having no effect on the initial exercise bronchoconstriction, prevented the development of exercise refractoriness in asthmatic subjects, a finding that was confirmed two years later by Margolskee and colleagues (Margolskee et al, 1988). The mechanism of the inhibitory prostaglandin release and subsequent development of exercise refractoriness is

unclear.

Since histamine, as indicated earlier, was shown to be involved in exercise bronchoconstriction, the initial hypothesis examined in this thesis was that histamine was involved in the development of exercise refractoriness. The rationale for this hypothesis was based on the findings from our previous studies demonstrating histamine tachyphylaxis in mild asthmatic subjects (Manning et al, 1987; Manning and O'Byrne, 1988). In these studies we demonstrated that a progressively reduced bronchoconstrictor response occurs to inhaled histamine and, like exercise refractoriness, the effect was inhibited by indomethacin. It was also specific for histamine (Manning et al, 1987) and occurred through histamine H₂-receptor stimulation (Jackson et al, 1988).

1.8 Histamine Tachyphylaxis

Histamine tachyphylaxis was first demonstrated in the late 1970s by Anderson and colleagues in-vitro using airway smooth muscle from dogs (Anderson et al, 1977, 1979^b, 1980) and monkeys (Krzanowski et al, 1980). These investigators demonstrated that repeated contraction of airway smooth muscle with the same dose of histamine produced progressively reduced constrictor responses. Tachyphylaxis comes from the Greek 'tachy' meaning 'fast' and 'phylaxis' meaning 'protection'. Histamine tachyphylaxis was associated with progressively increasing

release of prostaglandin E₂, from the airway smooth muscle in response to histamine stimulation. In 1985, Shore and Martin demonstrated that histamine tachyphylaxis occurred in-vivo in dogs (Shore and Martin, 1985) and that indomethacin blocked the effect. We and others, have shown that histamine tachyphylaxis occurs in human airway in-vivo and in-vitro. (Manning, 1987; Knight, 1992). Histamine tachyphylaxis in humans can be also be inhibited by indomethacin (Manning, 1987)

Histamine-induced airway smooth muscle contraction occurs through stimulation of airway H₁-receptors. However, release of prostaglandin E occurs in some animal species following airway H₂-receptor stimulation (Yen 1976, Mathé 1977). This suggests that stimulation of airway smooth muscle H₂-receptors by histamine may be important in the generation of tachyphylaxis to histamine. Recently we have demonstrated that pretreatment with cimetidine, a potent H₂-receptors antagonist, attenuates histamine tachyphylaxis in-vivo in asthmatic subjects, suggesting an important role for airway H₂-receptor stimulation to release prostaglandins leading to the subsequent development of histamine tachyphylaxis in these subjects (Jackson, 1988). This was subsequently confirmed in human airways in-vitro (Knight, 1992).

1.9 Prostaglandins

The existence of prostaglandins was first inferred by Kurzrok and Lieb in 1930, when they demonstrated that strips of human uterus relaxed and contracted when exposed to human semen (Kurzrok and Leib 1930). In the mid-1930s, Ulf von Euler in Sweden and Goldblatt in England, independently drew attention to specific substances derived from C₂₀ polyunsaturated fatty acids (Goldblatt 1933, 1935; von Euler 1934, 1937). Crude prostaglandins were extracted from the seminal fluid of man and vesicular glands of sheep and von Euler, believing these substances to originate from the prostate gland, named them 'prostaglandins'. Shortly after this the smooth muscle-contracting and vasopressor actions of these substances was shown.

In the late 1950s von Euler, together with Bergstrom and Sjovall isolated two prostaglandins, which seemed to be responsible for the biological activity of the crude prostaglandin extract (called prostaglandin E and F) and by the early 1960's chemical structure of the compounds were determined (Bergstrom and Sjovall, 1957, 1960^a, 1960^b, 1960^c). With the help of Samuelsson and Ryhage they managed to isolate and identify six prostaglandins (PGE₁, E₂, E₃, F_{1α}, F_{2α}, F_{3α}) from the crude extracts (Bergstrom et al, 1960^a, 1962^a, 1963^a; Bergstrom and Sjovall, 1960^b, 1960^c; Bergstrom and Samuelsson, 1962^b, 1963^b).

Samuelsson subsequently carried out extensive studies on the metabolism of prostaglandins and in 1965 along with Anggard, he demonstrated prostaglandin biosynthesis from arachidonic acid in guinea pig lung (Anggard and Samuelsson, 1965). Samuelsson and Bergstrom, simultaneously with van Dorp and colleagues at Unilever Laboratories in Holland and the scientists at Upjohn Laboratories in the United States, discovered the relationship between essential fatty acids and prostaglandin production. This enabled prostaglandins to be synthesized in vast quantities and made them readily available for scientific research.

By the early 1970s, Samuelsson and Hamberg identified unstable endoperoxide intermediaries in the prostaglandin biosynthetic pathway associated with platelet aggregating and thrombosis and termed them thromboxane (Hamberg and Samuelson 1973, 1974). Vane, Moncada and others, later described a platelet anti-aggregating prostaglandin produced by blood vessels called prostaglandin I₂ or prostacyclin (Moncada et al, 1976; Dusting et al, 1977).

Vane and Ferreira demonstrated, in the late 1960s, that prostaglandins were metabolized and inactivated in the lung (Ferreira and Vane, 1967). Piper and Vane also demonstrated that prostaglandins are released from the lungs and other tissues (Piper and Vane, 1971). In addition, at that time Vane and colleagues demonstrated that aspirin and aspirin-like drugs such as indomethacin or

flurbiprofen (a drug used in this thesis) prevent the biosynthesis of prostaglandins in homogenates of guinea-pig lung in-vitro (Vane 1971; Higgs et al, 1976)

Subsequently, Smith and Willis demonstrated a similar finding in human platelets ex vivo (Smith and Willis, 1971). These results therefore provided an explanation for the efficacy of aspirin in relieving some effects, particularly pain, associated with inflammation and were, according to Vane and Ferriera, a clear indication for the involvement of prostaglandins in these effects (Ferriera et al, 1971; Ferriera and Vane, 1973). The finding that prostaglandins play a role in inflammation opened the way for further research to examine the importance of these compounds in the inflammatory response in diseases such as asthma. Flurbiprofen has been shown to be a potent cyclooxygenase inhibitor and is used in this thesis as an inhibitor of prostaglandin synthesis (Mizushima et al, 1975; Higgs et al, 1983).

Prostaglandins are now known to be products of polyunsaturated fatty acid metabolism (Lands and Samuelsson, 1968) and in man, the most common prostaglandin precursor is arachidonic acid (Figure 2).

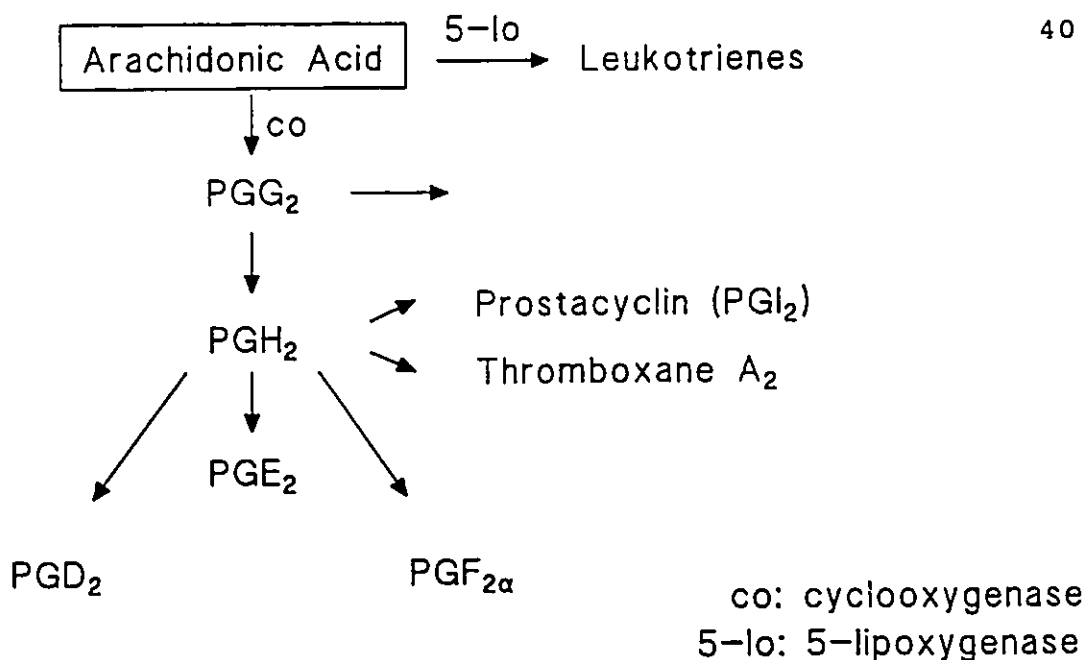


Figure 1.2 The cyclooxygenase pathway of arachidonic acid metabolism.

Prostaglandins are rapidly inactivated through the oxidation of the C15 hydroxyl group by 15-hydroxy-prostaglandin-dehydrogenase. This enzyme is widely distributed in many tissues, especially lung tissue. The lungs therefore play a major role in prostaglandin metabolism, with over 90% of prostaglandins, particularly prostaglandin E, being metabolized during a single passage through the lungs (Bakhle, 1983).

Although work continues in the area of prostaglandin function, it is of interest that the Noble prize for Physiology and Medicine was jointly awarded to Bergstrom,

Samuelsson and Vane jointly for their work on eicosanoid (cyclo-oxygenase and lipoxygenase products) research in 1982 (Check, 1982).

1.9.1 Prostaglandin E and Airway Function

The exact role of prostaglandin E in airway functioning is unclear but may include important local homeostatic control of bronchial tone (Grodzinska et al, 1975). Airway smooth muscle (Steele et al, 1979; Adkinson et al, 1980) and epithelium (Leikauf et al, 1985) are capable of producing prostaglandin E stimulated. In addition, prostaglandin E₁ and E₂ relax human airway smooth muscle in vitro and human airways in-vivo. Inhaled PGE₂ induces bronchodilation and a short lived improvement in histamine airway hyperresponsiveness in asthmatics (Herxheimer and Roetscher, 1971; Rosenthale et al, 1971; Smith and Cuthbert, 1972; Walters et al, 1982^a; Walters and Davies, 1982^b).

1.10 Purpose of the Thesis

The purpose of the studies described in this thesis was to evaluate the role of LTD₄ in the pathogenesis of exercise bronchoconstriction and of histamine or LTD₄-induced inhibitory prostaglandin release in causing exercise refractoriness.

1.10.1 General Objective of the Thesis

Due to the similarities of exercise refractoriness and histamine tachyphylaxis in asthmatic subjects, both of which are inhibited by indomethacin, one hypothesis for the development of exercise refractoriness, explored in this thesis, was that histamine release in the airways during exercise causes both the initial acute bronchoconstriction and the subsequent release of inhibitory prostaglandins, possibly through histamine H₂-receptor stimulation.

In the first three studies, described in this thesis, a role for histamine in the pathogenesis of exercise refractoriness was evaluated by two different approaches. The first approach examined whether exercise refractoriness and histamine tachyphylaxis occurred in the same individuals and whether cross refractoriness/tachyphylaxis occurred. The second examined whether ranitidine, a potent histamine H₂-receptor antagonist inhibited exercise refractoriness. The results from these studies did not support the hypothesis that histamine-stimulated inhibitory prostaglandin release is the mechanism underlying exercise refractoriness.

In view of these results with histamine, the involvement of another bronchoconstrictor mediator, LTD₄, was evaluated as a possible mediator causing exercise bronchoconstriction. The rationale for this was based on previous studies

which demonstrated that LTD₄ is a potent bronchoconstrictor of asthmatic airways and plays a role in the pathogenesis of other asthmatic responses including allergen-induced bronchoconstriction. The results from this study demonstrated that LTD₄ is the main mediator causing exercise bronchoconstriction. This led to the revised hypothesis that LTD₄-induced inhibitory prostaglandin release is the cause of exercise refractoriness. Once again several approaches were taken to address this issue. These included, investigating whether exercise refractoriness and LTD₄ tachyphylaxis occurred in the same individuals and whether cross refractoriness/tachyphylaxis occurred and if so, whether flurbiprofen blocked this effect.

1.10.2 Specific Questions

The work described in this thesis addressed the following specific questions on the involvement of histamine and LTD₄ in exercise bronchoconstriction and refractoriness:

A. Role of Histamine in Exercise Refractoriness

- Question (A). Does histamine tachyphylaxis occur in asthmatic subjects who develop exercise refractoriness ?
- Question (B). Does cross refractoriness/tachyphylaxis occur between exercise and histamine bronchoconstrictor responses in asthmatic subjects ?

Question (C). Does pretreatment with ranitidine, a potent histamine H₂-receptor antagonist inhibit exercise refractoriness in asthmatic subjects?

B. Role of LTD₄ in Exercise Bronchoconstriction

Question (D). Does the selective LTD₄ receptor antagonist, MK-571, inhibit exercise bronchoconstriction in asthmatic subjects.

C. Role of LTD₄ in Exercise Refractoriness

Question (E). Does exercise refractoriness and LTD₄ tachyphylaxis occur in asthmatic subjects and if so, is there a correlation between the degree of exercise refractoriness and LTD₄ tachyphylaxis in asthmatic subjects?

Question (F). Is tachyphylaxis to inhaled LTD₄ in asthmatic subjects attenuated by pretreatment with the cyclooxygenase inhibitor flurbiprofen?

Question (G). Does exercise cause tachyphylaxis to LTD₄-induced bronchoconstriction in asthmatic subjects and is the effect attenuated by flurbiprofen.

Question (H). Does LTD₄ cause refractoriness to exercise in asthmatic subjects and is the effect attenuated by flurbiprofen?

Chapter II

Methods

2.1 General Overview

This section will describe the characteristics of subjects enrolled in the studies and the experimental methods used in the exercise, histamine and LTD₄ tests in the studies. The specific methodology, study design and analytic methods employed in each separate study will be described in the following chapter.

2.2 Subject Characteristics

All the research subjects in these studies had mild asthma and were non-smokers. Subjects required only inhaled salbutamol occasionally as needed for control of asthma symptoms and abstained from this medication for at least 8 hours prior to each test. All subjects were atopic, as indicated by one or more wheal and flare responses to skin prick tests with 16 common allergen extracts. During each study, subjects were not exposed to relevant allergens, with the exception of the dust mite, or had symptoms of respiratory tract infection for at least 2 months before the studies.

At the beginning of each study, subjects had a baseline forced expired volume in

one second (FEV_1) greater than 70% of predicted (Crapo et al, 1981), which did not vary by more than 10% throughout the particular study period. All demographic information obtained from patients before each study was documented.

2.3 Methodology

In the various studies, subjects undertook challenges with either exercise alone or in combination with inhaled histamine or LTD_4 . Following the completion of the tests on each study day, subjects received inhaled salbutamol, if necessary, to relieve the induced bronchoconstriction prior to leaving the laboratory.

2.3.1 Pulmonary Function Test

The airway response to bronchoconstrictor stimuli such as histamine, LTD_4 or exercise can be measured in a variety of ways. The forced expired volume in 1 second (FEV_1) is the most common method used in clinical practice and was used in the studies described in this thesis to measure the response of the airways to these bronchoconstrictor stimuli. This was because the FEV_1 has been shown to be reproducible, easy to perform, acceptable to the subject and does not require expensive equipment to perform. These spirometric measurements were made using a Collins water spirometer (Warren E. Collins Inc. Braintree, Mass) for most tests except in the first two studies when a Vitalograph dry wedge spirometer (Vitalograph, U.K.) was used to measure the FEV_1 response to exercise.

2.3.2 Exercise Test

The exercise challenge test was performed on a stationary bicycle ergometer (Ergomed 740, Siemens, West Germany) by the method described by O'Byrne and Jones (O'Byrne and Jones, 1986). The exercise workload was determined as the workload which increased the maximal heart rate to 80% predicted normal during a stage 1 exercise test (Jones, 1988). Once established this exercise workload was kept constant for each subject.

During exercise, subjects wore noseclips and inhaled dry air at room temperature (22-23°C) from a Douglas bag reservoir through a mouthpiece. The minute ventilation achieved by each subject during the test was measured, as litres per minute, with a dry gasometer (Parkinson-Cowen CD4) and heart rate responses were continuously monitored using a three lead electrocardiogram. The maximal heart rate achieved during exercise was recorded in beats per minute.

The bronchoconstrictor response to exercise was determined by measuring the FEV₁ prior to exercise, (the mean of three best values was taken as the baseline value) and following exercise measured immediately, at 3 minutes, and subsequently every 2 minutes until the lowest FEV₁ was recorded, and then every 5 minutes until the FEV₁ had returned to baseline for a period of up to 60 minutes.

The pre- and post-exercise FEV₁ values were then compared. The percent fall index was used to quantify the degree of exercise bronchoconstriction (Figure 2.1) and was determined by calculating the percentage fall in FEV₁ from the baseline FEV₁ and the lowest post exercise FEV₁ value as follows:

$$\frac{\text{Baseline FEV}_1 - \text{lowest FEV}_1 \text{ post exercise}}{\text{Baseline FEV}_1} \times 100$$

The following is a typical response and is shown graphically in figure 2.1.

Time (Minutes) (post-exercise)	FEV ₁ (litres)	FEV ₁ (% Baseline)
Baseline	3.00	100
0	3.00	100
1	2.85	95
3	2.61	87
5	2.49	83
8	2.37	79
10	2.34	78
15	2.40	80

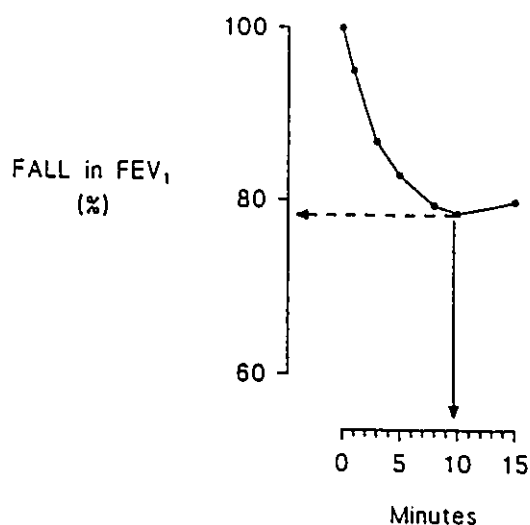


Figure 2.1 Exercise bronchoconstriction

A typical response is shown. The results are expressed as the maximum fall in the FEV₁ post exercise; as indicated by the arrow.

In general the maximal fall in FEV₁ occurs between 5-10 minutes post exercise and is usually back to baseline value ($\pm 5\%$) within 45 minutes. A fall of 10% or greater in FEV₁ post exercise is now accepted as significant exercise bronchoconstriction. In the studies described in this thesis all asthmatic subjects had a documented fall in FEV₁ of 15% or greater following exercise prior to entering the studies.

2.3.2.1 Repeated Exercise Tests

All subjects had a second exercise challenge test performed at least on 1 occasion, similar in workload and duration to the initial test. When subjects

performed repeated exercise tests within 1 hour, refractoriness occurred as demonstrated by a reduced fall in FEV₁ after exercise. The degree of refractoriness varied between subjects. This second test was performed when the FEV₁ values had returned to at least 5% of baseline FEV₁ value following the first test. The response was again measured by the fall in FEV₁ and subsequently after exercise at similar time points to that described in the first study (Figure 2). The % fall in FEV₁ between the first and second test was compared to obtain the degree of refractoriness as follows:

$$\frac{\text{Maximal \% fall in FEV}_1 \text{ for the first test} - \text{second test}}{\text{Maximal \% fall in FEV}_1 \text{ for the first test}} \times 100$$

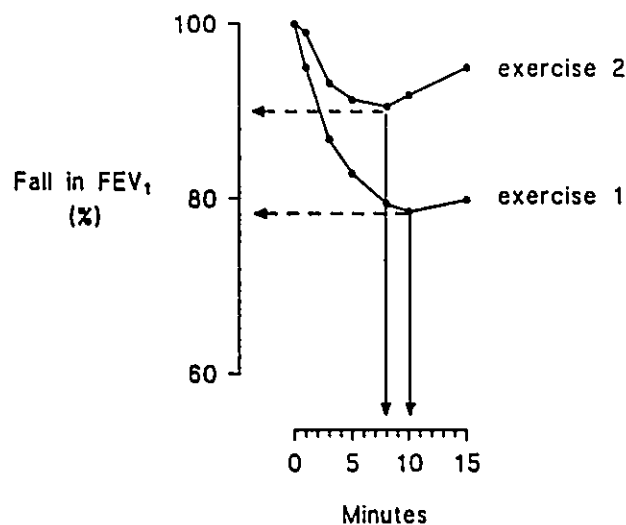


Figure 2.2 Exercise Refractoriness

A typical response is shown and the results expressed as the fall in FEV₁ post-exercise. The arrows demonstrate the maximal fall in FEV₁ for both challenges. The response is reduced during the second exercise test indicating the development of exercise refractoriness.

2.3.3 Histamine Inhalation Test

Histamine inhalation tests, performed in this thesis were carried out by the method described by Cockcroft and colleagues (Cockcroft et al, 1977^a). The test measures the concentration of histamine required to produce a specific change in the airway calibre, as measured by a fall in FEV₁. During the test, subjects first performed three baseline FEV₁s, to ensure reproducibility of the measurement and then inhaled nebulized saline diluent for 2 minutes, to determine the effect of the histamine diluent on airway function. Following diluent inhalation, subjects performed FEV₁ measurements at 30, 90, and 180 seconds. The lowest FEV₁ value was then chosen as the baseline FEV₁ for the inhalation test. Provided this FEV₁ post-diluent did not fall by more than 10%, the test continued.

After the diluent inhalation, increasing doubling concentrations of histamine acid phosphate from 0.03 mg/ml to 32.0 mg/ml were inhaled for 2 min at intervals of 5 min. The response to the histamine was measured by the fall in FEV₁. The FEV₁ was measured at 0.5, 1.5 minutes and then at 2 minute intervals, if necessary, to record the lowest value after each separate histamine inhalation. The test was stopped when the FEV₁ has fallen by 20%, or when the maximum concentration of histamine is inhaled. The percentage fall in FEV₁ was then calculated from the lowest post-saline FEV₁ and the lowest post-histamine acid phosphate FEV₁ value as follows:

$$\frac{\text{Lowest FEV}_1 \text{ post saline} - \text{lowest FEV}_1 \text{ post histamine}}{\text{lowest FEV}_1 \text{ post-saline}} \times 100$$

The results were expressed as the provocation concentration causing a fall in the FEV₁ by 20% (PC₂₀) (Figure 2.3).

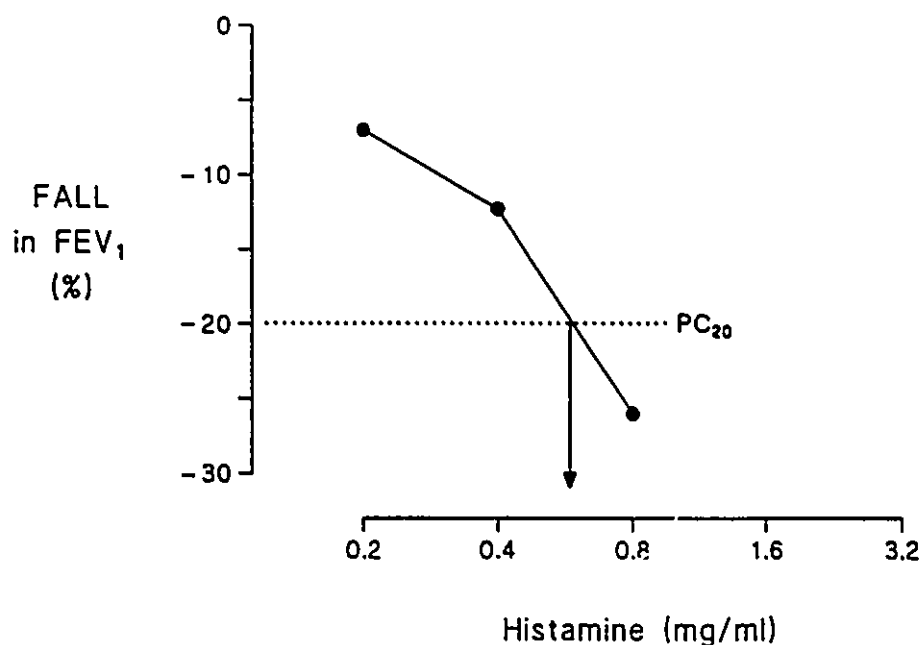


Figure 2.3 Histamine Bronchoconstriction

A typical response is shown and the results expressed as the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀). The arrow indicates the PC₂₀ histamine concentration.

The PC₂₀ histamine was obtained by linear interpolation of the last two data points on the log dose-response curve and this was done using a Hewlett-Packard 41CV Calculator (Hewlett-Packard Co., Oregon, USA). The important technical factors for inhalation tests, which can influence the response were standardized to ensure

that the results were interpreted accurately. These included, the nebulizer output, the particle size of the aerosol, the speed and volume of inspiration (Ryan et al, 1981^a) and the temperature of the solution. The aerosols of the histamine and diluent solution were generated in all cases by the same Wright nebulizer. The aerosols were delivered into a face mask and inhaled through the mouth by quiet tidal breathing for 2 min, the nose being closed by a clip.

2.3.3.1 Repeated Histamine Tests

Some subjects described in the initial part of the thesis had a second histamine test performed at least on one occasion to determine whether tachyphylaxis occurred. This second histamine test was performed 1 hour following the initial histamine test and no bronchodilator was given between the tests.

The second test was done when the FEV₁ values had returned to at least 5% of baseline FEV₁ value following the first test. An increase in the histamine PC₂₀ in the second test compared to the initial test was indicative of tachyphylaxis (Figure 2.4).

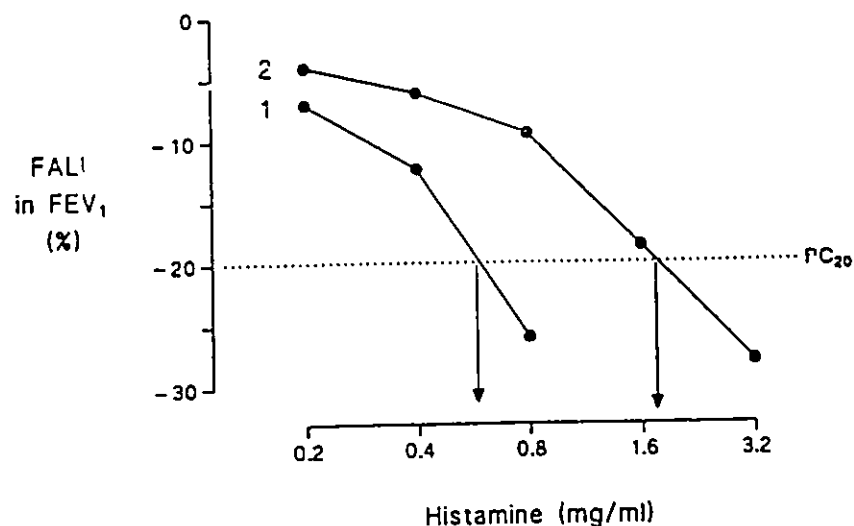


Figure 2.4 Histamine Tachyphylaxis

A typical response is shown. The arrows indicate the PC₂₀ histamine concentration for both tests. The increase in the second histamine PC₂₀ indicates the development of tachyphylaxis.

2.3.4 LTD₄ Inhalation Test

LTD₄ inhalation tests were carried out by the method described by Aderoth et al (Aderoth et al, 1986). The FEV₁ was used to measure the bronchoconstrictor response. Subjects first performed three FEV₁s and a vital capacity (VC) and the lowest FEV₁ was chosen as the baseline FEV₁ for the inhalation test. Subjects initially inhaled 10 breaths of nebulized LTD₄-diluent, a phosphate-buffered saline solution, in order to determine the effect of the diluent on the airway function. The inhalation test proceeded provided the FEV₁ did not fall by more than 10% post diluent. Subjects then performed a dose response inhalation challenge with LTD₄ diluted in saline. The LTD₄ aerosol were generated using a Rosenthal-French

dosimeter attached to a DeVilbiss 646 nebulizer as previously described (Ryan et al, 1981^a).

After the initial control solution of saline diluent, subjects inhaled 10 deep breaths of increasing doubling concentrations of LTD₄ from 0.025 to 12.5 µg/ml at intervals of 5 min through a mouthpiece attached to the nebulizer, the nose being closed by a clip. The response was measured by the FEV₁ which was performed at 0.5, 1.5 minutes and then at 2 minute intervals, if necessary, to record the lowest value after each inhalation. The test was stopped when the FEV₁ had fallen by at least 20% or when the maximum concentration had been inhaled. The percentage fall in FEV₁ was calculated from the lowest post-saline FEV₁ and the lowest post-LTD₄ FEV₁ value as follows:

$$\frac{\text{Lowest FEV}_1 \text{ post saline} - \text{lowest FEV}_1 \text{ post LTD}_4}{\text{lowest FEV}_1 \text{ post-saline}} \times 100$$

The results were expressed as the provocation concentration causing a fall in the FEV₁ by 20% (PC₂₀). The PC₂₀ LTD₄ was obtained by linear interpolation of the last two data points on the log dose-response curve using a Hewlett-Packard 41CV Calculator (Hewlett-Packard Co., Oregon, USA). The FEV₁ was then measured every 3 minutes until it had returned to within 5 % of the baseline FEV₁ (Figure 2. 5).

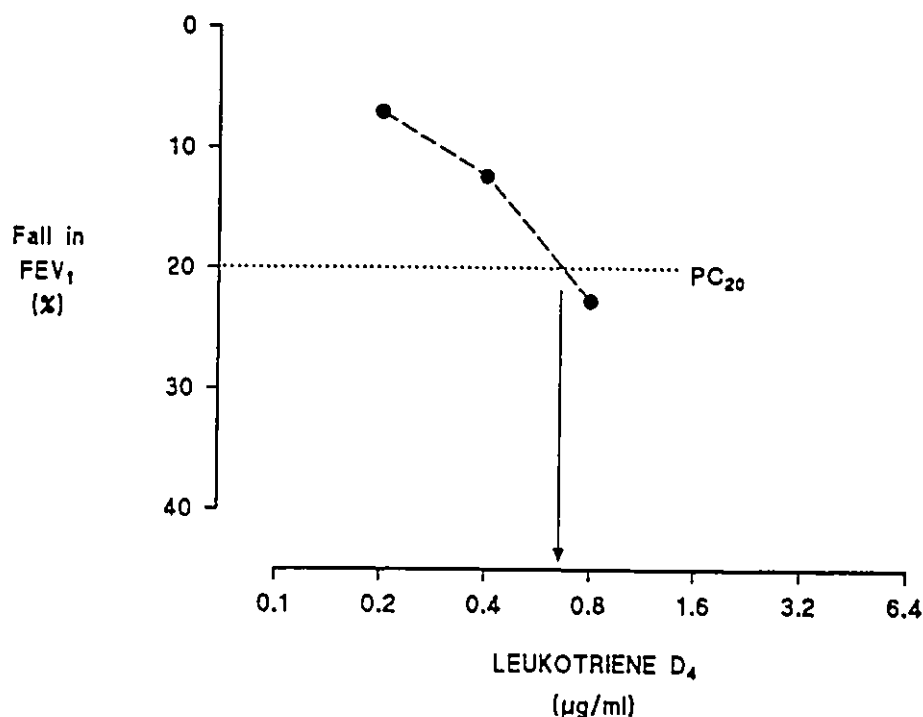


Figure 2.5 LTD₄ Bronchoconstriction

A typical response is shown and the results were expressed as the provocative concentration of LTD₄ causing a 20% fall in FEV₁ (PC₂₀). The arrow indicates the PC₂₀ LTD₄ concentration.

As with the histamine tests, the important technical factors were standardised in order to ensure an accurate interpretation of the results. These factors included, the nebulizer output, the particle size of the aerosol, the speed and volume of inspiration (Ryan et al, 1981^b) and the temperature of the solution.

Although the methods of inducing bronchoconstriction between the Rosenthal nebulizer used for the LTD₄ tests and the Wright nebulizer used for the histamine test are different, both methods are comparable in terms of the reproducibility of the responses. However, the reduced volume (1 millilitre) required for the DeVilbiss nebulizer was useful because only small volumes of LTD₄ were available.

2.3.4.1 Repeated LTD₄ Tests

Some subjects described in the latter part of the thesis had a second LTD₄ test performed at least on 1 occasion to determine whether tachyphylaxis occurred. This second LTD₄ test was performed 1 hour following the initial LTD₄ test and no bronchodilator was given between the tests (Figure 2.6).

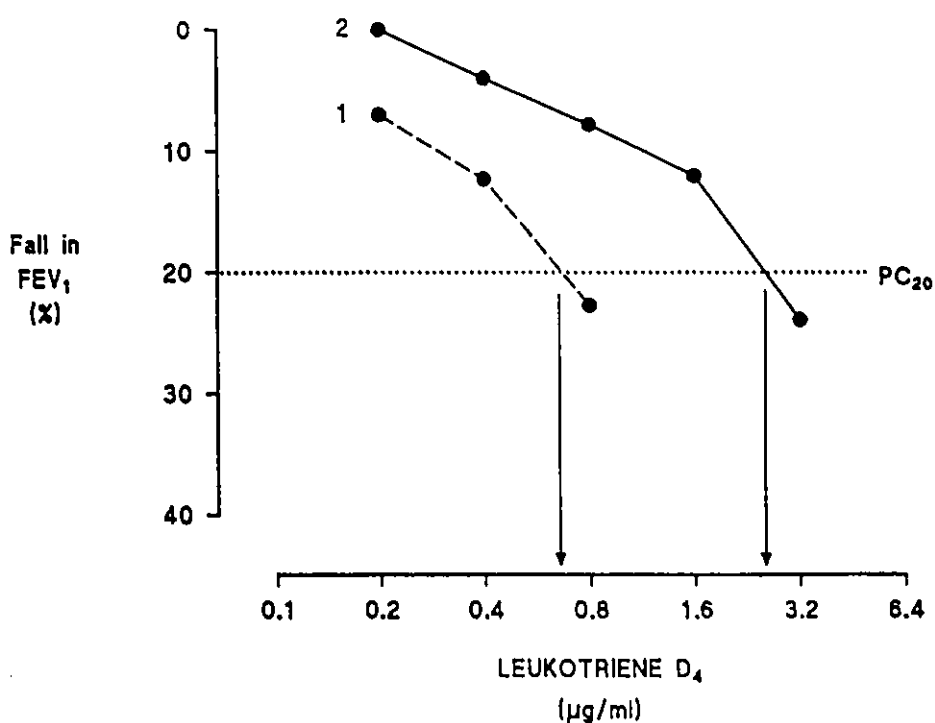


Figure 2.6 LTD₄ Tachyphylaxis

A typical response is shown. The arrows indicate the PC₂₀ LTD₄ concentration for both tests. The increase in the second LTD₄ PC₂₀ indicates the development of tachyphylaxis.

This test was done when the FEV₁ values had returned spontaneously to at least

5% of baseline FEV₁ value following the first test. In this second test the response was once again measured as the LTD₄ PC₂₀. An increase in the LTD₄ PC₂₀ in the second test compared to the initial test was indicative of tachyphylaxis.

2.4 Ethics Approval

Each study described in this thesis was approved by the Ethic's Committee at McMaster University Medical Centre and written informed consent for each study was obtained from the subjects prior to study start.

Chapter III

A. Role of Histamine in Exercise Refractoriness

3.1 Introduction

Histamine and exercise are potent stimuli for bronchoconstriction in asthma. As mentioned earlier, asthmatic subjects demonstrate refractoriness to repeated exercise challenges and tachyphylaxis to repeated histamine challenges. Both refractoriness and tachyphylaxis can be inhibited by indomethacin (O'Byrne and Jones, 1986; Manning et al, 1987; Margolske et al, 1988), suggesting the involvement of inhibitory prostaglandins in these effects. Histamine tachyphylaxis in asthmatic subjects is attenuated by cimetidine, a potent histamine H₂-receptor antagonist, thereby implicating airway H₂-receptor stimulation in this effect (Jackson et al, 1988). The hypothesis (Figure 3.1) investigated in this initial part of the thesis examined the possible role for histamine, through the mechanism of histamine tachyphylaxis, in the development of exercise refractoriness. We therefore hypothesised that cross refractoriness and tachyphylaxis should occur between histamine and exercise bronchoconstriction and exercise refractoriness should involve histamine H₂-receptor stimulation. Studies 1-3 of this thesis, were therefore undertaken to investigate the possible involvement of histamine in the development of exercise refractoriness.

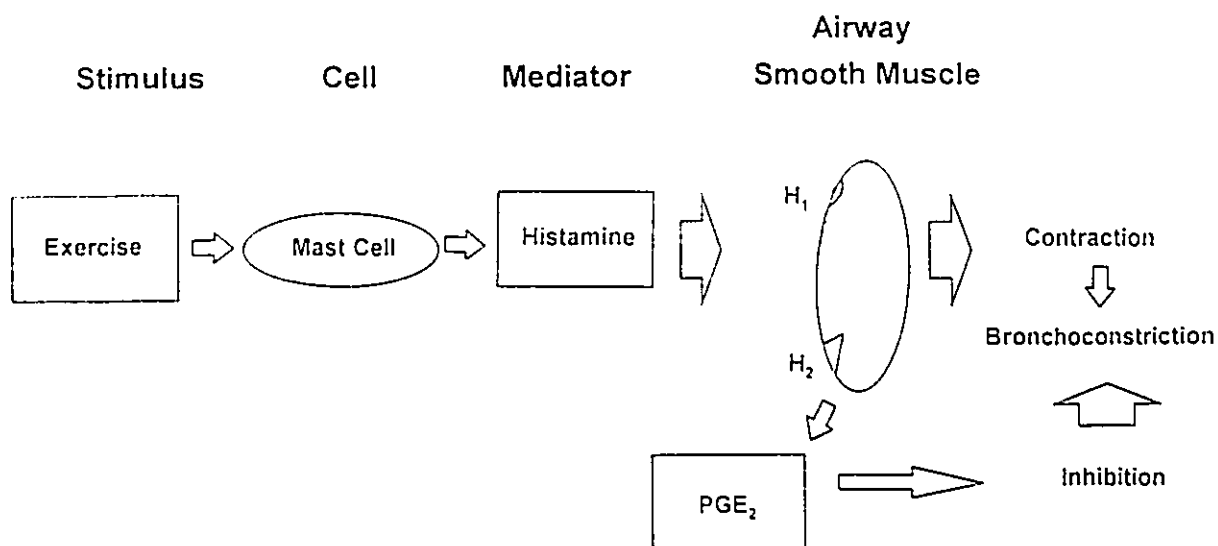


Figure 3.1 A possible mechanism for exercise refractoriness.

STUDY 1

3.2 Introduction

In order to investigate whether histamine tachyphylaxis plays a role in exercise refractoriness, the following initial question was addressed:

- Q. Does histamine tachyphylaxis occur in asthmatic subjects who develop exercise refractoriness ?

3.2.1 Subjects

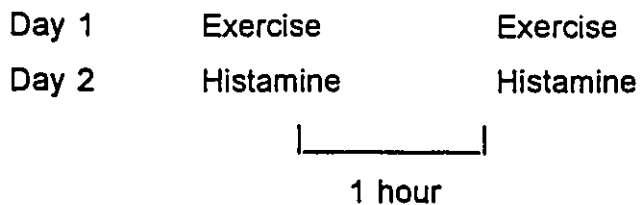
Eight asthmatic subjects with documented bronchoconstriction after exercise were studied (Table 3.1).

Table 3.1 Subject characteristics

Subject	Age (years)	Gender	Baseline FEV ₁ (%Pred.)	Baseline PC ₂₀ Histamine (mg/ml)
1.	17	Male	92	2.50
2.	36	Male	95	1.52
3.	19	Female	102	2.40
4.	38	Male	104	4.22
5.	30	Male	98	0.64
6.	16	Male	86	0.81
7.	45	Female	94	1.23
8.	22	Female	90	3.21

3.2.2 Study Design

Subjects attended the laboratory on two study days, separated by at least two days. On each day two consecutive challenges were carried out one hour apart as follows; Day 1, two exercise challenges to document exercise refractoriness; Day 2, two histamine challenges, to document the presence or absence of histamine tachyphylaxis.



3.2.3 Tests.

3.2.3.1 Exercise challenge

Exercise was performed on a stationary bicycle ergometer as previously described (Section 2.3.2).

3.2.3.2 Histamine Inhalation Test

Subjects performed a dose response inhalation challenge with histamine diluted in phosphate-buffered saline as previously described (Section 2.3.3).

3.2.4 Analysis of Results

Histamine PC_{20} values were log transformed prior to analysis and summary statistics are expressed as geometric mean and percent standard error (% SEM). The logarithmic transformation of the histamine PC_{20} values was required to eliminate the tendency for the standard deviation to be related to the mean. This is important because the standard deviation of repeated PC_{20} values are generally greater in subjects with high PC_{20} compared to low PC_{20} value. All other summary statistics are expressed as mean and standard error (SEM). Comparisons of the fall in FEV_1 after exercise and the histamine PC_{20} on different study days were made using t-tests for paired observations. Statistical significance was accepted as a probability of less than 0.05. Comparisons of prechallenge baseline FEV_1 , heart rate, and ventilation responses during exercise were made using an analysis

of variance (ANOVA).

3.2.5 Results

All subjects developed both bronchoconstriction and refractoriness after exercise on Day 1 (Figure 3.2). The mean fall in FEV₁ after the first exercise challenge was 22.1% (SEM 2.5%). The mean fall in FEV₁ after the second challenge was 11.1% (SEM 3.8%) ($p < 0.005$). This represents a refractory index of 50%.

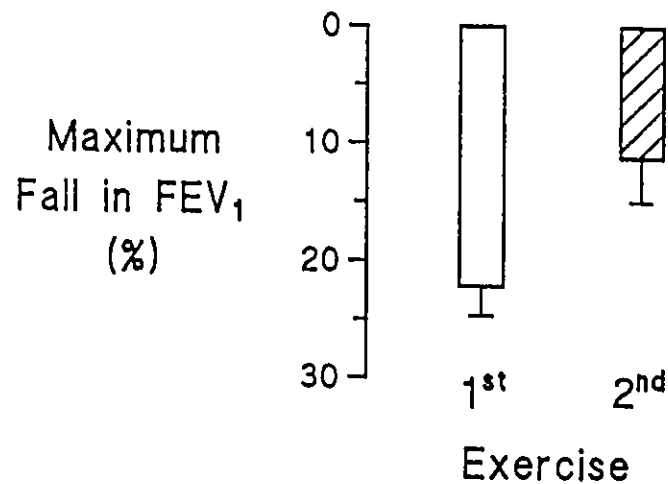


Figure 3.2 Mean (SEM) bronchoconstrictor responses (% fall the FEV₁), to two serial exercise challenges separated by one hour on study day 1.

In addition, the two exercise challenges were comparable with respect to maximum heart rate ($p=0.09$) and minute ventilation ($p=0.75$) (Table 3.2).

Table 3.2 Mean maximum heart rate (beats/minute) and ventilation (litres/minute) with exercise on study day 1.

Test	<u>Heart Rate</u>		<u>Ventilation</u>	
	1	2	1	2
Mean	168.5	171.6	76.4	72.3
SEM	2.8	2.4	6.9	8.7

In addition, all subjects developed tachyphylaxis to inhaled histamine on Day 2 (Figure 3.3). The mean histamine PC_{20} after the first challenge was 1.68 mg/ml (%SEM 1.3) and after the second challenge was 2.68 mg/ml (%SEM 1.3) ($p<0.005$).

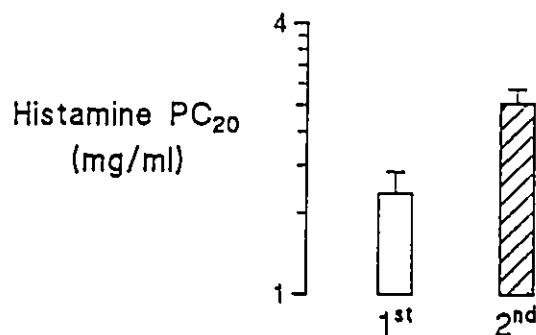


Figure 3.3 Mean (%SEM) bronchoconstrictor responses (PC_{20}) for two serial histamine challenges separated by a one hour interval on study day 2.

The mean baseline FEV₁ was not significantly different prior to each challenge on each study day (p=0.50) (Table 3.3).

Table 3.3 Mean baseline FEV₁ (Litres) before each challenge test.

Test	<u>Day 1</u>		<u>Day 2</u>	
	1*	2*	1	2
Mean	3.49	3.47	3.85	3.82
SEM	0.20	0.30	0.20	0.20

* Measurements made with a wedge spirometer.

3.2.6 Summary

In this study we demonstrated that histamine tachyphylaxis and exercise refractoriness occurs in same asthmatic subjects. This finding is supportive of the hypothesis that histamine tachyphylaxis is involved in exercise refractoriness. If this is so, then cross refractoriness/tachyphylaxis should occur between histamine and exercise and study 2 was therefore undertaken to address this question.

STUDY 2

3.3 Introduction

In order to investigate whether cross refractoriness/tachyphylaxis occurs between exercise and histamine the following question was addressed in this study:

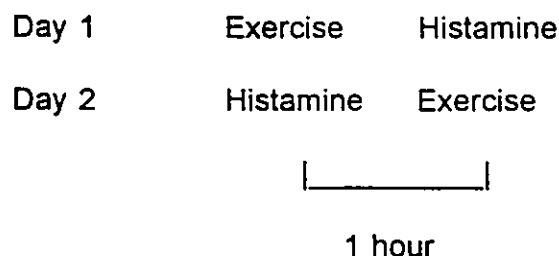
- Q. Does cross refractoriness/tachyphylaxis occur between exercise and histamine bronchoconstrictor responses in asthmatic subjects ?

3.3.1 Subjects

The same eight asthmatic subjects (Table 3.1) with documented exercise refractoriness and histamine tachyphylaxis from study 1 were entered into this study.

3.3.2 Study Design

Subjects attended the laboratory on two study days, separated by at least two days. On each day two consecutive challenges were carried out one hour apart as follows; Day 1, an exercise challenge followed by histamine, to examine whether exercise could induce tachyphylaxis to histamine and Day 2, a histamine challenge followed by exercise, to examine whether histamine could induce exercise refractoriness.



3.3.3. Tests.

3.3.3.1 Exercise challenge

Exercise was performed on a stationary bicycle ergometer as previously described (Section 2.3.2).

3.3.3.2 Histamine Inhalation Test

Subjects performed a dose response inhalation challenge with histamine diluted in phosphate-buffered saline as previously described (Section 2.3.3).

3.3.4 Analysis of Results

Histamine PC_{20} values were log transformed prior to analysis and summary statistics are expressed as geometric mean and percent standard error (% SEM). All other summary statistics are expressed as mean and standard error (SEM). Comparisons of the fall in FEV_1 after exercise and the histamine PC_{20} on different study days were made using t-tests for paired observations statistical significance was accepted as a probability of less than 0.05. Comparisons of prechallenge

baseline FEV₁, heart rate, and ventilation responses during exercise were made using an analysis of variance (ANOVA).

3.3.5 Results

All subjects developed bronchoconstriction after exercise on Day 1. The mean exercise-induced fall in FEV₁ before the histamine challenge on Day 1 was 23.1% (SEM 3.0%). However, on Day 2, a prior histamine challenge reduced the subsequent exercise-induced fall in FEV₁ in all subjects (Figure 3.4). The mean % fall in FEV₁ after exercise following histamine inhalation on Day 2 was 15.6% (SEM 3.5%) ($p < 0.005$). This indicates a refractoriness index of 32.4%.

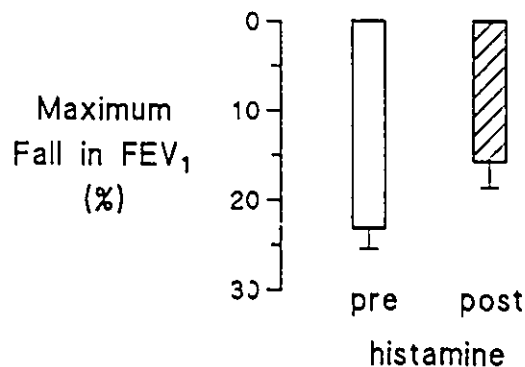


Figure 3.4 The mean (SEM) % fall in FEV₁ post exercise prior to, and 1 hour post histamine challenge on Days 1 and 2 respectively.

The two exercise challenges were comparable with respect to maximum heart rate ($p = 0.52$) and minute ventilation ($p = 0.26$) (Table 3.4).

Table 3.4 Mean maximum heart rate (beats/minute) and ventilation (litres/minute) with exercise on study days 1 and 2.

Day	<u>Heart Rate</u>		<u>Ventilation</u>	
	1	2	1	2
Mean	167.2	165.8	70.0	66.1
SEM	4.8	4.2	7.0	5.7

Prior exercise bronchoconstriction did not induce histamine tachyphylaxis. The mean histamine PC₂₀ following exercise on Day 1 was 1.88 mg/ml (%SEM 1.4) ($p>0.5$), which was not different from the mean histamine PC₂₀ before exercise on Day 2 which was 1.67 mg/ml (%SEM 1.3) ($p>0.05$) (Figure 3.5).

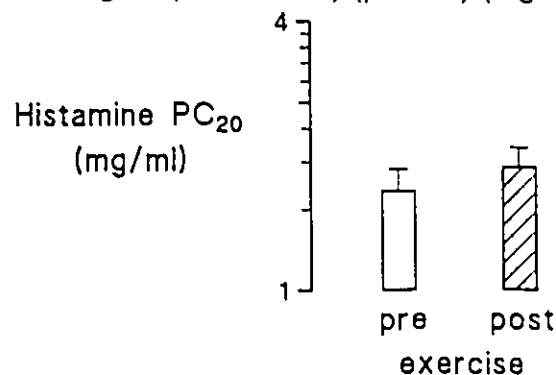


Figure 3.5 Mean (%SEM) bronchoconstrictor responses (PC₂₀) to histamine; prior to exercise on study day 2 and; 1 hour after exercise on study day 1.

The mean baseline FEV₁ was not significantly different prior to each challenge on each study day (p=0.56) (Table 3.5).

Table 3.5 Mean baseline FEV₁ (Litres) before each challenge.

Test	<u>Day 1</u>		<u>Day 2</u>	
	1*	2	1	2*
Mean	3.82	3.70	3.40	3.81
SEM	0.20	0.20	0.20	0.20

*Measurement made with wedge spirometer.

Since the same 8 subjects were involved in both this study and study 1, the reproducibility of the baseline exercise bronchoconstriction and histamine PC₂₀ were compared and were found to be reproducible when the tests were separated by up to 1 month. The mean % fall in FEV₁ post exercise on Day 1, in Studies 1 and 2, were 22.1% (%SEM 3.5%) and 23.1% (%SEM 3.0) (p>0.5) respectively. The mean histamine PC₂₀ on Day 2, in Studies 1 and 2, were 1.68 mg/ml (%SEM 1.3) and 1.67 mg/ml (%SEM 1.3) (p>0.5) respectively.

3.3.6 Summary

In this study, we have demonstrated that in the same asthmatic subjects with previously documented exercise refractoriness and histamine tachyphylaxis, a prior histamine bronchoconstriction induces exercise refractoriness. However, the converse is not true; prior exercise does not reduce subsequent histamine airway responsiveness. This results indicates that complete cross refractoriness/tachyphylaxis does not occur between exercise and histamine. These results are not consistent with the hypothesis that exercise refractoriness is caused through the mechanism of histamine tachyphylaxis.

STUDY 3

3.4 Introduction

To further investigate the possible role of histamine in exercise refractoriness, a third study was carried out to determine whether exercise refractoriness, like histamine tachyphylaxis in asthmatic subjects could also be attenuated by pretreatment with a histamine H₂-receptor antagonist. The following question was addressed in this study:

- Q. Does pretreatment with ranitidine, a potent histamine H₂-receptor antagonist attenuate exercise refractoriness in asthmatic subjects?

3.4.1 Subjects

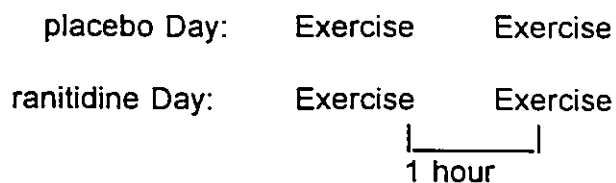
Eight asthmatic subjects with previously documented exercise-induced bronchoconstriction and refractoriness after exercise were studied (Table 3.6).

Table 3.6 Subject characteristics

Subject	Age (years)	Gender	Baseline FEV ₁ (%Pred.)
1.	22	Female	90
2.	23	Male	77
3.	37	Male	72
4.	18	Male	88
5.	21	Male	73
6.	22	Male	100
7.	32	Male	89
8.	38	Female	95

3.4.2 Study Design

Subjects attended the laboratory on 2 study days, at the same time of day and at least 1 week apart. On each study day, 2 exercise tests were performed separated by 1 hour. Subjects were pretreated with oral ranitidine (150 mg) or placebo taken twice daily for 3 days with the final dose taken 1 hour before the initial exercise test. This dose of ranitidine is used in clinical practice to inhibit histamine H₂-receptor effects in man (Brunton et al, 1990). Treatment medication and placebo were administered in a double-blind, randomized, cross-over fashion.



3.4.3 Test.

3.4.3.1 Exercise challenge

Exercise was performed on a stationary bicycle ergometer as previously described (Section 2.3.2).

3.4.4 Analysis of Results

Summary statistics were expressed as means and standard errors of the mean (SEM). Comparisons of the maximum fall in FEV₁ after exercise to baseline values and the absolute change in FEV₁ between the different study days were made using t-tests for paired observations. The differences were considered statistically significant when $p < 0.05$. The comparisons between the prechallenge baseline FEV₁ values, the maximum heart rate and ventilation responses to exercise on the placebo and treatment days were made using an analysis of variance (ANOVA).

3.4.5 Results

Ranitidine pretreatment did not alter baseline exercise-induced bronchoconstriction in these subjects. The mean initial fall in FEV₁ after exercise on the placebo day was 25.1% (SEM 2.9%) and on ranitidine was 23.4% (SEM 3.4%) (p=0.64) (Figure 3.6).

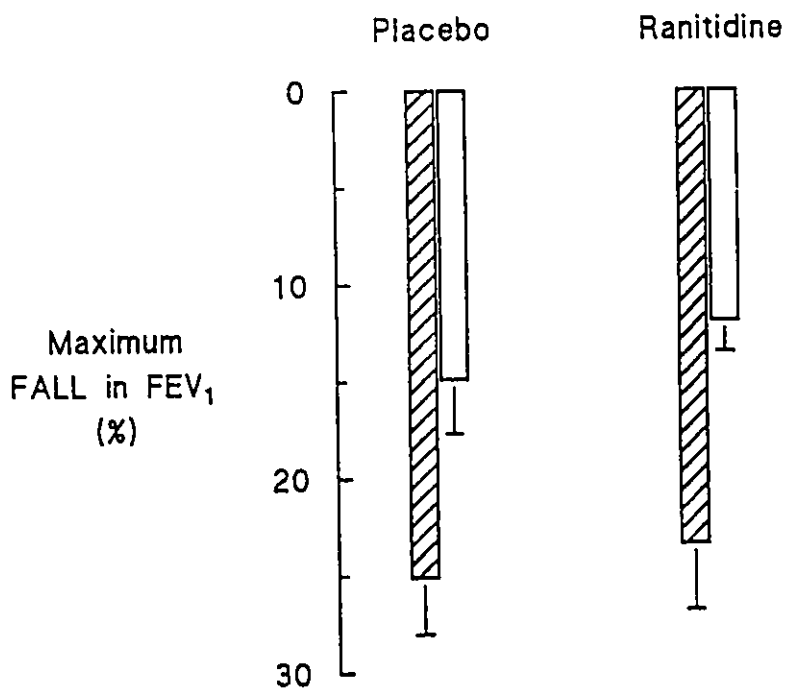


Figure 3.6 Mean (SEM) bronchoconstrictor responses (% fall in FEV₁) to two exercise challenges separated by 1 hour on the ranitidine or placebo treatment study days.

In addition, ranitidine pretreatment did not alter the development of exercise refractoriness in these subjects. On the placebo day, the mean fall in FEV₁ after exercise during the second exercise challenge was 14.9% (SEM 2.8%) and was 11.9% (SEM 1.6%) on ranitidine (p=0.19) (Figure 3.6). Thus, the magnitude of refractoriness after the second exercise challenge after placebo treatment was 44.0 (8.8)% inhibition and after ranitidine treatment was 42.9 (7.1)% inhibition (p=0.36).

The exercise challenges on the ranitidine and placebo study days were comparable with respect to maximum heart rate (p=0.9) and minute ventilation (0.9) (Table 3.7).

Table 3.7 Mean maximum heart rate (beats/minute) and ventilation (litres/minute) with exercise on each day.

Test	Heart Rate				Ventilation			
	Placebo		Ranitidine		Placebo		Ranitidine	
	1	2	1	2	1	2	1	2
Mean	154.7	157.5	152.9	155.1	69.7	70.0	69.1	70.2
SEM	6.3	6.5	4.9	5.7	7.9	9.1	8.6	9.9

Ranitidine pretreatment did not alter the baseline FEV₁ in these subjects (p=0.9)

(Table 3.8).

Table 3.8 Mean baseline FEV₁ (litres) on each study day.

Test	<u>placebo</u>		<u>ranitidine</u>		
	1	2	1	2	
Mean		3.10	3.07	3.17	3.15
SEM		0.20	0.20	0.20	0.20

3.4.6 Summary

This study has demonstrated that exercise refractoriness is not inhibited by pretreatment with ranitidine, a potent H₂-receptor antagonist, suggesting that histamine stimulation of airway H₂-receptors does not play an important role in the development of exercise refractoriness.

Conclusion

The result obtained in this study, in conjunction with the findings from the previous two studies demonstrating incomplete cross refractoriness/tachyphylaxis between exercise and histamine bronchoconstriction, suggests that a mechanism other than histamine tachyphylaxis is responsible for exercise refractoriness.

B. Role of LTD₄ in Exercise Bronchoconstriction

3.5 Introduction

The cysteinyl leukotrienes are known to be potent constrictor mediators in human airways and play an important role in bronchoconstrictor responses following acute asthma and allergen exposure. To date, definitive evidence of the role of the bronchoconstrictor leukotrienes, such as LTD₄, in this asthmatic response to exercise has not been available because of a lack of readily available potent and specific leukotriene antagonists to study in asthmatic subjects. A potent and highly selective LTD₄-receptor antagonist developed by Merck Pharmaceuticals, MK 571, recently became available for study in man. We therefore undertook to examine the effect of pretreatment with this drug on exercise bronchoconstriction in asthmatic subjects.

STUDY 4

3.6. Introduction

The question addressed in this study was;

- Q. Does the selective LTD₄ receptor antagonist, MK 571, inhibit exercise bronchoconstriction in asthmatic subjects ?**

3.6.1 Subjects

Twelve asthmatic subjects were studied (Table 3.9).

Table 3.9 Subject characteristics

Subject	Age (years)	Gender	Baseline FEV ₁ (%Pred.)
1.	22	Female	85
2.	23	Male	75
3.	37	Male	70
4.	18	Male	89
5.	21	Male	84
6.	22	Male	73
7.	32	Male	81
8.	38	Female	76
9.	21	Female	87
10.	22	Male	75
11.	21	Male	73
12.	21	Male	84

3.6.2 Study design

Subjects attended the laboratory on three study days, each separated by one week. The first day was a screening day, during which subject characteristics were documented, and a prestudy exercise challenge performed to demonstrate the presence of exercise-induced bronchoconstriction. Subjects with, at least, a 20% fall in FEV₁ after the exercise challenge, entered the double-blind crossover study during which they were pretreated with an intravenous infusion of MK-571,

change and %change in FEV₁ from the post-infusion, pre-exercise, baseline FEV₁, as well as the time to recovery to within 5% of baseline FEV₁. In subjects in whom the FEV₁ had not returned to within 5% of baseline by 45 minutes, a conservative approach was taken and the FEV₁ value at 45 minutes was used as the time to recovery.

Summary statistics are expressed as means and standard error of the mean (SEM). The analysis of variance (ANOVA) model of Grizzle (Grizzle, 1974) was used to compare between treatments for the changes in FEV₁ and the recovery time. The assumptions of the ANOVA, that the residuals are normally distributed with equal variances across treatments were verified with the Shapiro-Wilk test of normality (Shapiro and Wilk, 1965); and the homogeneity of variance was verified with Hartley's Maximum F-test (Hartley, 1950) on variance of the residuals and by visual inspection of the plots of residuals versus predicted values. A probability value of less than 5%, using a two-tailed test, was considered significant.

3.6.5 Results

Treatment with MK-571 attenuated the bronchoconstriction after exercise in all subjects. The mean maximal %fall in FEV₁ following exercise after treatment with placebo was 25.2% (SEM 3.5%) and after treatment with MK-571 was 9.2% (SEM 2.5%)($p < 0.001$)(Figure 3.7).

The degree of inhibition from exercise bronchoconstriction afforded by MK-571 varied among subjects, from 30% inhibition of the maximal fall in FEV₁ for one subject to 100% in three subjects. The mean % inhibition for the group was 64%.

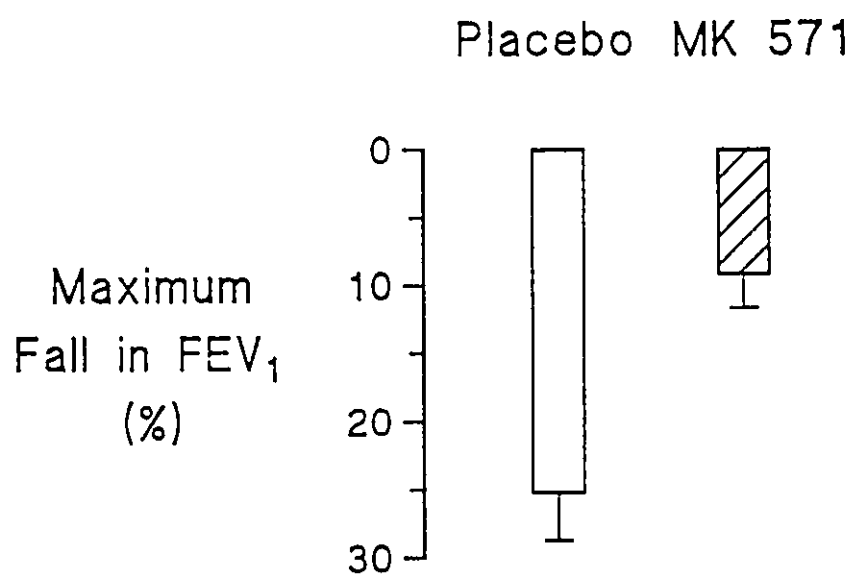


Figure 3.7 The maximal % fall in FEV₁ (mean and SEM) post exercise after treatment with placebo or MK-571.

The duration of the exercise-induced bronchoconstriction was significantly reduced after treatment with MK-571 ($p < 0.001$) (Figure 3.8).

The mean recovery time from exercise bronchoconstriction (to within 5% baseline FEV₁) was also significantly reduced after treatment with MK-571 ($p < 0.001$) (Table

3.10). Six subjects receiving placebo had not returned to within 5% baseline FEV₁ even by 45 minutes after exercise, while the longest recovery time after treatment with MK-571 in any individual was 25 minutes.

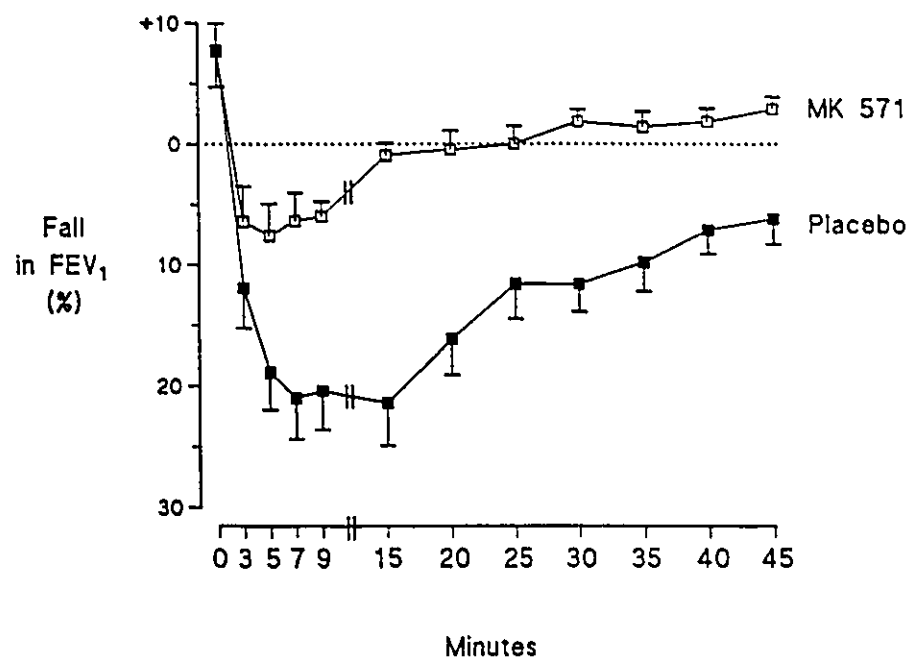


Figure 3.8 The %change in FEV₁ (mean and SEM) over time post-exercise after treatment with placebo (solid squares) or MK-571 (open squares).

Table 3.10 The mean recovery time (minutes) from exercise bronchoconstriction (to within 5% baseline FEV₁) after treatment with placebo and MK-571.

	Placebo	MK-571
Mean	33.4	8.4
SEM	4.0	2.5

The exercise workload was kept the same on the two study days. Consistent with this, the exercise challenges on the MK 571 and placebo study days were comparable with respect to maximum heart rate ($p=0.54$) and minute ventilation ($p=0.64$) (Table 3.11).

Table 3.11 Mean maximal heart rate (beats/minute) and ventilation (litres/minute) with exercise on each study day.

	Heart Rate		Ventilation	
	Placebo	MK-571	Placebo	MK-571
Mean	158.0	160.0	89.6	93.9
SEM	3.0	3.0	6.5	6.6

The baseline FEV₁ was similar prior to exercise on both study days and pretreatment with MK 571 did not alter the FEV₁ value in these subjects ($p=0.14$)(Table 3.12).

Table 3.12 Mean (SEM) baseline FEV₁ (litres) for subjects before and after treatment with MK 571 or placebo on each study day.

Test	Placebo		MK 571	
	Pre	Post	Pre	Post
Mean	3.49	3.45	3.49	3.59
SEM	0.20	0.30	0.20	0.20

Two subjects experienced possible adverse effects of the treatments administered

prior to exercise. One subject (subject 10) had three loose bowel movements within 12 hours of treatment with MK-571. The other subject (subject 12) complained of headache within 12 hours of treatment with placebo. Both adverse effects were mild and self-limiting, and did not require any treatment. No other side-effects occurred which would have allowed unblinding of the study drug.

3.6.6 Summary

This study has demonstrated that pretreatment with a selective LTD₄-receptor antagonist, MK-571, markedly attenuates exercise-induced bronchoconstriction in asthmatic subjects. These results demonstrate, for the first time, that release of LTD₄ and subsequent LTD₄-induced bronchoconstriction is an important component of exercise bronchoconstriction in asthma.

C. Role of LTD₄ in Exercise Refractoriness

3.7 Introduction

In the previous study we demonstrated, for the first time, that LTD₄ plays an important role in the development of exercise bronchoconstriction. Tachyphylaxis occurs to repeated challenges with inhaled LTD₄ in normal non-asthmatic subjects (Kern et al, 1986), however, to date, LTD₄ tachyphylaxis has not been demonstrated in asthmatic subjects. The studies detailed in this part of the thesis were undertaken to address the hypothesis that LTD₄ tachyphylaxis caused by

inhibitory prostaglandin release is the mechanism of exercise refractoriness in asthmatic subjects. In order to investigate this further, the following questions were addressed in studies 5-8:

- Q. Does exercise refractoriness and LTD₄ tachyphylaxis occur in asthmatic subjects and if so, is there a correlation between the degree of exercise refractoriness and LTD₄ tachyphylaxis in asthmatic subjects?

- Q. Is tachyphylaxis to inhaled LTD₄ in asthmatic subject attenuated by pretreatment with flurbiprofen, the cyclooxygenase inhibitor?

- Q. Does exercise cause tachyphylaxis to LTD₄-induced bronchoconstriction in asthmatic subjects and is the effect attenuated by flurbiprofen?

- Q. Does LTD₄ cause refractoriness to exercise in asthmatic subjects and is the effect attenuated by flurbiprofen?

STUDY 5

3.8 Introduction

Inhaled LTD₄ is a potent bronchoconstrictor mediator of human airways and tachyphylaxis occurs to repeated stimulation with inhaled LTD₄ in normal subjects although the mechanism of LTD₄ tachyphylaxis in normal subjects is not known (Kern et al, 1986). To date LTD₄ tachyphylaxis has not been demonstrated in asthmatics subjects. The purpose of this study was to determine whether LTD₄

tachyphylaxis occurs in asthmatics, and whether exercise refractoriness also occurs in these same subjects.

- Q. Does exercise refractoriness and LTD₄ tachyphylaxis occur in asthmatic subjects and if so, is there a correlation between the degree of exercise refractoriness and LTD₄ tachyphylaxis in asthmatic subjects?

3.8.1 Subjects

Fourteen subjects with current asthma and documented exercise bronchoconstriction were studied when their asthma was mild and controlled by inhaled B₂-agonists alone (Table 3.13).

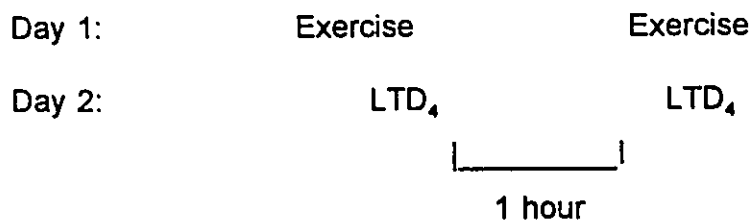
Table 3.13 Subject characteristics

Subject	Age (years)	Gender	Baseline FEV ₁ (%Pred.)	Baseline PC ₂₀ LTD ₄ (ug/ml)
1.	24	Male	92	5.86
2.	22	Male	97	0.50
3.	22	Male	98	0.44
4.	21	Female	87	0.67
5.	22	Female	98	0.14
6.	21	Female	84	0.32
7.	34	Male	68	0.66
8.	24	Female	86	1.10
9.	23	Female	71	0.07
10.	23	Female	87	0.15
11.	32	Female	75	0.06
12.	25	Male	72	0.32
13.	38	Male	77	0.20
14.	53	Male	71	0.15

3.8.2 Study design

Initially 14 subjects attended the laboratory on 4 study days at least 48 hours apart. The first day was a screening day, during which subject characteristics were

documented, and a prestudy exercise challenge performed to demonstrate the presence of exercise bronchoconstriction. Once established, the workload was kept constant for each subject on each study day. Only subjects with at least a 15% fall in FEV₁ after exercise were entered into the study. On the second day subjects underwent a challenge with increasing concentrations of inhaled LTD₄ to document the airway responsiveness to LTD₄. On the remaining two study days, subjects performed either two exercise challenges 1 hour apart to document the degree of exercise refractoriness or two LTD₄ challenges 1 hour apart to document LTD₄ tachyphylaxis.



3.8.3 Tests.

3.8.3.1 Exercise Challenge

Exercise was performed on a stationary bicycle ergometer as previously described (Section 2.3.2).

3.8.3.2 LTD₄ Inhalation Test

Subjects performed a dose response inhalation challenge with LTD₄ diluted in

phosphate-buffered saline as previously described (Section 2.3.4).

3.8.4 Analysis of results

As with histamine in the previous studies the LTD₄ PC₂₀ values were also log transformed prior to analysis and therefore summary statistics are expressed as geometric means and percent standard errors of the mean (% SEM). Other summary statistics are expressed as means and standard error of the mean (SEM). Exercise bronchoconstriction was determined as the maximal % change in FEV₁ from the pre-exercise baseline FEV₁.

Comparisons of the maximal fall in FEV₁ after exercise and the LTD₄ PC₂₀ on different study days were made using t-tests for paired values. Comparisons of baseline FEV₁, heart rate, and ventilation responses during exercise and baseline fall in FEV₁ after exercise and LTD₄ on different days were made using analysis of variance (ANOVA).

3.8.5 Results

Exercise caused bronchoconstriction in all 14 subjects. In addition, all subjects developed exercise refractoriness. The mean % fall in FEV₁ after the initial exercise challenge was 20.1% (SEM 1.8%) and after the second challenge, one hour later, was 9.4% (SEM 1.3%) (p<0.0001) (Figure 3.9), a refractory index of

53%.

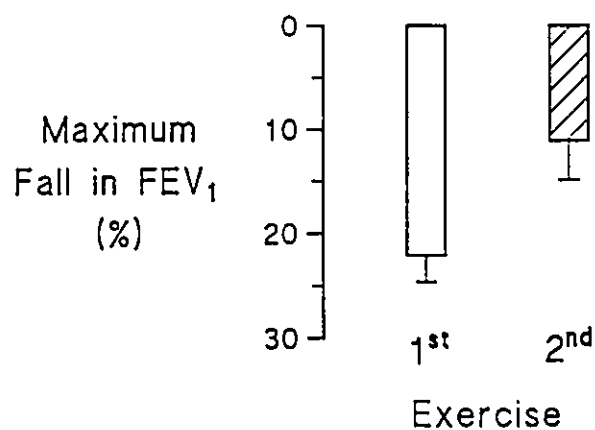


Figure 3.9 The mean % fall post exercise in the FEV₁ for two exercise challenges separated by 1 hour in 14 asthmatic subjects.

During the exercise challenges the workload was kept constant on all the study days and consistent with this, the maximum heart rate ($p=0.24$) and minute ventilation ($p=0.21$) achieved during the exercise challenges were similar for the 14 asthmatic subjects (Table 3.14).

Table 3.14 Mean maximal heart rate (beats/minute) and ventilation (Litres/minute) with each exercise test on Day 1.

Test	Heart Rate		Ventilation	
	1	2	1	2
Mean	164.4	165.8	70.0	69.0
SEM	4.3	4.1	6.3	6.4

Inhaled LTD₄ also caused bronchoconstriction in these subjects. In addition, there was an increase in the PC₂₀ LTD₄ when the two LTD₄ challenges are also separated by 1 hour indicating LTD₄ tachyphylaxis. The mean initial LTD₄ PC₂₀ was 0.31 µg/ml (%SEM 1.2), and the subsequent LTD₄ PC₂₀ 1 hour later was 0.96 µg/ml (%SEM 1.3) (p<0.0001) (Figure 3.10).

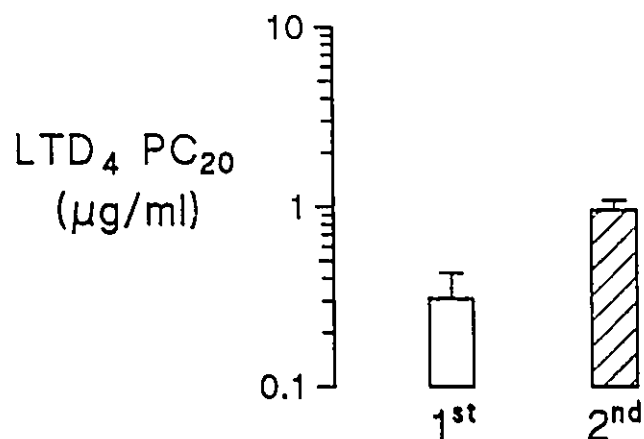


Figure 3.10 The mean PC₂₀ LTD₄ for two LTD₄ challenges separated by 1 hour in 14 asthmatic subjects.

The baseline FEV₁ values, were similar prior to all exercise and LTD₄ challenges (p=0.9)(Table 3.15).

Table 3.15 Mean baseline FEV₁ (litres) for subjects prior to all exercise and LTD₄ challenges on each study day.

Test	Exercise		LTD ₄	
	1	2	1	2
Mean	3.25	3.17	3.16	3.10
SEM	0.20	0.20	0.20	0.30

The magnitude of exercise refractoriness was correlated with the magnitude of LTD₄ tachyphylaxis ($r=0.72$, $p=0.005$)(Figure 3.11).

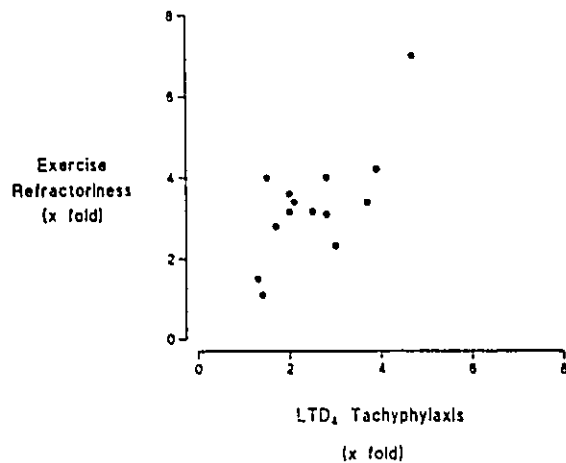


Figure 3.11 Correlation between the fold exercise refractoriness and LTD₄ tachyphylaxis in 14 asthmatic subjects.

3.8.6 Summary

This study demonstrates for the first time that exercise refractoriness and LTD₄ tachyphylaxis occur in the same asthmatic subjects and that the degree of refractoriness and tachyphylaxis between these subjects is closely correlated. This result supports the hypothesis that LTD₄ tachyphylaxis is the mechanism of

exercise refractoriness in asthma.

In order to investigate the mechanism of LTD₄ tachyphylaxis and the likely involvement of this effect in exercise refractoriness, subjects 7-14 with documented exercise refractoriness and LTD₄ tachyphylaxis, took part in a series of three separate double-blind, randomized, placebo-controlled studies (Studies 6-8).

STUDY 6

3.9 Introduction

Since exercise refractoriness is caused by inhibitory prostaglandin release, the purpose of this study was to examine whether LTD₄ tachyphylaxis is also caused by inhibitory prostaglandin release. Therefore the following question was addressed in this study:

- Q. Is tachyphylaxis to inhaled LTD₄ in asthmatic subjects attenuated by pretreatment with the cyclooxygenase inhibitor flurbiprofen?

3.9.1. Subjects

All 8 subjects from study 4 (Table 3.9) and study 5 with current asthma (Table 3.13 subjects 7-14) also took part in this study.

The results of repeated exercise and LTD₄ challenges from study 5, indicated that

these subjects demonstrated exercise refractoriness (Figure 3.12) and LTD₄ tachyphylaxis (Figure 3.13). In these subjects, the mean % fall in FEV₁ after the initial exercise challenge was 22.2% (SEM 3.4%) and after the second challenge, one hour later, was 8.9% (SEM 1.9%). In addition, the mean initial LTD₄ PC₂₀ was 0.23 µg/ml (%SEM 1.2), and the subsequent LTD₄ PC₂₀ one hour later was 0.58 µg/ml (%SEM 1.1).

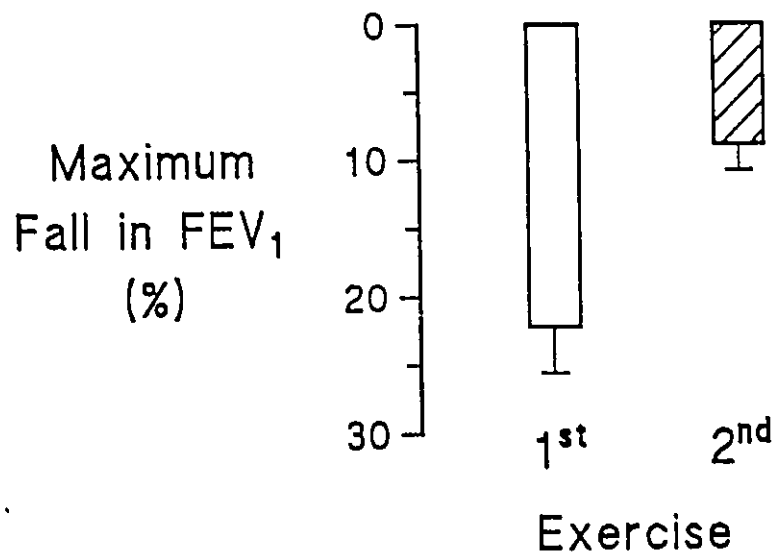


Figure 3.12 The mean % fall post-exercise in the FEV₁ for two exercise challenges separated by one hour in the last eight asthmatic subjects from study 5.

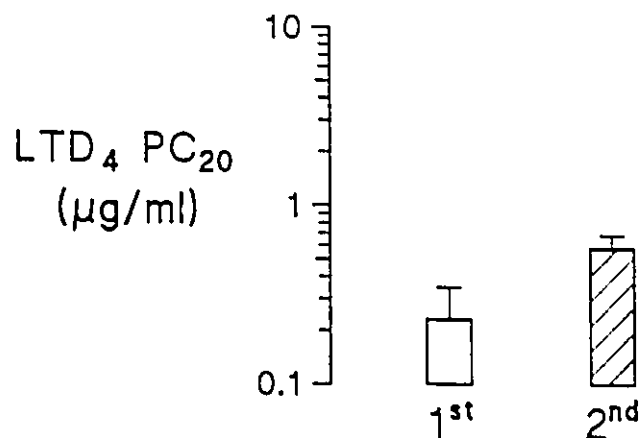
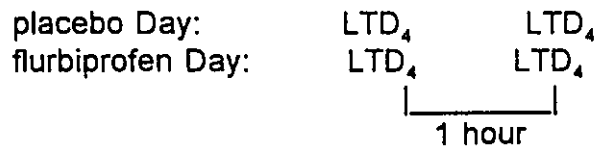


Figure 3.13 The mean PC₂₀ LTD₄ for two LTD₄ challenges separated by one hour in the last eight asthmatic subjects from study 5.

3.9.2 Study design

The study was a double-blind, randomized, crossover study, in which subjects were pretreated orally with either flurbiprofen 50 mg three times daily for three days or placebo. This dose of flurbiprofen is used clinically to inhibit cyclooxygenase in man. The final dose was taken one hour before the first challenge. On each study day, two consecutive LTD₄ challenges were performed separated by one hour and the study days were separated by at least one week.



3.9.3 Test.

3.9.3.1 LTD₄ Inhalation Test

Subjects performed a dose response inhalation challenge with LTD₄ diluted in phosphate-buffered saline as previously described (Section 2.3.4).

3.9.4 Analysis of Results

LTD₄ PC₂₀ values were log transformed prior to analysis and therefore summary statistics are expressed as geometric means and percent standard errors of the mean (% SEM). Other summary statistics are expressed as the mean and standard error (SEM).

Comparisons between the initial PC₂₀ LTD₄ on the placebo and flurbiprofen days were made using a t-test for paired values. The assessment of placebo and flurbiprofen pretreatment on LTD₄ tachyphylaxis involved comparison of the log difference (the arithmetic ratio) of the two LTD₄ PC₂₀ values separated by one hour on these two days. A log difference of 0 signifies no change in airway responsiveness. Comparisons of the log differences were again made with t-test for paired values. A probability value of less than 5% was considered significant.

Comparisons of baseline FEV₁ on the different study days were made using an

analysis of variance (ANOVA).

3.9.5 Results

Flurbiprofen pretreatment had no effect on the initial bronchoconstrictor response to LTD₄. The baseline LTD₄ PC₂₀ was 0.20 (%SEM 1.2) µg/ml on placebo and was 0.22 (%SEM 1.3) µg/ml on flurbiprofen (p=0.29) (Figure 3.14).

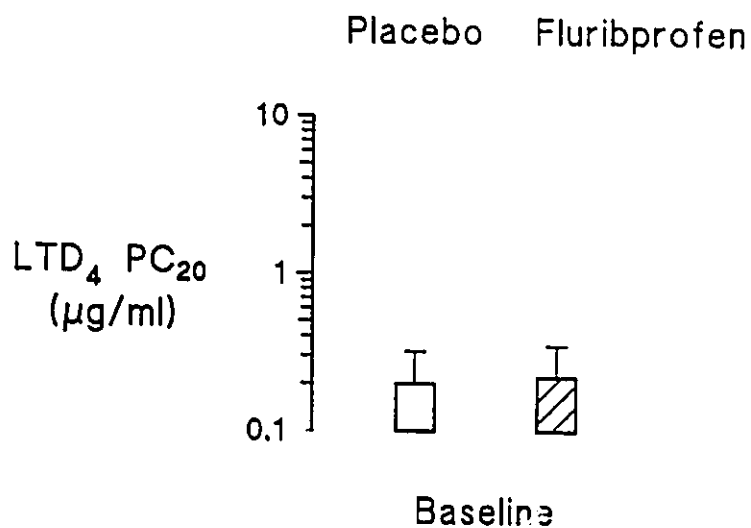


Figure 3.14 The mean PC₂₀ LTD₄ for the baseline LTD₄ challenges on the placebo and flurbiprofen treatment days.

On placebo treatment, the $LTD_4 PC_{20}$ values increased in all subjects indicating the development of tachyphylaxis. The mean $LTD_4 PC_{20}$ for the second LTD_4 0.71 $\mu\text{g/ml}$ (%SEM 1.4) $\mu\text{g/ml}$ ($p=0.0001$)(Figure 3.15).

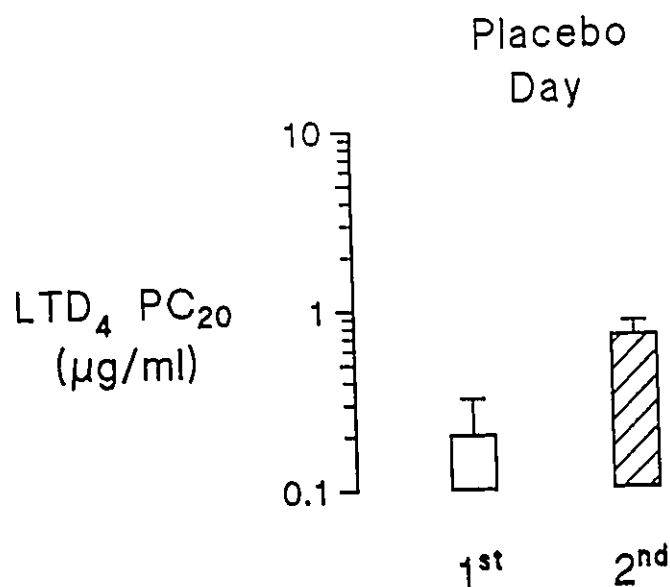


Figure 3.15 The mean $PC_{20} LTD_4$ for two LTD_4 challenges on the placebo day separated by one hour.

Flurbiprofen pretreatment significantly inhibited the development of LTD_4 tachyphylaxis. During flurbiprofen treatment, the mean $LTD_4 PC_{20}$ for the first and second challenges were 0.21 (%SEM 1.6) $\mu\text{g/ml}$ and 0.47 (%SEM 1.4) $\mu\text{g/ml}$.

On placebo there is an increase in the log difference indicating tachyphylaxis in these eight subjects and flurbiprofen pretreatment attenuates this effect. The mean log difference for the $LTD_4 PC_{20}$ during placebo treatment was 0.56, and during

flurbiprofen treatment was 0.34 ($p=0.017$) (Figure 3.16).

The baseline FEV₁ values were similar prior to all LTD₄ challenges ($p=0.9$) (Table 3.16).

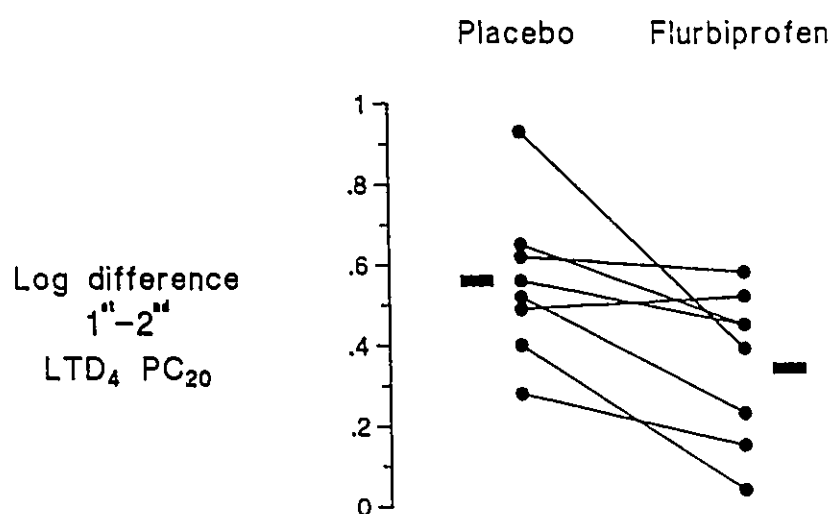


Figure 3.16 The individual and mean log differences for two LTD₄ challenges at baseline and on placebo and flurbiprofen pretreatment.

Table 3.16 Mean baseline FEV₁ (litres) for the subjects prior to all LTD₄ challenges on each study day.

Test	placebo		flurbiprofen	
	LTD ₄	LTD ₄	LTD ₄	LTD ₄
Mean	2.95	2.87	2.92	2.90
SEM	0.20	0.20	0.30	0.20

No side-effects occurred which would have allowed unblinding of the study drug.

3.9.6 Summary

This study demonstrates that LTD₄ tachyphylaxis in asthmatic subjects is inhibited by flurbiprofen suggesting the involvement of inhibitory prostaglandins in this effect.

STUDY 7

3.10 Introduction

The results from study 4 suggested that LTD₄ released following exercise, is responsible in large part for exercise bronchoconstriction. In study 5, we demonstrated that LTD₄ tachyphylaxis occurs in asthmatic subjects with exercise refractoriness and that the degree of refractoriness/tachyphylaxis correlate. In study 6, we demonstrated that LTD₄ tachyphylaxis effect is caused by inhibitory prostaglandin release. In order to investigate further the role of LTD₄ in exercise refractoriness, we determined whether cross refractoriness/tachyphylaxis occurs with exercise and LTD₄-induced bronchoconstriction. The following question was asked:

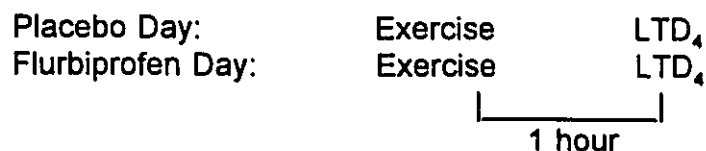
- Q. Does exercise tachyphylaxis to LTD₄-induced bronchoconstriction in asthmatic subjects and is the effect attenuated by flurbiprofen?

3.10.1 Subjects

The same eight subjects as in study 6 entered this study, and at least one week separated the two studies. As indicated previously from the values obtained in study 5, these subjects had documented LTD₄ tachyphylaxis. The initial and subsequent mean LTD₄ PC₂₀ values were 0.23 µg/ml (% SEM 1.2) and 0.58 µg/ml (%SEM 1.2) one hour later.

3.10.2 Study design

The subjects took part in a double-blind randomized placebo controlled cross-over study with flurbiprofen and placebo. Subjects were pretreated orally with either flurbiprofen 50 mg three times daily for three days or placebo. The final dose was taken 1 hour before the first challenge. On each study day, two consecutive challenges were performed separated by one hour and each day was separated by at least one week. On each day an exercise challenge was followed by an LTD₄ challenge.



3.10.3 Tests

3.10.3.1 Exercise Challenge

Exercise was performed on a stationary bicycle ergometer as previously described

(Section 2.3.2).

3.10.3.2 LTD₄ Inhalation Test

Subjects performed a dose response inhalation challenge with LTD₄ diluted in phosphate-buffered saline as previously described (Section 2.3.4).

3.10.4 Analysis of Results

LTD₄ PC₂₀ values were log transformed prior to analysis and therefore summary statistics are expressed as geometric means and percent standard errors of the mean (% SEM). Other summary statistics are expressed as means and standard error of the mean (SEM).

Exercise bronchoconstriction was determined as the maximal % change in FEV₁ from the pre-exercise baseline FEV₁. Comparisons of the maximal fall in FEV₁ after exercise, the LTD₄ PC₂₀ and heart rate and ventilation responses during exercise on different study days were made using t-test for paired values. A probability value of less than 5% was considered significant. Comparisons of baseline FEV₁ were made using a analysis of variance (ANOVA).

3.10.5 Results

Flurbiprofen pretreatment had no effect on the initial bronchoconstrictor response

to exercise. On placebo, the maximum % fall in FEV₁ after the first exercise challenge was 20.4% (SEM 3.2%) and was 22.1% (SEM 3.7%) (p=0.13) on flurbiprofen (Figure 3.17).

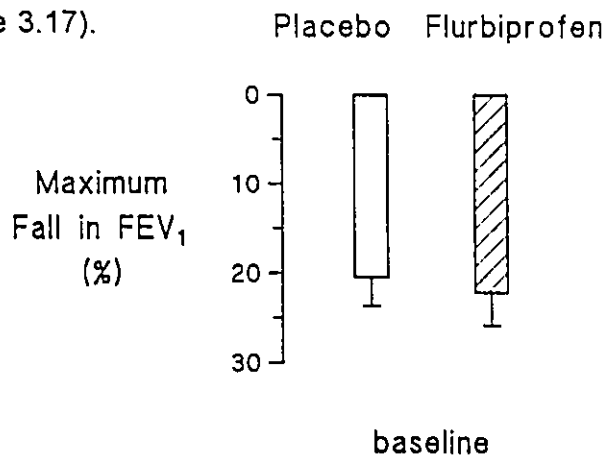


Figure 3.17 Mean (SEM) % fall in FEV₁ post exercise on placebo and flurbiprofen study days.

Prior challenge with exercise caused an increase in the subsequent LTD₄ PC₂₀ value and flurbiprofen pretreatment attenuated this effect. Following exercise on placebo treatment, the LTD₄ PC₂₀ increased to 0.73 (%SEM 1.4) µg/ml, and this was reduced to 0.30 (%SEM 1.8) µg/ml on flurbiprofen treatment (p=0.026) (Figure 3.18).

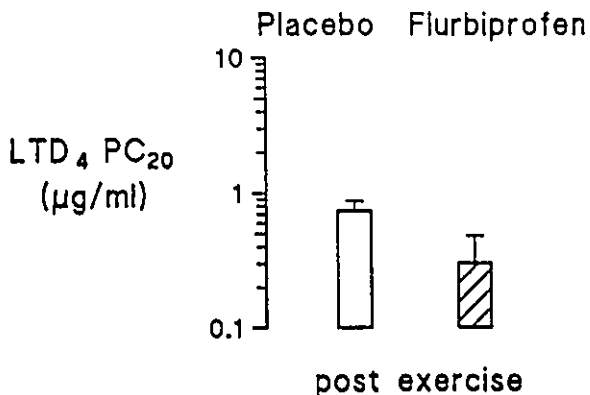


Figure 3.18 Mean (SEM) LTD₄ PC₂₀ values after an initial exercise challenge on placebo and flurbiprofen treatment days.

During the exercise challenges, the workload was kept constant on all the study days and consistent with this, the maximum heart rate ($p=0.56$) and minute ventilation ($p=0.44$) achieved during the exercise challenges were similar for the eight asthmatic subjects (Table 3.17).

Table 3.17 Mean maximal heart rate (beats/minute) and ventilation (litres/minute) with each exercise test.

	<u>Heart Rate</u>		<u>Ventilation</u>	
	placebo__flurbiprofen	flurbiprofen__placebo	placebo__flurbiprofen	flurbiprofen__placebo
Mean	154.7	153.3	62.3	62.1
SEM	3.1	4.5	5.7	6.1

The baseline FEV₁ values, were similar prior to all exercise and LTD₄ challenges ($p=0.9$)(Table 3.18).

Table 3.18 Mean baseline FEV₁ (Litres) for subjects prior to all exercise and LTD₄ challenges on each study day.

Test	<u>placebo</u>		<u>flurbiprofen</u>	
	Exercise__LTD ₄	LTD ₄ __Exercise	Exercise__LTD ₄	LTD ₄ __Exercise
Mean	2.90	2.87	2.84	2.77
SEM	0.20	0.20	0.20	0.30

No side-effects occurred during the study which would have allowed unblinding of the study drug.

3.10.6 Summary

This study has demonstrated that an initial bronchoconstrictor challenge with exercise in asthmatic subjects will attenuate subsequent bronchoconstrictor responses to inhaled LTD₄. Flurbiprofen significantly inhibited this effect, suggesting an important role for inhibitory prostaglandins. These results are in keeping with previous studies implicating inhibitory prostaglandin release in exercise refractoriness, and support the hypothesis that this effect is caused by exercise-induced LTD₄ release which subsequently causes inhibitory prostaglandin release.

STUDY 8

3.11 Introduction

As exercise, in study 7, was shown to cause tachyphylaxis to LTD₄, this study was undertaken to investigate whether LTD₄ could cause a similar effect on exercise bronchoconstriction. The following question was addressed in this study:

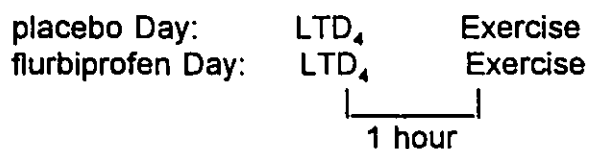
- Q. Does LTD₄ cause refractoriness to exercise in asthmatic subjects and is the effect attenuated by flurbiprofen?

3.11.1 Subjects

The same eight subjects from the previous two studies entered this study, with at least one week separating the studies. As indicated previously from the values obtained in study 5, these subjects had documented exercise refractoriness, with the initial and subsequent %fall in FEV₁ post exercise challenge being 22.2% (SEM 3.4%) and 8.9% (SEM 1.9%) respectively one hour later.

3.11.2 Study design

The subjects took part in a double-blind, randomized placebo-controlled cross-over study with flurbiprofen and placebo. Subjects were pretreated orally with either flurbiprofen 50 mg three times daily for three days or placebo. The final dose was taken one hour before the first challenge. On each study day, two consecutive challenges were performed separated by one hour and each day was separated by at least one week. On each study day, an LTD₄ challenge was followed by an exercise challenge.



3.11.3 Tests.

3.11.3.1 Exercise Challenge

Exercise was performed on a stationary bicycle ergometer as previously described (Section 2.3.2).

3.11.3.2 LTD₄ Inhalation Test

Subjects performed a dose response inhalation challenge with LTD₄ diluted in phosphate-buffered saline as previously described (Section 2.3.4).

3.11.4 Analysis of Results

LTD₄ PC₂₀ values were log transformed prior to analysis and therefore summary statistics are expressed as geometric mean and percent standard error (%SEM). Other summary statistics are expressed as mean and standard error (SEM).

Exercise bronchoconstriction was determined as the maximal %change in FEV₁ from the pre-exercise baseline FEV₁. Comparisons of the maximal fall in FEV₁ after exercise, the LTD₄ PC₂₀, the heart rate, and ventilation responses during exercise, on different study days were made using t-tests for paired values. Comparisons of baseline FEV₁ heart rate prior to each test were made using an analysis of variance (ANOVA).

3.11.5 Results

Flurbiprofen pretreatment had no effect on the initial bronchoconstrictor response to LTD₄ (Figure 3.19). The baseline LTD₄ PC₂₀ was 0.20 (%SEM 1.2) µg/ml on placebo and was 0.22 (%SEM 1.3) µg/ml on flurbiprofen (p=0.29).

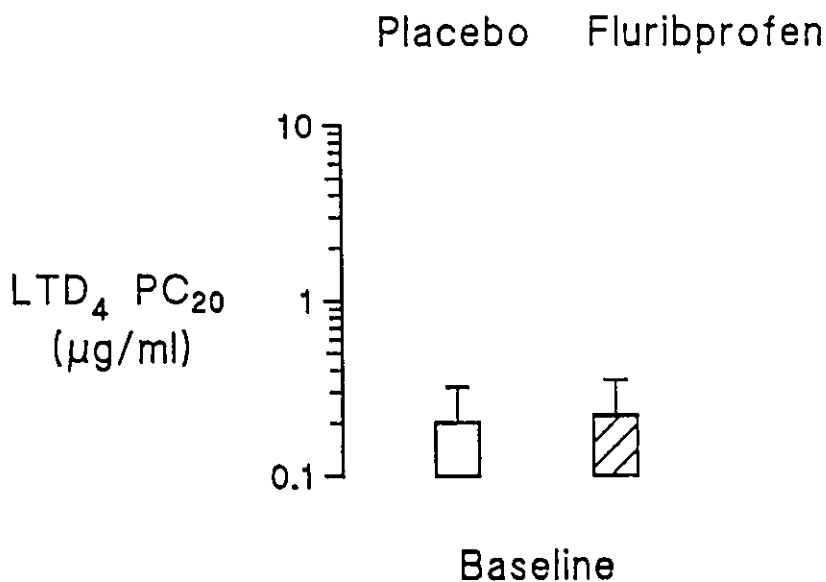


Figure 3.19 Mean (SEM) baseline LTD₄ PC₂₀ values on placebo and flurbiprofen treatment days for these eight subjects.

Prior challenge with LTD₄ caused refractoriness to a subsequent exercise challenge and flurbiprofen pretreatment attenuated this effect (Figure 3.20). Following an initial LTD₄ challenge, the mean %fall in FEV₁ following exercise was 12.0% (SEM 3.7%), on placebo treatment and on flurbiprofen treatment was 17.1% (SEM 3.8%)(p=0.027).

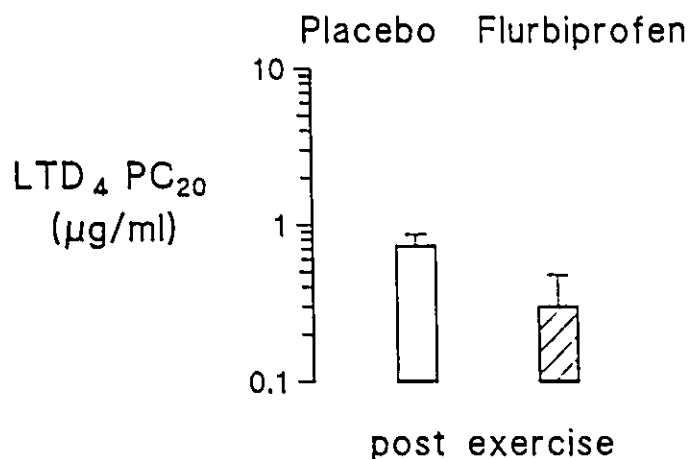


Figure 3.20 Mean (SEM) % fall in FEV₁ values post exercise after an initial LTD₄ challenge on placebo and flurbiprofen study days for these eight subjects.

During the exercise challenges the workload was kept constant on all the study days and consistent with this, the maximum heart rate ($p=0.10$) and minute ventilation ($p=0.12$) achieved during the exercise challenges were similar for the 8 asthmatic subjects (Table 3.19).

Table 3.19 Mean maximal heart rate (beats/minute) and ventilation (litres/minute) with each exercise test.

	Heart Rate		Ventilation	
	placebo	flurbiprofen	placebo	flurbiprofen
Mean	149.0	146.0	63.9	62.1
SEM	2.6	2.9	6.5	6.5

The baseline FEV₁ values, were similar prior to all exercise and LTD₄ challenges

($p=0.9$)(Table 3.20).

Table 3.20 Mean baseline FEV₁ (litres) for subjects prior to all exercise and LTD₄ challenges on each study day.

Test	placebo		flurbiprofen	
	LTD ₄	Exercise	LTD ₄	Exercise
Mean	2.86	2.73	2.87	2.81
SEM	0.20	0.20	0.30	0.20

No side-effects occurred during the study which would have allowed unblinding of the study drug.

3.11.6 Summary

This study has demonstrated that an initial bronchoconstrictor challenge with inhaled LTD₄ in asthmatic subjects, will attenuate subsequent bronchoconstrictor responses to exercise. This inhibitory mechanism was significantly attenuated by treatment with the prostaglandin synthesis inhibitor, flurbiprofen, suggesting an important role for inhibitory prostaglandins in causing this effect.

Chapter IV

Discussion

4.1 Summary of Results

The body of work detailed in this thesis utilized challenges with exercise as well as histamine and LTD₄, to investigate the importance of these bronchoconstrictor mediators, in causing exercise bronchoconstriction and refractoriness in asthmatic subjects.

These studies have demonstrated that MK 571, a potent and selective LTD₄ receptor antagonist (Jones et al, 1989), inhibited exercise bronchoconstriction and thus indicating for the first time, that LTD₄ plays an important role in exercise bronchoconstriction in asthmatic subjects. When exercise, LTD₄ or histamine challenges tests were repeated at hourly intervals refractoriness was demonstrated to exercise and tachyphylaxis to LTD₄ and histamine. Complete cross refractoriness and tachyphylaxis occurred between exercise and LTD₄ stimuli, but not between exercise and histamine. In addition, unlike histamine tachyphylaxis (Jackson et al, 1988; Knight et al, 1992), pretreatment with ranitidine, a histamine H₂-receptor antagonist, did not inhibit exercise refractoriness. This work is therefore

supportive of the involvement of LTD₄, but not histamine, in the mechanism of exercise refractoriness.

The results from the remaining studies detailed in this thesis supports this hypothesis. Firstly, there was a correlation between the degree of LTD₄ tachyphylaxis and exercise refractoriness in the asthmatic subjects studied. Secondly, like exercise refractoriness, LTD₄ tachyphylaxis was attenuated by pretreatment with the cyclooxygenase inhibitor, flurbiprofen, implicating inhibitory prostaglandin release in this mechanism. Thirdly, the cross refractoriness/tachyphylaxis between exercise and LTD₄ bronchoconstrictor challenges was also attenuated by flurbiprofen pretreatment. This chapter will critically examine these results outlined in this thesis.

4.2 Exercise Bronchoconstriction

All subjects investigated in this thesis developed exercise bronchoconstriction. In the subjects studied, the baseline responses to exercise (measured as the % fall in FEV₁) were reproducible from day to day when the tests were carried out, in a standardised way, on a stationary cycle ergometer and at a predetermined constant workload for each subject. The reproducibility of this bronchoconstriction is consistent with the results of previous work from this laboratory (O'Byrne and

Jones, 1986). When the tests were repeated at hourly intervals, refractoriness occurred in these subjects as demonstrated by a reduced bronchoconstrictor response following the second test. However, the degree of exercise refractoriness varied between the subjects and this is in keeping with the observation that exercise refractoriness usually occurs in about 50% of asthmatics with exercise bronchoconstriction (Lee and Anderson, 1985). The reason why some asthmatics have a reduced capacity to develop refractoriness is not known, but this effect may be related to the reduced capacity of these subjects to release inhibitory prostaglandins following exercise. The observation, in this thesis, that the degree of exercise refractoriness correlates to LTD₄ tachyphylaxis, an effect caused by inhibitory prostaglandin release, is consistent with this.

4.3 Histamine Airway Responses

Subjects in the first two studies of the thesis, demonstrated reproducible histamine bronchoconstrictor responses and also histamine tachyphylaxis. The results of the baseline histamine inhalation tests between studies 1 and 2, confirm the reproducibility of the test from day to day. The presence of tachyphylaxis depends on this reproducibility which was ensured by using the same test method and Wright nebulizer throughout the study, with an output of 0.13 ml/min and with an effective particle size for inhaled histamine of 1.5 µM aerodynamic mass median

diameter (MMD) for all tests. However, when two histamine inhalation tests were carried out one hour apart, the resulting PC₂₀ values of the second test were increased and were significantly outside this range of variability. This result is consistent with the studies from our laboratory, and others demonstrating histamine tachyphylaxis in asthmatic subjects (Manning et al, 1987; Jackson et al, 1988; Connolly et al, 1989).

4.4 Histamine and Exercise Refractoriness

As histamine was shown previously to play a role in exercise bronchoconstriction (Anderson et al, 1981; Barnes and Brown, 1981; Hartley et al, 1981; Lee et al, 1982; Belcher et al, 1988^a, 1988^b) and inhibitory prostaglandins caused histamine tachyphylaxis (Manning et al, 1987), it was postulated that histamine-induced inhibitory prostaglandin release was the cause of exercise refractoriness. The demonstration that ranitidine, a potent histamine H₂-antagonist, did not block the development of exercise refractoriness, in conjunction with the lack of complete cross refractoriness/tachyphylaxis between histamine and exercise indicates that histamine-induced prostaglandin release is not the cause of exercise refractoriness.

The interpretation of these results depends on the selectivity and potency of the

H₂-receptor antagonist. At the doses used, ranitidine has been demonstrated to be a potent and selective H₂-receptor antagonist in man (Brunton, 1990). It is unlikely that the inability of ranitidine to inhibit exercise refractoriness was due to the kinetics of administration of the drugs which were given for 72 hours prior to exercise with the final dose administered one hour before. Peak blood levels of ranitidine following oral administration are attained between 1-2 hours after oral administration, the duration of the study (Brunton, 1990).

Previous studies have implicated histamine in the pathogenesis of exercise bronchoconstriction (Anderson et al, 1981; Barnes and Brown, 1981; Hartley et al, 1981; Lee et al, 1982; Belcher et al, 1988^a, 1988^b). However in studies described in this thesis, we have shown that exercise bronchoconstriction does not cause histamine tachyphylaxis in asthmatic subjects. The reason for this is unclear. One explanation may be that the dose of histamine released with exercise is not sufficient to induce tachyphylaxis to histamine. Studies in canine airways in-vitro and in dogs in-vivo, have shown that histamine tachyphylaxis occurs at high doses of histamine but not low doses (Bradley and Russell 1983; Shore and Martin, 1985). We have also shown a similar effect in asthmatic subjects (Strban et al, 1992). In addition other studies described in this thesis suggest that LTD₄, rather than histamine plays the more important role in exercise bronchoconstriction and

that the effective dose of histamine released in the airway with exercise may therefore be too low to induce histamine tachyphylaxis.

4.5 Exercise Bronchoconstriction and LTD₄ Release

The role for LTD₄ in causing exercise bronchoconstriction is based on the demonstration in this thesis that pretreatment with MK-571, a selective LTD₄-receptor antagonist, markedly attenuates exercise bronchoconstriction in asthmatic subjects without significantly altering baseline airway calibre. This indicates that release of LTD₄ and subsequent LTD₄-induced bronchoconstriction is an important component of exercise bronchoconstriction in asthma.

The interpretation of these results depends on the potency and selectivity of the LTD₄-receptor antagonist. MK-571 is a potent and selective LTD₄-receptor antagonist *in-vitro* with pA₂ values of 9.4 and 8.5 for LTD₄-induced contractions of human and guinea-pig tracheal strips (Jones et al, 1989). MK-571 has been demonstrated to be a very potent and selective LTD₄-antagonist in several animal species and in human subjects (Jones et al, 1989; Kips et al, 1991) *in-vivo*. In guinea pigs intravenous administration of MK-571 (3µg/kg) inhibited LTD₄-induced bronchoconstriction, shifting the LTD₄ dose-response curve by 38-fold, while a higher dose (10µg/kg) completely abolished the response (Jones et al, 1989). In

conscious squirrel monkeys, oral or inhaled MK-571 completely inhibited the bronchoconstriction caused by LTD₄ (Jones et al, 1989). A similar effect was seen in normal subjects when the drug was given intravenously (Kips et al, 1991). In mild asthmatic subjects, two doses of intravenous MK-571 (28 mg and 277 mg) shifted the dose-response curve to inhaled LTD₄ by 44-fold and 84-fold respectively (Kips et al, 1991).

The selectivity of MK-571 has been shown in guinea pigs where up to 3 mg/kg intravenously did not influence the bronchoconstriction caused by histamine, acetylcholine, serotonin, a thromboxane mimetic or arachidonic acid (Jones et al, 1989). In view of the selectivity of MK 571 for the LTD₄ receptor, it is likely therefore that the inhibitory effect of MK-571 on exercise bronchoconstriction documented in this thesis, is due to LTD₄-receptor antagonism.

An important role for LTD₄ in causing exercise bronchoconstriction is supported by the recent observations that other LTD₄-receptor antagonists markedly reduce exercise bronchoconstriction (Finnerty et al, 1992; Robuschi et al, 1992). Efforts to measure LTD₄ release after exercise, as indicated by increased levels LTD₄ in bronchoalveolar lavage (BAL) fluid after exercise, have not been successful (Broide et al, 1990). However, this may reflect a lack of sensitivity of BAL to

measure the levels of LTD₄ released. Increases in urinary LTE₄, a LTD₄ metabolite, after exercise have been demonstrated in one study (Kikawa et al, 1992) but not another (Taylor et al, 1992).

The degree of protection provided by MK-571 varied among subjects from almost complete protection (<5% fall in FEV₁) in 5 subjects, to minor protection (>20% fall in FEV₁) in 2 subjects. These results suggest that either the potency of MK-571 varied between subjects, or, more likely, that in some subjects other mediators, such as histamine, may play an important role in causing exercise bronchoconstriction.

4.6 LTD₄ Airway Responses

In this thesis the development of LTD₄ tachyphylaxis was demonstrated in asthmatic subjects. LTD₄ is a very potent bronchoconstrictor mediator, indeed it is the most potent yet studied in both normals and asthmatics (Adelroth et al, 1986). The demonstration of LTD₄ tachyphylaxis in these studies is dependent on the LTD₄ inhalation test being reproducible, which was ensured by using the same test method, employing a DeVilbiss (Model 646) jet nebulizer with a Rosenthal-French dosimeter device, with an output of 0.098 ml/min and an effective aerosol particle size of approximately 1.5 μm aerodynamic mass median diameter (MMD) for the

nebulized LTD₄ for all the studies. This is the first time that LTD₄ tachyphylaxis has been demonstrated in asthmatic subjects. This finding is consistent with the demonstration of LTD₄ tachyphylaxis in normal subjects (Kern et al, 1986). Unlike the results in this thesis, it is not known whether LTD₄ tachyphylaxis in normal subjects also involves inhibitory prostaglandin release.

4.7 Mechanism of LTD₄ Tachyphylaxis

The mechanism underlying inhibitory prostaglandin release by LTD₄ and the associated tachyphylaxis in normal and asthmatic subjects is unclear. However, as knowledge underlying the cellular signalling processes following LTD₄ receptor stimulation increases, this will help to elucidate this mechanism underlying LTD₄ tachyphylaxis.

Crooke and colleagues, detailed the current knowledge of these cellular processes (Crooke et al, 1990). LTD₄ communicates with the interior of the cell through specific receptors coupled to G-proteins in the cellular plasma membrane and phosphoinositidase C isoenzymes. Enhanced production of inositol 1,4,5-triphosphate (IP₃) and 1,2-diacylglycerol (DAG) occurs through hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP₂). The intracellular increase of these second messengers, IP₃ and DAG, leads an increase in calcium (Ca²⁺) levels from

external sources and internal stores, and protein phosphorylation. Activation of phospholipase A₂, with release of arachidonic acid from cell phospholipids and the production of cyclo-oxygenase and lipoxygenase metabolites, including prostaglandins follows.

The precise mechanism by which inhibitory prostaglandins cause LTD₄ tachyphylaxis in human airways is currently not known. However, prostaglandin E₂ may cause heterologous desensitization in isolated cell systems. This effect is mediated, in part, through alterations in the level of intracellular guanine nucleotides and can lead to a heterospecific reduction in ligand affinity for a variety of receptors in the same cell (Garrity et al, 1983; Sibley and Lefkowitz, 1985). It is possible therefore that LTD₄ tachyphylaxis may be occurring through a similar mechanism.

An alternative mechanism may be that LTD₄ receptor down-regulation is occurring through activation of intracellular protein kinase C. Activation of this enzyme can lead to down-regulation of receptors by a process of transmodulation. This process prevents the agonist from activating the appropriate receptor and leads to a decreased cellular response. For example, muscarinic receptor activation in bronchial smooth muscle (Grandordy et al, 1987) and heart muscle (Limas and

Limas, 1985) results in transmodulation of cell surface β -adrenergic receptors. In addition, LTD₄ receptors may also be subject to homologous desensitization through activation of protein kinase C and alterations of intracellular Ca²⁺ levels (Crooke et al, 1990). However, at present, there is no evidence to suggest that any of these mechanism is involved in LTD₄ tachyphylaxis in humans.

4.8 LTD₄ and Airway Control

The cysteinyl leukotrienes are the most potent known bronchoconstrictor mediators in asthmatic airways (O'Byrne 1988). In recent years, studies, using specific receptor antagonists, including MK-571, and lipoxygenase inhibitors have demonstrated that cysteinyl leukotrienes may play an important role in ongoing airway bronchoconstriction in asthma (Cloud et al, 1989; Gladys et al, 1990; Kips et al, 1991; Hui and Barnes, 1991; Gaddy et al, 1992). However, in the subjects studied in this thesis, treatment with MK-571 did not result in bronchodilation in the subjects studied. This result differs from previous reports in which MK-571 treatment improved baseline pulmonary function in mild asthmatic patients as measured by specific airway conductance (Kips et al, 1991), and in moderately severe patients as measured by FEV₁ (Gaddy et al, 1992). The subjects in the present study were selected to be stable asthmatics with little or no resting bronchoconstriction. In this population, spirometry may have been too insensitive

to detect an increase in airway calibre after drug administration. Nonetheless, even in those subjects with baseline FEV₁ values of less than 80% predicted normal, treatment with MK-571 did not reverse this mild bronchoconstriction. However, the power of our study to detect a significant effect was weak, only 31% for the magnitude of the effect seen (a mean increase in FEV₁ of 0.10 liter).

4.9 Importance of Refractoriness and Tachyphylaxis

The importance of exercise refractoriness and LTD₄ tachyphylaxis in human airway function is unclear. O'Byrne and Jones (O'Byrne and Jones, 1986) and others (McNeill et al, 1966^b), have shown that the degree of refractoriness is usually between 40-50%. In addition, with repeated sequential exercise tests the bronchoconstrictor response become progressively less (McNeill et al, 1966^b). The clinical relevancy of this is not clear, however, it is known that warm-up exercises may inhibit bronchoconstriction during subsequent exercise in asthmatics (Morton et al, 1979).

The magnitude of LTD₄ tachyphylaxis was a 2-3 fold increase in the LTD₄ PC₂₀. Asthmatic subjects demonstrate airway hyperresponsiveness to a variety of inhaled bronchoconstrictor stimuli including, histamine, methacholine, and LTD₄. However, Adelroth and associates (Adelroth et al, 1986) and others (Weiss et al, 1983;

Griffen et al, 1983; Bisgaard et al, 1985) have shown that asthmatic subjects fail to demonstrate the same relative degree of airway hyperresponsiveness to inhaled LTD₄ compared to that shown to methacholine and histamine. The reason may be that because of ongoing endogenous release of LTD₄ in the airways, tachyphylaxis occurred to inhaled LTD₄.

4.10 Prostaglandins and Airway Control

Prostaglandins, are known to play an important regulatory role in various organs, including the gastrointestinal tract, the kidney, and the cardiovascular system (Dunn, 1987; Isselbacher, 1987; Goodman, 1987). In human airways, prostaglandins may also have an important homeostatic role in the control of bronchial tone. Some prostaglandins, such as PGD₂ and PGF_{2α}, contract smooth muscle while others such as PGE₁ and PGE₂ relax human airway smooth muscle in vitro and human airways in-vivo (Gardiner and Collier, 1980; Herxheimer Roetscher, 1971; Rosenthale et al, 1971; Smith and Cuthbert, 1972; Smith et al, 1975; Walters et al, 1982^a, Walters and Davies, 1982^b). Inhaled PGE₂ induces bronchodilation (Walters and Davies, 1982^b). In addition, airway hyperresponsiveness to histamine and methacholine may be improved following a single dose of either PGE₁ or PGE₂ in asthmatics (Manning et al, 1989; Walters and Davies, 1982^b).

We, and others, have demonstrated that acute airway responses to allergen, in asthmatic subjects, involves the release of cysteinyl leukotrienes (Dahlen et al, 1983; Manning et al, 1990; Bel et al, 1990; Wenzel et al, 1990). Inhaled prostaglandin E₂ attenuates these allergen-induced responses (Pavord et al, 1993). Recently, we have also shown that a similar dose of inhaled PGE₂ also attenuates exercise bronchoconstriction (Melillo et al, 1993). These results suggest the importance of PGE₂ as a potential protective mediator against bronchoconstriction in asthmatic subjects. The effect of inhaled PGE₂ on airways responses to inhaled LTD₄ in asthmatic subjects is not known.

Homeostatic control of airway function by inhibitory prostaglandins is believed to be particularly important in asthmatic subjects with aspirin intolerance (Szczeklik, 1975). This idiosyncratic reaction develops to cyclooxygenase drugs, including indomethacin, flurbiprofen and aspirin in up to 19% of asthmatics. Aspirin intolerance may be associated with acute exacerbations of asthma which can be life-threatening (Barnes and Thomson, 1992). Recent research suggests that inhibitory prostaglandin and cysteinyl leukotriene release may play an important role in the control of airway function in aspirin sensitive asthmatics (Barnes and Thomson, 1992). Leukotrienes have been implicated in the mechanism of aspirin

intolerance in these individuals through; the demonstration of enhanced LTE₄ release following aspirin exposure; an increased bronchoconstrictor responses to inhaled LTE₄; and the demonstration that MK-0679, a leukotriene-receptor antagonist, inhibited the bronchoconstrictor responses following aspirin exposure (Arm et al, 1989; Ferreri et al, 1988; Kumlin et al, 1992; Knapp et al, 1992; Dahlen et al, 1993).

Since subjects with a history of aspirin intolerance were specifically excluded from the studies in this thesis, is not surprising that treatment with flurbiprofen did not alter baseline airway function; change the airway responsiveness to LTD₄ or exercise; or cause exacerbation of asthma symptoms in the asthmatic subjects studied.

4.11 General Summary and Future Directions

The studies described in this thesis suggests that exercise refractoriness and LTD₄ tachyphylaxis are potential inhibitory mechanisms in the airways, which when activated, protect the airways against the effects of repeated bronchoconstriction to exercise and LTD₄. The studies also demonstrate that exercise bronchoconstriction is mainly caused by LTD₄ release and that LTD₄-induced inhibitory prostaglandin release is the cause of exercise refractoriness.

In summary, the studies in this thesis have shown, for the first time, new and important information regarding LTD₄ release and subsequent effects in the airways of asthmatic subjects in that :

- (1) LTD₄ is the major cause of exercise bronchoconstriction in asthmatic subjects.
- (2) The presence of tachyphylaxis, a potential protective mechanism occurs in the airways of asthmatic subjects following repeated LTD₄ inhalation.
- (3) LTD₄ tachyphylaxis in humans, like exercise refractoriness is caused by inhibitory prostaglandin release, likely prostaglandin E₂.
- (4) Cross refractoriness/tachyphylaxis occurs between exercise- and LTD₄-induced bronchoconstriction, and this is also caused by inhibitory prostaglandin release.

While illuminating the mechanism underlying inhibitory prostaglandin release associated with exercise refractoriness and LTD₄ tachyphylaxis *in-vivo*, many questions are left unanswered. The events underlying the inhibitory prostaglandin release needs to be clarified at a cellular level and the time course of the effect needs to be determined. The LTD₄ receptor-mediated events, the subsequent enzyme induction processes, the metabolic pathways leading to the release of inhibitory prostaglandins and the mechanism of LTD₄ tachyphylaxis will need to be identified. Clinically, the importance of these inhibitory effects on airway control in normal airway function and on airway hyperresponsiveness in asthmatic subjects

will need to be examined further. In particular, it needs to be determined whether severe asthma is associated with loss of these protective mechanisms.

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