THE EFFECT OF ACUTE ALTERATIONS IN OXYGEN TENSION ON BRONCHOCONSTRICTOR AND BRONCHODILATOR STIMULI *IN-VITRO* IN MAN AND *IN-VIVO* IN PATIENTS WITH ASTHMA

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A thesis submitted to the University of Glasgow for the degree of Doctor of Medicine
September 2001

CONTENTS

ABSTRACT

An acute severe exacerbation of asthma is characterised by the release of inflammatory mediators, reduction in airway calibre and ultimately hypoxaemia. The initial management of such patients includes the administration of high concentrations of inspired oxygen and both nebulised and intravenous bronchodilators. Little however is known about the influence of oxygen tension on the responsiveness of airways to bronchodilator drugs and to bronchoconstrictor stimuli. Recent studies undertaken in our own laboratory have suggested that acute alterations in oxygen tension have profound effects on responses evoked by bronchodilators and bronchoconstrictors in isolated bronchial rings. Similar findings have been made in animals. Such observations in man may have relevance to the management of patients with acute exacerbations of asthma. The purpose of our studies was to investigate the influence of acute alterations in oxygen tension on bronchodilator and bronchoconstrictor stimuli both *in-vitro* in man and in-patients with asthma.

Our initial *in-vitro* studies suggested that the ability of salbutamol to relax human bronchial rings is significantly attenuated by hypoxia (O_2 tension 4%) when compared to normoxia (O_2 tension 20%) and hyperoxia (O_2 tension 95%). We also found that acute hypoxia (O_2 tension 4%) significantly attenuated the ability of both histamine and methacholine to constrict human isolated bronchial rings when compared to normoxia (O_2 tension 20%) and hyperoxia (O_2 tension 95%). In-patients with mild asthma we found that hypoxia (FiO_2 15%) potentiated and hyperoxia (FiO_2 100%) had no effect on methacholine induced bronchoconstriction. In a series of studies we have also found that airway responses to both inhaled histamine and salbutamol were unaffected by both hypoxia (FiO_2 15%) and hyperoxia (FiO_2 100%) in-patients with mild stable asthma. We would conclude from these studies that acute alterations in

ambient oxygen tension have significant effects on airway responses to constrictor and dilator stimuli both *in-vitro* in man and in-patients with asthma. The finding that hypoxia potentiates both salbutamol induced relaxation *in-vitro* in man and methacholine induced bronchoconstriction in patients with asthma may have relevance to the management of patients with acute exacerbations asthma who as part of their routine treatment receive high inspired oxygen tensions and both nebulised and intravenous bronchodilators. Further studies may determine the mechanism of these effects and lead to the development on novel asthma therapies.

ACKNOWLEDGEMENTS

I would particularly like to thank Professor Neil Thomson for his support and encouragement throughout this project. His advice and helpful appraisal of this thesis and the manuscripts arising from the various studies have been invaluable in the completion of this project. I would also like to take the opportunity to thank my colleague, Dr S Ramsay, for his support and assistance during our time together in the Asthma Research Unit. I am particularly indebted to Sister Lorna Thomson our Asthma Research Nurse. She gave an enormous amount of her own time to help with the delivery of the inspired gases and the blinding of those gases. Her endless enthusiasm and energy was an inspiration and without her assistance the completion of this project would not have been possible. I am grateful to Dr R Clayton and Dr J Nally who so willingly allowed me to collaborate with them in carrying out the *in-vitro* studies. I would like to thank Miss Wendy Fallon and Miss Dawn Farmer of our pharmacy department who blinded the studies and were responsible for all of the drug preparation for the inhalational studies and also Mr Gordon Burnside from the Department of Medical Physics who assisted in the design and construction of the closed breathing circuit. The hormone assays were undertaken by Dr J Morton from the Department of Medicine and Therapeutics. I would also like to acknowledge the large number of normal volunteers and patients with asthma who willingly gave of their time and without whom these studies would not have been possible. I will always be in debt to the British Lung Foundation, who provided a grant, which enabled me to undertake a period of full time research leading to the completion of this thesis.

DECLARATION

I am the sole author of this thesis and I have personally consulted all of the references listed. This work was undertaken by myself in the Department of Respiratory Medicine, The North Glasgow Hospitals University NHS Trust, Glasgow. The *in-vitro* work was performed in collaboration with Drs R Clayton and J Nally in the Respiratory Physiology Laboratory, Western Infirmary, Glasgow. This thesis has not previously been submitted for a higher degree.

ABBREVIATIONS

BAL	bronchoalveolar lavage
BHR	bronchial hyperresponsiveness
BP	blood pressure
CCRC's	cumulative concentration-response curves
FEV_1	forced expiratory volume in one second
FiO_2	inspired oxygen tension
Hist	histamine
HR	heart rate
$inspCO_2\%$	percentage inspired carbon dioxide level
$inspO_2\%$	percentage inspired oxygen level
MAP	mean arterial blood pressure
MCh	methacholine
O_2	oxygen
$PaCO_2$	partial pressure of carbon dioxide (units kPa)
PaO_2	partial pressure of oxygen (units kPa)
PC_{20}	provocation concentration causing 20% fall in FEV_1
PEFR	peak expiratory flow rate
$PETCO_2\%$	percentage end-tidal carbondioxide level
$PETO_2\%$	percentage end-tidal oxygen level
RR	respiratory rate
$SaO_2\%$	oxygen saturation
SD	standard deviation
SEM	standard error of the mean

CHAPTER 1

INTRODUCTION

1.1 Asthma

1.1.1 Epidemiology

Asthma is the commonest chronic disease to affect both adults and children in the developed world[1] and it is estimated that in the United Kingdom alone, there are more than 3 million people with the disease.[2] The clinical syndrome of asthma does not readily lend itself to a specific definition because of overlap with smoking related lung disorders which may also cause airflow obstruction and increased bronchial hyperresponsiveness. In 1986 however, the board of directors of the American Thoracic Society ratified a definition of asthma as "a clinical syndrome characterised by increased responsiveness of the tracheo-bronchial tree to a variety of stimuli. The major symptoms of asthma are paroxysms of dyspnoea, wheezing and cough, which may vary from mild and almost undetectable to severe and unremitting (status asthmaticus). The primary physiological manifestation of this hyperresponsiveness is variable airways obstruction, which can take the form of spontaneous fluctuations in the severity of obstruction, following bronchodilators or corticosteroids, or increased obstruction caused by drugs or other stimuli".[3]

Despite considerable improvements in our understanding and management of asthma over the last two decades, studies from around the world would suggest that the prevalence of this illness continues to rise at an alarming rate.[4] Asthma is responsible for considerable morbidity and mortality within the community, much of which is thought to be preventable.[5] It is estimated that in the United Kingdom almost one in three patients suffer 3 or more exacerbations of asthma per year leading to absences from school or work which cost the country an estimated £400 million pounds per annum.[2] During 1993 acute exacerbations of asthma accounted for more than 100

000 acute admissions to hospital in Scotland and England.[2] The British Thoracic Association confidential inquiry into asthma deaths in the 1980's suggested that severe exacerbations of asthma accounted for more than 1500 deaths per annum in the United Kingdom and that avoidable factors were present in more than 80% of these deaths.[6] A recent Scottish confidential inquiry into asthma deaths investigated 95 deaths between January 1994 and December 1996. This study found a significant fall in the calculated "deaths assessed as due to asthma" and detected significant improvements in overall asthma management when compared with the British Thoracic Association study of the 1980's.[7]

The improvements in asthma mortality which have been observed reflect an increased understanding of the pathogenesis of this disease and with this improvements in the emphasis of asthma therapy and its delivery. Despite these improvements severe exacerbations of asthma remain a common reason for admission to hospital and are still associated with avoidable deaths.

1.1.2 Pathology

Early theories relating to the pathophysiology of asthma suggested that it was intrinsic abnormalities of autonomic neural innervation of the bronchial tree in patients with asthma, which resulted in variable airway obstruction and increased bronchial hyperresponsiveness. However within the last two decades it has emerged through intensive research that asthma is an inflammatory condition largely confined to airways, particularly the bronchi and smaller bronchioles.[8] Early evidence for this hypothesis came from pathological studies of patients dying in 'status asthmaticus'. The airway lumens of patients with asthma contained odema fluid, cellular debris and

inflammatory cells which had coalesced to form mucus plugs. These plugs found throughout the bronchial tree caused a reduction in airway calibre which contributed to the airflow obstruction observed in patients with asthma.[9] Microscopy of pathological specimens confirmed the presence of increased smooth muscle bulk, glandular hyperplasia, separation of the superficial columnar cells from basal cells, odema of the airway walls and thickening of the basement membrane.[9,10] In addition to these striking structural changes, the airway walls themselves contained an intense inflammatory cell infiltrate. This cellular infiltrate included eosinophils which were found in large numbers both within the bronchial wall and migrating through it into the airway lumen.[9] Degranulated mast cells were also found in abundance within the airway wall and lumen.[11] Recent intense interest has focused on the role of lymphocytes in the pathogenesis of asthma. This interest has been provoked by observations from biopsy studies in patients with asthma which have suggested that lymphocytes are found in the airway walls in even higher numbers than eosinophils, once thought to be the major effector cells of the asthmatic inflammatory response.[12] Further classification of these lymphocytes has confirmed an abundance of T-helper cells within the airway walls with specific activation of the Th2 subgroup.[12] Recent biopsy studies from asymptomatic,[13] mild,[14] moderate and severe[15] asthmatics have shown similar histological changes to those observed in patients dying from acute asthma. The only difference between these groups appears to be in the degree of histological change.[8] This would suggest that inflammation is the main abnormality in patients with asthma. Studies examining biopsy samples from patients with extrinsic and intrinsic asthma have also shown little in the way of histological difference between these so called sub-groups.[8]

The pathological studies described above, in patients with asthma of varying severity, have confirmed that inflammation within the airway wall has a fundamental role to play in the pathophysiology of asthma. The association between inflammation and the development of airflow obstruction and more importantly bronchial hyperresponsiveness is further considered in the next section on the pathogenesis of asthma.

1.1.3 Pathogenesis

Many attempts have been made to classify patients with asthma into subgroups based on aetiology. These subgroups include extrinsic, intrinsic, occupational, exercise and aspirin induced asthma. While aetiologically a rather heterogenous group of conditions it has become increasingly apparent that they share common physiological, pathological and immunological mechanisms. Atopic asthma, the commonest form of extrinsic asthma with a readily identifiable aeroallergen, typically develops in childhood and early adulthood.[8] It is the investigation of this form of asthma which provides the basis for much of our understanding of the pathogenesis of asthma today.

1.1.3.1 Cellular response

Inhalation of a specific aeroallergen in a sensitised individual, results in cross linking of IgE molecules bound to high-affinity receptors on mast cells.[16] This process results in degranulation of mast cells and the release of a variety of preformed mediators into the airways. These mediators include histamine, prostaglandins, thromboxanes, leukotrienes and cytokines.[8] These mediators have a wide range of functions but effectively trigger a cellular and molecular cascade which results in airway inflammation. Within 15-20 minutes of mast cell degranulation vascular leakage and

smooth muscle constriction are observed, a process which reverses spontaneously within hours and constitutes the early asthmatic response.[17] In many patients a second phase of bronchoconstriction occurs and is characterised by the recruitment of eosinophils, lymphocytes and macrophages to the inflammatory response.[18] This occurs between 6 and 12 hours after the initial challenge and may recur on subsequent days. This is termed the late asthmatic response and is associated with further bronchoconstriction and the development of increased airway responsiveness which is so typical in patients with asthma.

Pathological studies have already confirmed that a variety of cells are involved in the development and maintenance of the asthmatic inflammatory response. Degranulation of mast cells seem to provide the initial stimulus to the development of this response. Other cell types involved include eosinophils, once thought to be airway protective because of a capacity to degrade proinflammatory mediators such as histamine[8] They are now recognised as one of the major effector cells of the inflammatory response in asthma. They are found in considerable numbers in the airways of asthmatic patients both spontaneously[19] and in response to allergen challenge.[20] They secrete a number of toxic substances including eosinophil peroxidase, major basic and eosinophil cationic proteins which cause considerable disruption to airway epithelium.[20] Treatment of atopic asthmatics with regular inhaled corticosteroids results in a significant reduction in airway eosinophil counts, improved control of asthma symptoms and a reduction in airway hyperresponsiveness.[21]

Macrophages, the commonest cell type found in airways, have low affinity IgE receptors on them suggesting a role in allergic mediated asthma.[22] Recovery of mast

cells from bronchoalveolar lavage fluid following allergen challenge show increased production of eicosanoid mediators and GM-CSF which may prolong eosinophil survival.[23] Macrophages may also have a regulatory role in the inflammatory cascade altering the activity of lymphocytes and controlling recruitment of eosinophils and neutrophils to the airway inflammatory response.[24]

In recent years increasing interest has focused on the role of lymphocytes in the asthmatic inflammatory response. Activated lymphocytes have been found in large numbers in the airway mucosa of patients with asthma[21] and are found in direct proportion to the number of eosinophils.[25] The production of specific IgE by B-lymphocytes is a well recognised feature of atopic asthma. The role of T-lymphocytes is less well understood. It is thought they may act as immune modulators in the asthmatic inflammatory response. T-helper lymphocytes have been divided into Th1 and Th2 subgroups. These groups appear mutually inhibitory[26,27] It is thought that Th2 lymphocytes on the basis of their cytokine profile (secretion of IL-3, IL-4, IL-5 and GM-CSF) promote the inflammatory response in asthma by stimulating IgE synthesis and potentiating mast cell and eosinophil function.[28] Th1 lymphocytes appear to oppose these actions. The role of neutrophils and platelets in the production and propagation of the acute inflammatory response and subsequent development of bronchial hyperresponsiveness remains controversial and poorly understood in patients with asthma.[8]

1.1.3.2 Inflammatory mediators

It is becoming increasingly clear that the development and maintenance of the asthmatic inflammatory response involves a complex interaction between a number of

different cell types and the mediators they secrete. These mediators have a number of functions. They are thought to act directly on airway smooth muscle causing bronchoconstriction, increase microvascular leakage from airway capilliaries, enhance mucus secretion from mucosal glandular cells, promote chemotaxis and activate other inflammatory cells.[8] It is beyond the scope of this introduction to discuss all of the inflammatory mediators which have been implicated in the asthmatic inflammatory response. The role of several of the more important mediators are discussed below.

Histamine is released following degranulation of mast cells and basophils. Levels of histamine have been found to be elevated in BAL fluid obtained from asthmatic patients at rest and in response to allergen challenge.[29-32] Histamine causes airway smooth muscle contraction,[33] potentiates microvascular leakage producing airway odema[34] and stimulates mucus secretion into the airway lumen.[35]

The cysteinyl leukotrienes are formed from the arachidonic acid pathway by the action of the enzyme 5-lipoxygenase. They are potent inflammatory mediators causing cell chemotaxis, neural bronchoconstriction, microvascular leakage and mucus hypersecretion.[36] Cysteinyl leukotrienes are found in high levels in the BAL fluid and urine obtained following allergen challenge in patients with asthma.[37] Recently developed cysteinyl-leukotriene receptor antagonists have been shown to attenuate both allergen,[38,39] exercise,[36] and aspirin-induced asthma.[40,41] Clinical studies with these antagonists in patients with mild to moderate asthma have shown significant improvements in asthma symptoms and lung function when compared to

placebo.[42-45] These studies would suggest an important role for leukotrienes in propagating the asthmatic inflammatory response.

Prostaglandins produced by the enzyme cyclo-oxygenase are also found to be elevated in the BAL fluid obtained from asthmatic patients at rest and following allergen challenge.[31,46] PGD2 and PGF2α are potent bronchoconstrictors[47,48] and potentiate airway hyperresponsiveness to histamine and methacholine.[49] Interestingly however cyclo-oxygenase inhibitors have little clinical effectiveness in asthma suggesting a limited role for these mediators in the propagation of the asthmatic inflammatory response.[50]

Cytokines and chemokines are a group of more than 50 peptide mediators, secreted by inflammatory cells which seem to have an important role in cell signalling. These mediators appear to have both pro and anti-inflammatory activity. They are principally involved in the maintenance of chronic inflammation in patients with asthma. They include the Interleukins 1L-8, Tumour necrosis factor-α, Granulocyte-macrophage colony-stimulating factor and the interferons. Their actions, which are diverse and not yet fully understood, include promotion of IgE production from B lymphocytes,[51] enhancement and suppression of antigen presentation via macrophages,[52,53] increased cell chemotaxis and activation of inflammatory cells within the airways. Considerable research efforts continue to be made into determining the exact role of cytokines and chemokines in the development and maintenance of the inflammatory response. It is clear however that they are an

extremely important group of inflammatory mediators and improved understanding of their actions may open the door to the development of novel anti asthma therapies.

1.1.3.3 Neural innervation

Neural innervation of the airways is complex and still poorly understood in relation to the development and maintenance of asthmatic symptoms. Airway smooth muscle derives its innervation from several sources including vagal afferents, post ganglionic non-cholinergic vagal efferents and a non-adrenergic non-cholinergic supply.[54] Autonomic innervation of the lungs has influences on smooth muscle tone, vascular permeability, mucus secretion and blood flow. It has been postulated that abnormal neural innervation of the airways contributes to the bronchoconstriction seen in asthma.[55] It is thought however that this represents a secondary effect of the inflammatory process rather than a primary defect within the autonomic nervous system as was previously thought. Several mechanisms resulting in neural bronchoconstriction have been proposed including stimulation of vagal afferent C-fibres within the airways leading to cough and bronchoconstriction,[56] increased acetylcholine release from post ganglionic nerve endings[57] and intrinsic abnormalities of muscarinic receptors on cholinergic nerves. Others have hypothesised that the non-adrenergic non-cholinergic innervation may contain intrinsic defects in patients with asthma which result in the loss of a natural bronchodilator pathway.[58] That neural mechanisms have a role in the bronchoconstriction seen in asthma is generally agreed, the mechanisms however remain poorly understood.

1.1.3.4 Summary

The last two decades have seen significant advances in the understanding of the pathogenesis of asthma. The development of flexible fibre optic bronchoscopy allowing ready access to the lower airways for biopsy and bronchoalveolar lavage allied to advances in molecular biological techniques and the use of induced sputum have significantly increased our knowledge of the pathogenesis of asthma. Early beliefs that asthma occurred as a consequence of imbalances between inhibitory and excitatory autonomic nervous innervation of airways have now been shown to be somewhat simplistic. Asthma is an inflammatory condition affecting the airways of sensitised individuals. Triggers of asthma produce a complex inflammatory cascade within the airways involving a wide range of inflammatory cells and the mediators they secrete. It is the inflammatory process described above which produces the variable airflow obstruction and increased bronchial hyperresponsiveness that is so typical of asthma. The clinical symptoms that this inflammation process produces may vary from mild and almost unnoticeable in some patients to severe and occasionally fatal in others.

1.2 Acute Severe and Life Threatening Asthma

1.2.1 Introduction

While evidence now exists which suggests that mortality from asthma is declining[7] the number of admissions to hospital with acute severe and life threatening exacerbations of asthma appears unchanged. Studies have suggested that avoidable factors can be detected in more than 75% of these admissions.[59] Patients themselves often underestimate the severity and duration of severe attacks and may delay seeking medical help. Later questioning of patients who survive their hospital admission may reveal asthma symptoms which have been troublesome for several days and in some cases several weeks.[60] Other factors associated with the development of life threatening exacerbations of asthma include additional psychosocial problems, previous hospital admission, previous severe attacks, being an adolescent or young adult, high bronchodilator demand or oral corticosteroid use, intermittent nebuliser use and patients showing marked diurnal variation on peak flow recording.[61]

1.2.2 Presentation and assessment

Patients usually recognise the symptoms of an acute exacerbation which may include increasing breathlessness, wheeze, cough, chest tightness, reduced effort capacity, troublesome nocturnal symptoms and failure to respond to their usual medication.[61] They are however less able to determine the severity of their exacerbation, possibly due to tolerance of chronic symptoms or blunted perception to developing airflow obstruction.[62,63] It is well recognised that both hospital doctors[64] and family practitioners[65] faced with a patient suffering from an acute exacerbation of asthma will also underestimate the severity of the exacerbation and as a consequence will under prescribe anti-inflammatory and rescue bronchodilator medication,

occasionally with fatal consequences.[6] Proper assessment of the severity of an acute exacerbation requires objective measurements to be made. With this in mind the British Thoracic Society in conjunction with the British Paediatric Association, National Asthma Campaign and others produced guidelines for the recognition and assessment of patients with acute severe and life threatening exacerbations of asthma.[66]

It is recommended that objective measurements of respiratory rate, heart rate, peak expiratory flow rate (PEFR), blood pressure, gas exchange and general observations about the patient are made. An acute severe exacerbation should be suspected when the patient is unable to complete sentences, has a PEFR less than 50% of predicted or best, a respiratory rate > than 25 breaths/minute and a heart rate > than 110 beats/minute. Life threatening features of acute asthma include PEFR less than 33% of predicted or best; silent chest, cyanosis or feeble respiratory effort; bradycardia or hypotension; exhaustion, confusion or coma.[66]

The majority of patients admitted to hospital with acute severe and life threatening asthma will be hypoxaemic and approximately one third will have a PaO_2 of less than 8.0 kPa when breathing room air.[67,68] A minority, approximately 10% of patients will have severe hypoxaemia with a PaO_2 of less than 6.0 kPa.[61] Patients who are admitted to hospital usually have a low $PaCO_2$ as a consequence of hyperventilation. In those patients with a normal $PaCO_2$ the possibility of a more severe exacerbation is raised and the potential to develop hypoventilation should be documented.[61] Approximately 5-10% of patients present with hypercapnia. These patients have already developed hypoventilation as a consequence of severe airflow obstruction and are at greatest risk of dying.[69] Patients who have any life threatening feature and

those with an oxygen saturation (SaO_2%) of less than 92% should therefore have their blood gases measured and documented.

1.2.3 Management

The management of acute severe and life threatening exacerbations of asthma are contained within guidelines issued by the British Thoracic Society and other interested parties.[70] Patients should immediately receive high inspired oxygen tensions with an FiO_2 in excess of 60% in an attempt to raise PaO_2 above 8.0 kPa and ideally to between 10 and 14 kPa. It is rare for patients with asthma to develop hypoventilation as a consequence of oxygen therapy.[61] Nebulised bronchodilators should be administered and nebulisers should be driven by oxygen with a flow rate of 7 litres per minute. Salbutamol or terbutaline are the most commonly used bronchodilators. Nebulised ipratropium bromide can also be administered, although some physicians delay its use for 15-30 minutes and only give it to patients not improving with their initial treatment. Oral corticosteroids or intravenous hydrocortisone, in those patients unable to tolerate oral drugs, should be given immediately on admission.

The objective measurements made during the initial assessment should be repeated within 15-30 minutes of admission to hospital. Patients not improving or who have evidence of deterioration should receive further nebulised β_2-agonists at 15 minute intervals and ipratropium bromide if they have not already done so. Intravenous infusions of aminophylline, salbutamol or terbutaline should be commenced with bolus injections where appropriate. Once patients have stabilised transfer to a suitable medical ward should be arranged and patients continued on oxygen therapy,

oral or intravenous corticosteroids and 4 hourly nebulised bronchodilators as well as any intravenous bronchodilators that have been commenced.

Patients who continue to deteriorate despite the above measures should be considered for admission to the intensive care unit.[66] Current indications for referral to such a unit include persisting hypoxia ($PaO_2 < 8.0$ kPa) despite an inspired $FiO_2 > 60\%$, hypercapnia ($PaCO_2 > 6$ kPa), exhaustion, feeble respiration, confusion, drowsiness, coma or following a respiratory arrest.

It is recommended that patients are discharged from hospital following resolution of nocturnal symptoms, when PEFR has risen to greater than 75% of predicted or best and twice daily PEFR shows less than 25% diurnal variation. Patients should be established on their discharge medication for 24-48 hours prior to discharge and the dose of inhaled corticosteroids is usually higher than admission. Inhaler technique should have been assessed and arrangements should be in hand for asthma clinic review within one month of discharge.

1.2.4 Summary

Despite an apparent fall in the mortality of acute asthma in recent years severe and life threatening exacerbations remain a common reason for admission to hospital. A significant number of such patients will be hypoxaemic and as part of their routine in-hospital management will receive high inspired oxygen tensions to inhale as well as nebulised and occasionally intravenous bronchodilators.

1.3 The effect of acute alterations in oxygen and carbon dioxide tension on airway tone in isolated bronchial rings

1.3.1 Effects on resting tone

Hypoxia and hypercapnia are associated with a variety of both acute and chronic cardio-respiratory illnesses in man. As part of the management of these illnesses patients often have elevated inspired oxygen tensions administered in an attempt to correct hypoxia. It is not surprising therefore that researchers have, for some time, been interested in the effects of both acute and chronic alterations in oxygen (PaO_2) and carbon dioxide ($PaCO_2$) tension on smooth muscle resting tone, and on subsequent contraction of that smooth muscle following both electrical and pharmacological challenge. The effect of acute alterations in oxygen and carbon dioxide tension on smooth muscle responses from a variety of organs including lung, heart, vasculature, kidney and uterus have previously been examined and it is apparent that the responses between these organs vary quite considerably.[71-74]

Stephens et al[71] have previously demonstrated that in canine isolated bronchial rings, obtained from the first intrapulomonary division of the left lower lobe of mongrel dogs, suspended in vertical organ baths a reduction in ambient PaO_2 from 630 mm Hg to 60 mm Hg whilst maintaining a constant pH and $PaCO_2$ is associated with a small but significant ($p<0.05$) rise in resting tone in the bronchial rings of approximately 5% when compared to a control group. In isolated pulmonary arterial rings from several species including rats, sheep and pigs a similar reduction in ambient oxygen tension also causes a significant increase in resting tension.[74] This increase causes pulmonary arterial constriction and is thought to represent a reflex response within the pulmonary vasculature to hypoxia which improves ventilation perfusion matching.

This effect should however be contrasted with skeletal, gastrointestinal and cerebral vascular rings where a reduction in PaO_2 below 40 mm Hg causes a marked reduction in resting tension[74] and results in vasodilation. Stephens et al have also examined the effect of acute alterations in carbon dioxide levels on canine isolated bronchial rings.[71] They observed a small but significant rise ($p<0.05$) in resting tension following a reduction in $PaCO_2$ from 42 mm Hg to 25 mm Hg while PaO_2 was maintained at 630 mm Hg and pH at 7.41. Conversely a rise in $PaCO_2$ from 42 mm Hg to 76 mm Hg while maintaining a pH of 7.41 and PaO_2 of 630 mm Hg was associated with a small but significant ($p<0.05$) fall in resting tension. It would appear therefore that acute alterations in both oxygen and carbon dioxide tensions alone have significant, although variable, effects on smooth muscle tone in isolated rings from different tissues and species.

1.3.2 Effects on electrical stimulation

Studies *in-vitro* have examined the effect of supramaximal electrical stimulation, in different oxygen tensions, on the development of maximum active tetanic tension, maximum rate at which tension develops and the time to development of maximum tension in smooth muscle from canine isolated tracheal rings.[72,73] Stephens et al have suggested that in tracheal rings maintained at a constant length (L_{max}) the maximum active tetanic tension (AP_{max}) produced following supramaximal electrical stimulation is significantly reduced by hypoxia (PaO_2 60 mm Hg) when compared to control tracheal rings with an ambient oxygen tension of 630 mm Hg. Hypoxia also resulted in a significant reduction in the rate of development of active tetanic tension $(dP/dt)_{max}$ and an increase in the length of time to the development of maximum tension (t_{APmax}). The authors have postulated that hypoxia impairs mitochondrial

function reducing intracellular energy release and reducing the ability of smooth muscle to constrict in response to electrical stimulation. This effect does not appear to relate to the development of intracellular acidosis or impaired depolarisation of potassium channels.[72,73]

1.3.3 Effect on pharmacological challenge

Recent studies carried out in our own laboratories have examined the effect of acute alterations in oxygen tension on responses evoked by a variety of bronchoconstrictor and bronchodilator stimuli in isolated bovine and rat isolated bronchial rings.[75-77] The acute effects of three different oxygen tensions; 95% O_2 (hyperoxia), 20% O_2 (normoxia) and 4% O_2 (hypoxia) on methacholine induced tone in bovine isolated bronchial rings were examined.[75] Cumulative concentration-response curves to methacholine (10^{-9} - 3 x 10^{-4} M) were constructed in each of the oxygen tensions. Results for subsequent statistical analysis were expressed as a percentage of the maximum response in 95% O_2. The constrictor response to methacholine was significantly greater ($p<0.001$) in the 20% and 4% O_2 when compared to 95% O_2. There was no significant difference between 2% and 4% O_2. When a single concentration of methacholine (3 x 10^{-6} M) was used to constrict the bovine isolated bronchial rings similar results were found. The resultant contraction was greater in 20% and 4% O_2 when compared to 95% O_2 ($p<0.001$) but again no significant difference in contractions was found between oxygen tensions of 20% and 4%. Similar studies[76] have also been performed looking at the effect of acute changes in oxygen tension (95%, 20%, 4%) on concentration-response curves (10^{-10} - 3 x 10^{-7} M) to endothelin-1 in bovine isolated bronchial rings. Hypoxia (O_2 4%) significantly ($p<0.001$) potentiated and hyperoxia (O_2 95%) significantly attenuated ($p<0.001$) the

contractions evoked by endothelin-1 when compared to 20% O_2. It is interesting to note that the effect of hypoxia on endothelin-1 mediated contractions was completely abolished by the addition of indomethacin, a cycloxygenase inhibitor.[76]

Our laboratory has also examined the effect of acute alterations in oxygen tension (95%, 20%, 4%) on methacholine-induced contractions in rat isolated bronchial rings.[78] The results from this work differs quite markedly from what we have previously described in bovine isolated bronchial rings. In a similar series of studies cumulative concentration-response curves were constructed to methacholine (10^{-9} - 3 x 10^{-4} M) for rings of rat bronchi suspended in vertical organ baths. Changing the oxygen tension from 95% to 20% had no effect on responses evoked by methacholine. However when the oxygen tension was further reduced to 4% the constrictor response to methacholine was significantly attenuated ($p<0.001$). This observation was also found in rat isolated bronchial rings from chronically hypoxic rats which had been reared in a hypoxic chamber for 14 days.[78] These findings differ significantly from the observations we have made in bovine isolated bronchial rings but are in keeping with results reported by other groups.[79-81]

Vannier et al have also studied the effect of acute alterations in oxygen tension on KCl and carbachol induced constriction in third and fourth order porcine isolated bronchial rings.[80] Their group has reported that a reduction in oxygen tension from 95% to 0% caused a highly significant shift of the concentration-response curves for both carbachol ($p<0.005$) and KCl ($p<0.0001$) to the right indicating a reduction in tone generated during hypoxic conditions. On returning to hyperoxic conditions (O_2 95%) tone was restored to initial resting levels. In carbachol constricted rings this

observation was not significantly altered by removal of bronchial epithelium or the addition of indomethacin (a cyclooxygenase inhibitor), methylene blue (a soluble guanylate cyclase inhibitor) or propranolol (a ß-adrenoceptor antagonist). In a second series of experiments, the effect of a stepwise reduction in ambient oxygen tension (95%, 50%, 28%, 10% and 3%) on carbachol and KCl generated tone in porcine isolated bronchial rings was examined. On this occasion bronchial rings both with and without an intact epithelium were studied. They found that the degree of hypoxic relaxation of porcine rings pre-constricted with carbachol and KCl (O_2 95%) was proportional to the reduction in oxygen tension and that this relationship was linear. The presence or absence of an intact epithelium did not influence this observation suggesting that bronchial epithelium does not secrete a relaxant factor as had previously been suggested. In a later series of studies exploring the possible mechanisms of hypoxic relaxation[80] following pharmacological challenge Vannier et al have examined the effect that BAY K 8644 (a L-type Ca^{2+} channel agonist) has on hypoxic relaxation of porcine isolated bronchial rings pre-constricted with carbachol and KCl. BAY K 8644 completely abolished hypoxic relaxation in porcine rings pre-constricted with KCl (40 mM) at all oxygen tensions (5%, 28%, 10% and 3%) but had no effect on porcine rings constricted with the cholinergic agonist carbachol. This would suggest that a possible mechanism to explain the observation of hypoxic relaxation may be inhibition of calcium entry into cells.

Gao and Vanhoutte[82] have also considered the importance of an intact bronchial epithelium on hypoxic relaxation of canine isolated bronchial rings pre-constricted with carbachol (3×10^{-6} M) [10]. In their studies the magnitude of relaxation of canine isolated bronchial segments was dependent on both the level of hypoxia used (600

mm Hg, 140 mm Hg, 80 mm Hg and 40 mm Hg) in the organ bath and also on the presence of an intact bronchial epithelium. This clearly differs from the findings of Vannier et al.[80] The preparations in which the epithelium had been removed showed no significant relaxation at any oxygen tension when compared to controls. They have suggested that bronchial epithelium in response to hypoxia may release an unknown relaxing factor to explain the observation of hypoxic relaxation following pharmacological challenge. In keeping however with the results of Vannier et al they also found that indomethacin, methylene blue, propranolol and trodotoxin (a sodium channel blocker) did not alter the magnitude of hypoxic relaxation in the epithelium intact preparations.

We have up until now considered only the effect of acute alterations in oxygen tension on constrictor stimuli in a variety of tissues *in-vitro*. Our laboratory has recently undertaken studies to examine the effect of hypoxia, normoxia and hyperoxia on the ability of bronchodilator drugs to reverse airway tone in bovine isolated bronchial rings pre-constricted with methacholine (3×10^{-6} M).[75,77] The drugs used: salbutamol, atrial natriuretic peptide and isosorbide dinitrate were selected on the basis of their postulated mechanisms of action. Salbutamol is a β_2-adrenoceptor agonist which stimulates intracellular adenylate cyclase causing a rise in cyclic adenosine monophosphate (cAMP) as well as opening K^+ channels. Atrial natriuretic peptide (ANP) stimulates particulate guanylate cyclase causing a rise in cyclic guanosine monophosphate (cGMP). Isosorbide dinitrate also causes a rise in cGMP but by stimulating soluble guanylate cyclase. Each of these actions results in smooth muscle relaxation. Cumulative concentration-response curves were constructed for each of these dilators: salbutamol (10^{-8} - 3×10^{-6} M), atrial natriuretic peptide (10^{-9} -

10^{-6} M) and isosorbide dinitrate (10^{-7} - 3 x 10^{-5} M) in oxygen tensions of 95%, 20% and 4% for bovine isolated bronchial rings pre-constricted with methacholine (3 x 10^{-6} M). The ability of salbutamol to reverse tone in bovine bronchial rings constricted with methacholine was significantly reduced ($p<0.001$) by an oxygen tension of 4% when compared to 20%. Conversely atrial natriuretic peptide ($p<0.05$) and isosorbide dinitrate ($p<0.001$) caused significantly greater reversal of tone at an oxygen tension of 4% when compared to an oxygen tension of 20%. There was no significant difference for salbuatmol or atrial natriuretic peptide when oxygen tensions of 95% and 20% were compared. Isosorbide dinitrate caused greater relaxation ($p<0.01$) at an oxygen tension of 20% when compared to 95%.

1.3.4 Summary

It is apparent that acute alterations in oxygen tension have quite profound effects on the ability of smooth muscle to contract, alone, following electrical stimulation and pharmacological challenge. Smooth muscle responses however are unpredictable and vary significantly depending on the species, type of tissue used and the ambient oxygen tension within the organ bath. These observations may however have important implications for patients with acute exacerbations of asthma.

1.4 The effect of acute alterations in oxygen and carbon dioxide tension on airway tone in animals *in-vivo*

1.4.1 Effects on resting tone

The effect of acute alterations in ambient oxygen and carbon dioxide tensions on airway tone in animals has been of interest since the late nineteenth century, but to date no consensus about their effect has been reached by scientists. The earliest studies by Roy and Brown in 1885[83]and Dixon and Brodie in 1903[84] suggested that asphyxia, hypoxia and hypercapnia caused tracheal and bronchial constriction and that these effects were dependent on intact vagal innervation.

These observations were later revisited by Nadel and Widdicombe who in 1962 again examined the effect of changes in oxygen tension on the lung mechanics of anaesthetised dogs.[85] They showed that in dogs ventilated at a constant rate and tidal volume, lowering the inspired oxygen tension from 90 mm Hg to 37 mm Hg (FiO_2 10%) resulted in a significant increase in total lung resistance ($p<0.01$) and reduction total tracheal volume ($p<0.01$). These changes were not associated with any significant change in pulmonary compliance. In a separate series of studies Nadel and Widdicombe have also considered the effect of sequentially ventilating the dogs with different oxygen tensions (FiO_2 21%, 15% and 10%) to form a dose response curve. From this study they were able to show that the increase in total lung resistance observed with hypoxia was linear and was inversely proportional to the inspired oxygen tension administered to the dogs.[85]

In a similar series of studies the effect of hypercapnia, inhalation of an $FiCO_2$ 80%, was examined.[85] Raising the mean $PaCO_2$ from 32 mm Hg to 66 mm Hg resulted in a

significant increase in total lung resistance and fall in tracheal volume but no change in pulmonary compliance. This was similar to the pattern seen with hypoxia but of a lesser magnitude. It was also shown at this time that the degree of bronchoconstriction was proportional to the level of hypercapnia and that the relationship was linear.[85]

In a further series of studies Nadel and Widdicombe observed that the changes in pulmonary dynamics seen with hypoxia were abolished by vagosympathetic cooling, which resulted in a physiological denervation, and tying off the glossopharyngeal nerves. They suggested that bronchoconconstriction was initiated by hypoxic stimulation of carotid chemoreceptors which subsequently stimulated motor efferents in the vagus nerve causing an increase in smooth muscle tone. The bronchoconstriction seen with hypercapnia was also abolished by cooling the vagosympathetic nerves but unaffected by tying off the glossopharyngeal nerve. Nadel and Widdicombe suggested that hypercapnic constriction was mediated via the vagosympathetic chain. However this cannot be the entire explanation as isolated bronchial rings deprived of their neural innervation and circulating humoral factors also display changes in smooth muscle tone with different oxygen and carbon dioxide tensions.

The findings of Nadel and Widdicombe are in agreement with those of Green and Widdicombe[86] and Loofbourrow et al[87] who also demonstrated an increase in total lung resistance, reduction in tracheal volume and reduction in the airway anatomical dead space when ventilating dogs with an inspired oxygen tension of 10%. They were also unable to demonstrate any significant difference in pulmonary compliance. In

addition they also examined the effect of raising the inspired oxygen tension to 100% and showed a small but significant bronchodilation, observing a reduction in total lung resistance and a rise in both tracheal volume and anatomical dead space. This however was not the findings of Vidruk and Sorkness[88] who were unable to demonstrate any change in the airway tone of dogs ventilated with 10% oxygen. They did however find that approximately 50% of the dogs in their study breathing an inspired oxygen tension of 10% showed an increase in total lung resistance in keeping with bronchoconstriction. In the late eighties and early nineties, Ahmed and Marchette examined the effect of inhaling 13% oxygen on specific lung resistance (SRL) in conscious sheep. Eleven conscious sheep inhaled 13% oxygen balanced with nitrogen or an air sham for 30 minutes. Measurements of airway resistance made at baseline were then repeated. There was no significant difference in specific lung resistance between the groups suggesting that in conscious sheep hypoxia does not affect resting airway tone.[89]

To add to the confusion Wetzel et al[90] examined the effect of inhaling 7% oxygen on airway calibre in anaesthetised minipigs. In this study minipigs were anaesthetised and paralysed before having a tracheostomy fashioned. They were then ventilated sequentially with both hypoxic and normoxic gas mixtures for 15 minutes each, with a period of recovery in between. Airway calibre was assessed using high resolution computed tomography scanning. They confirmed hypoxia by measuring arterial blood gases. In 70 out of 76 airways examined hypoxia resulted in an increase in airway calibre which returned to normal during normoxia. They calculated that this represented a mean fall in airway resistance of 45%. These observations are clearly different from those already reported in earlier studies.

1.4.2 Summary

It is apparent once again that acute alterations in ambient oxygen and carbon dioxide levels have significant effects on airway tone *in-vivo* in animals. There is however considerable variation in the results found by different groups. There appear to be a number of confounding variables between the studies which include differences in the inspired oxygen tension used, differences in the species of animal studied and the presence or absence of drugs used for anaesthesia and paralysis in the animal models. It is therefore difficult to reach firm conclusions about the effect of acute alterations in oxygen and carbon dioxide levels *in-vivo* in animals.

1.4.3 Effects on bronchial reactivity *in-vivo* in animals.

A number of studies in animals have considered the effect of acute alterations in ambient airway oxygen tension on non-specific bronchial reactivity. Vidruk and Sorkness[88] examined the effect of both hyperoxia (PaO_2 344 mm Hg) and hypoxia (PaO_2 45 mm Hg) on histamine induced constriction of tracheal segments in 22 anaesthetised and paralysed mongrel dogs. They found that an inspired oxygen tension of 100% significantly attenuated ($p<0.005$) histamine induced tracheal constriction and that an inspired oxygen tension of 12% significantly potentiated tracheal constriction ($p<0.01$). In subsequent studies following combined cervical vagotomy and bilateral transection of the superior laryngeal nerve in anaesthetised and paralysed mongrel dogs the changes in airway reactivity seen with alterations in inspired oxygen tension were abolished. In a similar study using awake sheep Denjean et al[91] showed a significant increase in bronchial reactivity to metahcholine when sheep inhaled a hypoxic gas mixture (FiO_2 15%) compared to a control group

breathing an air sham (FiO_2 21%). Following peripheral surgical chemodervation the effect of hypoxia on methacholine induced bronchial reactivity was lost.[91] Denjean et al and Vidruk and Sorkness have concluded from their studies that the changes in bronchomotor tone following inhalation of methacholine and histamine at different oxygen tensions are due to reflex interactions between sensory c-fibres and carotid chemoreceptors rather than a direct effect of histamine or methacholine and oxygen on airway smooth muscle tone. Interestingly these observations are at odds with the *in-vitro* findings where isolated bronchial rings deprived of their neural innervation continue to display changes in smooth muscle tone following pharmacological challenge. It is perhaps worth noting that a variety of agents were used in these animals to induce and maintain anaesthesia and paralysis including ketamine, chloralose, urethane and gallamine triethiodide. These drugs may themselves have direct effects on smooth muscle tone and neuromuscular transmission which may have influenced their results.

D'Brot and Ahmed have performed similar studies in conscious sheep which had not been paralysed.[92] They have compared the effect of breathing a hypoxic (FiO_2 13%) and normoxic (FiO_2 21%) gas mixture for 30 minutes on non-specific bronchial reactivity to both inhaled histamine and carbachol. In their first study they found that specific lung resistance (SRL) in sheep after inhaling histamine increased by 337% breathing air and 621% breathing a hypoxic gas mixture ($p<0.01$). Likewise carbachol induced bronchoconstriction was potentiated by hypoxia. Specific lung resistance increased by 342% breathing air and 646% breathing the hypoxic gas mixture ($p<0.01$). In later studies they were able to demonstrate that infusion of cromolyn sodium,[89] which acts to stabilise mast cells and reduce histamine release,

abolished the additional bronchoconstriction produced by hypoxia. In a similar study pretreatment of the sheep with FPL 57231 a novel leukotriene receptor antagonist was also capable of abolishing the effect of hypoxia on histamine induced bronchoconstriction.[92] Potentiation of bronchoconstriction by hypoxia was unaffected by pretreatment of sheep with oral indomethacin suggesting that this mechanism is not mediated via the cycloxygenase pathway.[93] They suggested from these investigations that alveolar hypoxia may stimulate degranulation of mast cell and release of mediators which in turn increases non-specific bronchial reactivity.

1.4.4 Summary

In animals *in-vivo* hypoxia appears to potentiate and hyperoxia attenuate non-specific bronchial reactivity. The mechanism of these effects are less well understood, but both release of local inflammatory mediators and reflex bronchoconstriction following stimulation of carotid chemoreceptors have been suggested as possible causes. These observations may have relevance in the management of patients with acute exacerbations of asthma.

1.5 The effect of acute alterations in inspired oxygen and carbon dioxide tension on airway tone *in-vivo* in man

1.5.1 Effects on resting airway tone

The development and maintenance of airway tone in man is complex.[94] It depends on a number of variables which include circulating humoral factors, neurotransmitters released from nerve endings, substances released locally within the airways which may effect smooth muscle tone and the physical properties of inspired gases. The physical properties of the lungs and airways themselves will also influence airway tone. These properties include the thickness of the airway wall, forces applied by surrounding structures which oppose smooth muscle shortening and the length-tension relationships of the smooth muscle itself. This complex relationship makes airway calibre difficult to measure *in-vivo* and despite considerable research efforts over the years the effect of acute alterations in inspired oxygen tension on airway tone in man remain controversial. This controversy exists because of the wide variation in protocols that have been used to examine this hypothesis. These include different techniques used to induce hypoxia, variable control of hypocapnia, variable levels of inspired oxygen tension administered to subjects and most importantly different techniques used to measure airway calibre.

The earliest studies examining the effect of acute hypoxia on airway tone were stimulated by the prospect of early flight and the effect that hypoxia might have on both pilots and passengers. From studies performed in the 1950's by Milic-Emili and Petit[95] it was observed that acutely lowering the level of inspired oxygen from 50% to 21% to 10% had no effect on airway resistance or dynamic compliance in the lungs of trained healthy volunteers. These observations were in keeping with those of

Goldstein et al[96] who examined the effect of acute hypoxia on lung volumes and maximum expiratory flow volume curves in 15 normal subjects (9 male). In order to determine the effect of hypoxia on small airway function as well he compared the effect of four different gases including air, nitrogen-hypoxia, helium-normoxia and helium-hypoxia. Using a rebreathing method hypoxia was induced and the hypoxic studies were commenced once an oxygen saturation of 75% had been reached. In all studies the end-tidal carbon dioxide levels were maintained at a constant level. In their study Goldstein et al, like Milic-Emili and Petit were also unable to show any significant change in static lung volumes or maximum expiratory flow volume curves when comparing hypoxic and normoxic study days in their subjects. In a study looking at ten mild asthmatic patients Denjean et al,[97] using an inspired oxygen tension of 15% under isocapnic conditions, was also unable to demonstrate any change in dynamic pulmonary compliance or airway calibre in their subjects after they had inspired a hypoxic gas mixture for ten minutes.

These results differ however from the findings of Sterling[98] who has also examined the effect of acutely lowering the inspired oxygen tension, on pulmonary physiology in normal subjects, from 21% through 12% and 10%. Using whole body plethysmography in six normal subjects (3 smokers), who were familiar with the operation of a body box, he demonstrated that lowering the inspired oxygen tension from 21% to 12% and 10% over a period of twenty minutes caused a statistically significant fall in specific airway conductance (*SG*aw) and rise in thoracic gas volume (*V*tg) in keeping with bronchoconstriction. This effect was abolished within minutes of the subjects starting to inspire room air instead of the hypoxic gas mixture. Sterling[98]subsequently showed in a second series of studies that the

bronchoconstrictor effect observed when normal subjects inspired a hypoxic gas mixture (FiO_2 12% and 10%) was completely abolished by the inhalation of nebulised orciprenaline prior to the study but not by the administration of 1.2mg of intravenous atropine. He concluded from these observations that hypoxia must cause bronchoconstriction by a direct action on airway smooth muscle cells of the bronchi as orciprenaline is a smooth muscle relaxant. The failure of atropine to prevent hypoxic bronchoconstriction suggests that the effect is not mediated via vagal nerve fibres within the bronchial tree although it is acknowledged by the authors that 1.2mg of atropine may have been insufficient to completely abolish vagal input. The changes in airway resistance observed in these studies although statistically significant are relatively small and of debatable clinical significance. The findings by Sterling are in keeping with those of Saunders et al[99] who have also investigated the effects of acute hypoxia on lung mechanics. In seven normal subjects (3 smokers) they examined the effect of acute isocapnic hypoxia (FiO_2 40-50 mm Hg) on lung volumes and specific conductance of the lung over a twenty minute period employing a rebreathing method to produce hypoxia in their subjects. Saunders et al like Sterling observed a significant fall in specific lung conductance in keeping with a bronchoconstrictor effect. This was reversed when subjects began to inspire room air again. In addition to the changes in airway resistance they also found significant increases in total lung capacity, residual volume and functional residual capacity in their subjects breathing the hypoxic gas mixture. This observation may reflect premature airway closure in these subjects. It has been suggested by some groups that the apparent increase in airway resistance associated with acute hypoxia can be accounted for by an increase in vocal cord abduction during expiration rather than reflecting a true change in intrathoracic airway calibre. England et al[100] suggested

that in normal subjects isocapnic hypoxia caused a significant increase in expiratory glottic narrowing when compared to normoxic breathing and that this increase in laryngeal airflow resistance was mistakenly interpreted as bronchoconstriction.

To add to the confusion, recent studies have even suggested that acute isocapnic hypoxia may cause tracheal and bronchial dilation in normal subjects. Julia-serda et al[101] examined the effect of acute isocapnic hypoxia on the cross sectional area of the pharynx, extrathoracic trachea, intrathoracic trachea and main bronchi using an acoustic reflection technique in 15 normal volunteers. They sequentially lowered the subjects oxygen saturation to between 80-85% and then 70-75% using a rebreathing method whilst maintaining constant end-tidal carbon dioxide levels. The cross sectional areas of the intra and extra thoracic trachea and the main bronchi increased significantly ($p<0.001$) on the hypoxic study days at both oxygen saturations when compared to the normoxic study days. No significant changes were found in the cross sectional measurements of the pharyngeal and glottic regions. This effect could not be reproduced by acute isocapnic hyperventilation suggesting that changes in the ventilatory pattern alone do not account for these findings.

Very few studies have examined the effect of acute hyperoxia on airway tone in normal subjects. The majority of studies examining the effects of hyperoxia on pulmonary physiology have focused on the pulmonary toxicity of hyperoxia over prolonged periods rather than its acute effects on airway tone. Wollner et al[102] and Inoue et al[103] were unable to demonstrate any significant change in FEV_1 after inspiring oxygen tensions of 100% and 30% respectively over a period of 10 minutes in patients with asthma. Libby et al[104] in a study of 10 asymptomatic non-smokers

were also unable to demonstrate any change in maximum expiratory flow volume curves after breathing 30% oxygen over a period of twenty minutes. A study examining the effect of inhaling a hyperoxic gas mixture ($FiO_2 > 95\%$) for twelve hours, in eight healthy non-smoking males, found a significant fall in forced vital capacity at 4, 8 and 12 hours during exposure which persisted for 12 hours after exposure when compared to the same group breathing air.[105]

Studies have however examined the effect of acute hyperoxia on airway tone in patients with chronic hypoxia. Libby et al[104] studied 12 patients with severe chronic obstructive pulmonary disease (mean FEV_1 25% of predicted) and chronic hypoxia (mean PaO_2 at rest 61 mm Hg) all of whom had a history of heavy cigarette use. All of the patients experienced a significant improvement in their mean expiratory flow volume curves in keeping with bronchodilation after breathing 30% oxygen for a period of twenty minutes. The main conclusions from this work were that chronic hypoxaemia acted as a bronchoconstrictor in patients with chronic obstructive pulmonary disease and that reversal of hypoxia led to significant increases in airway calibre. This confirmed the earlier work of Astin[106] who studied 13 male patients with chronic hypoxia from chronic bronchitis and emphysema. He showed a significant fall ($p<0.001$) in airway resistance after breathing a gas mixture with 30% oxygen for 20 minutes and that the fall in airway resistance correlated strongly with the rise in oxygen saturation and not to changes in arterial carbon dioxide levels. He thought it was likely that this changes was due to a reflex mechanism rather than any direct action of oxygen on smooth muscle itself.

1.5.2 Summary

Once again it is apparent that acute alterations in oxygen tension have effects on resting airway tone in man, but that the observations from a number of studies have produced rather conflicting results. It is difficult to make comparisons between studies because of the differences in study protocols, inspired oxygen tensions and methods used to measure airway calibre.

1.5.3 Effects on non-specific bronchial reactivity

Interest in the effects of acute alterations in oxygen tension on bronchial reactivity to non-specific pharmacological and physical challenges in patients with asthma have been stimulated by observations made in animals that hypoxia may potentiate and hyperoxia attenuate bronchial reactivity. The small number of studies which have examined this effect in patients with asthma have provided conflicting results. The effect of hypoxia and hyperoxia on constrictor and dilator stimuli in patients with asthma may be of importance during acute exacerbations.

Recent studies examining the effect of hyperoxia on methacholine induced bronchoconstriction have provided very different results. Inoue et al[103] suggested that in patients with asthma, breathing an inspired oxygen tension of 30% significantly attenuated the effect of methacholine on airway constriction whereas Wollner et al[102] concluded that an inspired oxygen tension of 100% had no effect on methacholine induced bronchoconstriction. It is difficult to compare these studies as they have used different inspired oxygen tensions and examination of their patient characteristics shows differences in the severity of asthma and the treatment that patients were

receiving. In addition the study by Inoue et al was performed in a single blind fashion and 25% of patients had resting hypoxaemia.

Hyperoxia has also been shown to attenuate exercise induced bronchoconstriction in patients with asthma. The mechanism of this effect remanis controversial. Schiffman et al[107] found that in asthmatic patients who had previously undergone bilateral carotid body resection hyperoxia did not attenuate airway constriction as had been found in asthmatic patients with intact carotid bodies. They suggested that hyperoxia acted directly on carotid chemoreceptors to reduce airway tone and hence oppose the typical bronchoconstrictor effect of exercise. Resnick et al[108] however observed that in asthmatic patients who exercised whilst breathing oxygen (FiO_2 100%) the rise in minute ventilation was smaller than that seen in the same group who exercised breathing air (FiO_2 21%). They concluded that the reduction in airway constriction seen following exercise in patients with asthma who were breathing oxygen was due to a reduction in respiratory heat loss as a direct result of the fall in minute ventilation.

The results of studies examining the effect of acute hypoxia on non-specific bronchial reactivity have also provided conflicting results. In 1988 Denjean et al[97] reported that lowering the inspired oxygen tension to 15%, whilst maintaining isocapnia in ten adults with asthma (4 female), significantly increased airway reactivity to methacholine. Hypoxia itself had no effect on baseline airway tone nor did it alter plasma catecholamine levels. The mechanism of this effect remains unexplained although Denjean et al suggested that hypoxia may act directly on smooth muscle cells to cause constriction, may precipitate release of constrictor mediators from cells

within the airways or cause airway constriction as part of a reflex arc mediated via the carotid chemoreceptors. Interestingly however Tam et al[109] were unable to demonstrate any differences in bronchoconstrictor responses to dry air in 15 patients with asthma whilst breathing a hypoxic gas mixture. It is worth noting however in this study an inspired oxygen tension of 8% was used to produce an oxygen saturation in their subjects of less than 80%. They observed in their subjects a significant increase in heart rate on the hypoxic study day in keeping with a rise in sympathetic output. This rise in sympathetic output to the airways causing bronchodilation may have offset any bronchoconstrictor effect of hypoxia alone which may have been observed with dry air challenge in their subjects.

1.5.4 Summary

In summary, studies have suggested that the properties of inspired gases, particularily oxygen tension, may influence the effects of non-specific bronchial challenge on airway tone in patients in asthma although the results of limited studies performed to date are somewhat controversial. There is evidence to suggest that hyperoxia may well attenuate and hypoxia potentiate bronchial reactivity in patients with stable asthma. Such an observation could have significant implications for patients with asthma who become hypoxaemic during an acute exacerbation.

1.6 Hypothesis and Aims of Current Studies

Ambient oxygen tension has an important role in modulating the effects of constrictor and dilator stimuli on airway smooth muscle tone *in-vitro*[72,73,75-77,80,82] and *in-vivo* in animals.[88,91-93] In patients with asthma little is known about the effect of altering inspired oxygen tension on airway responses to bronchoconstrictor and bronchodilator stimuli and what reports are available have provided conflicting results. In patients with asthma hyperoxia appears to relieve exercise induced bronchoconstriction.[107,108] Airway hyperresponsiveness to methacholine may be attenuated[103] or unaffected by hyperoxia.[102] Eucapnic hypoxia has been shown to enhance airway hyperresponsiveness to methacholine[97]but have no effect on airway response to dry air.[109] To date no consensus has been reached and any comparison between these studies is confounded by differences in study protocols, patients studied and inspired oxygen tensions used. In addition most studies have failed to control adequately for the potential influences of circulating humoral factors or neural innervation of airway smooth muscle.

The hypothesis underlying this thesis is that acute alterations in oxygen tension will have an important role in modulating airway responses to constrictor and dilator stimuli in patients with asthma. Such observations may have relevance in patients admitted to hospital with acute exacerbations of asthma who are often hypoxaemic[67,68] and are given high inspired oxygen tensions to inhale in addition to both nebulised and intravenous bronchodilators. Using a common study design, in our *in-vivo* studies, we will investigate the interaction between oxygen tension and airway responses to constrictor and dilator stimuli in patients with mild stable asthma. We

will investigate the potential influences of circulating humoral factors, carotid chemoreceptor activity, hypocapnia and altered ventilation patterns in our patients.

The aims of our current studies are:

1. To develop a closed breathing circuit capable of delivering tightly controlled inspired oxygen tensions to patients with mild asthma.
2. To determine a level of inspired oxygen which produces moderate hypoxaemia, in our patients with asthma, without the unwanted effects of increased minute ventilation or altered carotid chemoreceptor activity which may accompany breathing low and high inspired oxygen tensions.
3. To investigate the effects of hypoxia (O_2 tension 4%), normoxia (O_2 tension 20%) and hyperoxia (O_2 tension 95%) on airway tone in human isolated bronchial rings following incubation with histamine, methacholine and salbutamol.
4. To investigate the effects of breathing high and low inspired oxygen tensions on methacholine and histamine induced bronchoconstriction in patients with mild stable asthma.
5. To investigate the effects of breathing high and low inspired oxygen tensions on salbutamol induced relaxation of airway tone in patients with asthma.

As we have stated such observations may have relevance in the management of patients with acute exacerbations of asthma who are often hypoxaemic and receive high inspired oxygen tensions to breathe in addition to both nebulised and intravenous bronchodilators following admission to hospital.

CHAPTER 2

GENERAL METHODS AND MEASUREMENTS

2.1 Subjects

Normal subjects were recruited from the medical staff within the Department of Respiratory Medicine of the West Glasgow Hospitals University NHS Trust. All were non smokers and none were receiving any routine medication. None of the normal subjects had any significant cardiovascular or respiratory past medical history. Asthmatic patients were recruited directly from the asthma clinic of Professor N C Thomson or from a patient database held within the Asthma Research Unit of the West Glasgow Hospitals University NHS Trust. Summary details of normal subjects and patients for each study are provided in table form within the relevant chapters.

2.2 Spirometry

Measurements of forced expiratory volume in one second (FEV_1) were made using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) which was calibrated prior to use on each study day. With the patient sitting comfortably the best FEV_1 from three attempts was recorded. Volumes were corrected for body temperature, pressure and saturation of water vapour (BTPS). Predicted values were taken from Knudsen et al.[110]

2.3 Measurement of heart rate, oxygen saturation, respiratory rate, inspired and expired oxygen and carbon dioxide levels

Heart rate and oxygen saturation were measured using a pulse oximetry probe placed on the subjects index finger (Datex Division of Instrumentarium Corp, Helsinki, Finland). A side port on the facemask worn during the study, allowed continuous sampling of the subjects inspired and expired gases and measurement of respiratory rate. The gases were analysed using an OSCARoxy TM multigas monitor (Datex of

Instrumentarium Corp, Finland, Helsinki). The multigas gas analyser was calibrated during each study using gases of predetermined concentration supplied by Datex of Instrumentarium Corp. Recordings of each of the above observations were made every ten seconds for one minute at predetermined time points during study days. The recordings were automatically printed by a Hewlett Packard Think Jet printer in a blind fashion for analysis after the study had been completed.

2.4 Blood pressure

Systolic, diastolic and mean arterial blood pressure were measured using a semi-automatic sphygmomanometer (Dinamap®, 1846 FX vital signs monitor, Critikon, Berkshire, UK). The results of three similar readings at each time point were averaged to give a single value which was subsequently used for statistical analysis. Recordings were made every 3 minutes throughout the study in chapter 4.

2.5 Methacholine and histamine inhalation challenges

Patients performed methacholine and histamine inhalational challenges at the same time of day during each study. Patients were asked to withhold their short acting β_2-agonists for 8 hours and long acting β_2-agonists for 24 hours, prior to the study day. Slow release oral theophyllines were withheld for 48 hours before each day. Inhaled corticosteroids were continued as normal throughout the study period. Bronchial reactivity to methacholine and histamine was measured according to the technique described by Cockcroft et al and Hargreaves et al.[111,112] Baseline FEV_1 was measured until reproducible within 5%. Thereafter a saline inhalation challenge was administered and FEV_1 measured at 30, 90 and 180 seconds. The lowest post-saline

value was taken as a baseline from which subsequent falls were measured. Doubling doses of nebulised histamine [histamine diphosphate monohydrate (Fluka Chemie, Gillingham, Dorset) made up in phosphate buffered normal saline] or methacholine [acetyl-ß-methyl choline chloride (Sigma Chemical Company, Poole, Dorset, UK) made up in phosphate buffered normal saline] were then administered at five minute intervals. Each concentration was given for two minutes via a nebuliser (Micro-cirrus nebuliser, Intersurgical Ltd, Crane House, Wokingham, Berkshire) driven at a flow rate of 6 or 7 litres per minute by oxygen (100%), air (21%) or a hypoxic gas mixture (15%), from dedicated cylinders (British Oxygen Corporation, Medical gases) with an output of 0.12 or 0.11 mls/min. The aerosol solution from the micro-cirrus nebuliser (Intersurgical Ltd, crane House, Wokingham, Berkshire) was delivered to the patient through a port in the tight fitting aircraft face mask worn on all study days by the patients. The FEV_1 was measured at 0.5, 1.5 and 3 minutes after each nebulisation until a fall in FEV_1 of at least 20% was achieved as determine by linear interpolation from the logarithmic dose-response curve. This result was then expressed as the histamine or methacholine PC_{20} (provocation concentration 20%). At the end of each inhalation challenge patients received nebulised salbutamol to reverse any persisting bronchoconstriction. The histamine and methacholine solutions were made up by the sterile unit of the West Glasgow Hospitals University NHS Trust pharmacy department.

2.6 Oxygen breathing circuit

Precise inspired oxygen tensions for the patients to breathe were generated by passing oxygen (G size cylinders 3400 litres capacity from British Oxygen Corporation, Medical gases) and nitrogen (British Oxygen Corporation, Medical gases) contained

in separate cylinders, through a Quantiflex air/oxygen flowmixer (model A-O, Cyprane Ltd, Keighly, Yorkshire). The gases generated from the flowmixer were passed via elephant tubing to a calibrated flowhead and a five litre rebreathing bag. Elephant tubing then ran from the rebreathing bag to a two way breathing valve attached to a tight fitting aircraft facemask (Thomas Respiratory Systems, London) which the patient breathed from throughout the study day. Dry gases which had not been humidified were used for all of the *in-vivo* studies. On study days patients, prior to performing an FEV_1, were asked to take a full inspiratory breath from the 5 litre rebreathing bag. The facemask was removed and patients blew into the spirometer before having the facemask reattached before performing the next FEV_1. This minimised the amount of time patients were breathing ambient air.

2.7 Nebuliser output

The nebuliser output was calculated at different flow rates for each oxygen tension (O_2 100%, 21% and 15%) used in the *in-vivo* studies, to ensure that the nebuliser output was the same for each study gas. The nebuliser (Micro-cirrus nebuliser, Intersurgical Ltd, Crane House, Wokingham, Berkshire) was driven by oxygen of different tensions from dedicated cylinders (British Oxygen Corporation, Medical gases). Prior to the *in-vivo* studies the nebuliser output was determined at flow rates of 5, 6, 7 and 8 litres per minute for oxygen tensions of 100%, 21% and 15%. The nebuliser was filled with 5 mls of normal saline and weighed. After 2 minutes of nebulisation the nebuliser was weighed again and the output in mls/min was calculated. This manoeuvre was repeated for each flow rate at each oxygen tension (figure 2.1).

2.8 Adrenaline and noradrenaline assays

All hormone assays were performed by Dr J Morton of the Department of Medicine and Therapeutics in the West Glasgow Hospitals University NHS Trust. 5mls of venous blood were withdrawn, from a venous catheter, into chilled lithium heparin tubes. These samples were kept on ice until centrifugation at 4° centigrade following completion of the study day. Plasma adrenaline and noradrenaline levels were then measured using reverse phase high performance liquid chromatography and electrochemical detection after extraction of adrenaline and noradrenaline from plasma using activated alumina as described by Goldstein et al.[113] The coefficient of variation for this technique is less than 10% and normal values are < 5.0 nmol/l and < 0.4 nmol/l for noradrenaline and adrenaline respectively.

2.9 Drugs

Salbutamol: Ventolin® solution for nebulisation. Concentrations of 0.05mg/ml, 0.17mg/ml and 5.0mg/ml were prepared by the sterile supplies unit of the West Glasgow Hospitals University NHS trust in 3.0ml ampuoles.

Histamine: histamine diphosphate monohydrate (Fluka Chemie, Gillingham, Dorset, UK) for inhalational challenge made up in phosphate buffered saline to pH 7.4 was prepared by our pharmacy sterile supplies department.

Methacholine: acetyl-ß-methyl choline chloride (Sigma Chemical Company, Poole, Dorset, UK) for inhalational challenge made up in phosphate buffered saline to pH 7.4 was prepared by our pharmacy sterile supplies department.

2.10 Statistical analysis

Statistical analysis for the *in-vivo* and *in-vitro studies* were performed using an Apple Mackintosh LC II computer. For the *in-vivo* studies a Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabasas) was used. For the *in-vitro* studies statistical analysis was performed using a minitab statistical package. For all studies a p value of < 0.05 was accepted as significant. Specific details relating to the statistical analysis are contained within the methods section of each paper.

2.11 Ethical approval

All of the study protocols described in this thesis had prior approval of the ethical committee for the West Glasgow Hospitals University NHS Trust. All of the patients gave written informed consent to the studies they participated in.

The raw data for this chapter is included in the Appendix (table 1A)

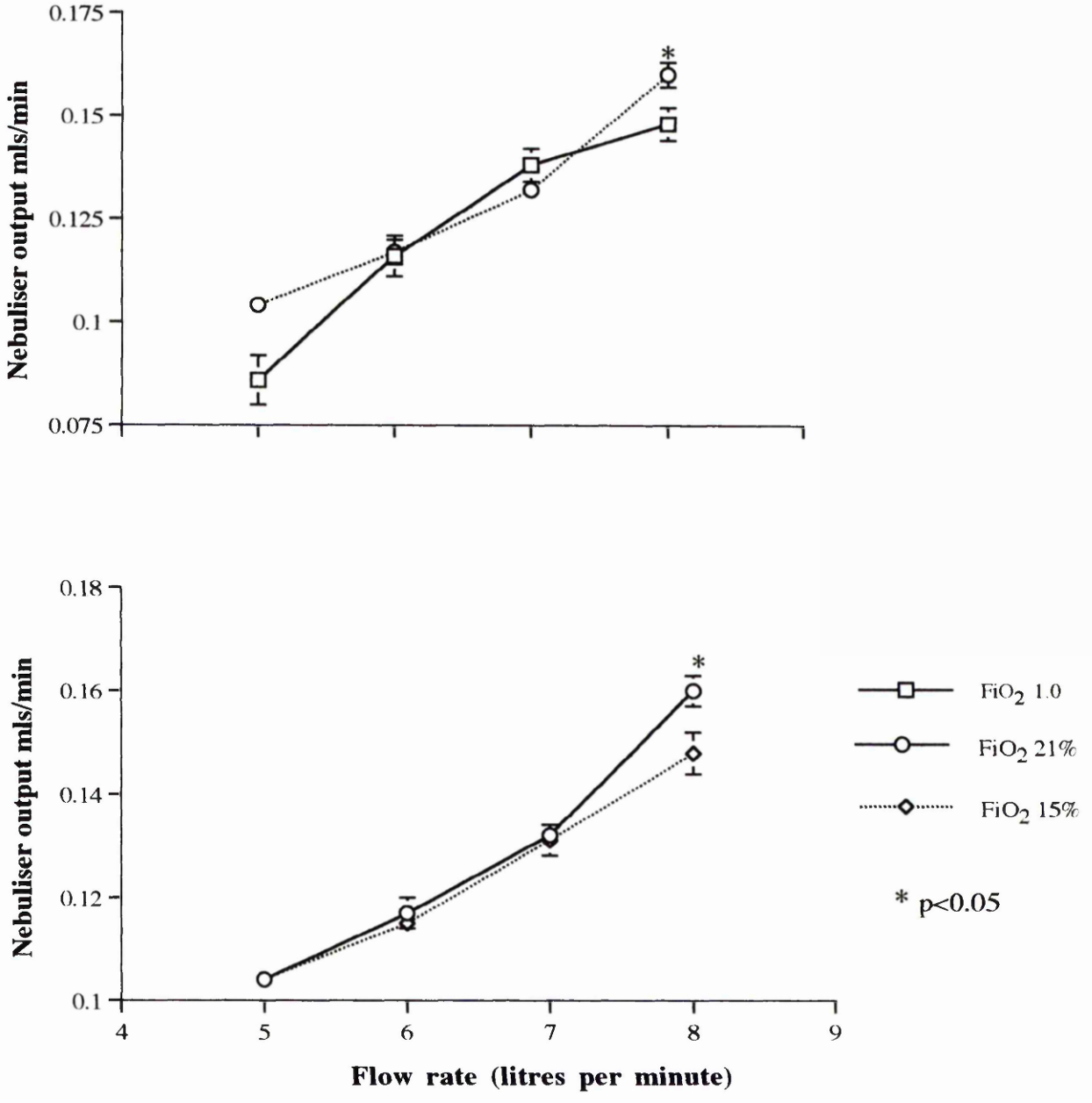

Fig 2.1 The nebuliser output (mls/min) of a micro-cirrus nebuliser at flow rates of 5, 6, 7 and 8 litres per minute using oxygen tensions of 15%, 21% and 100%

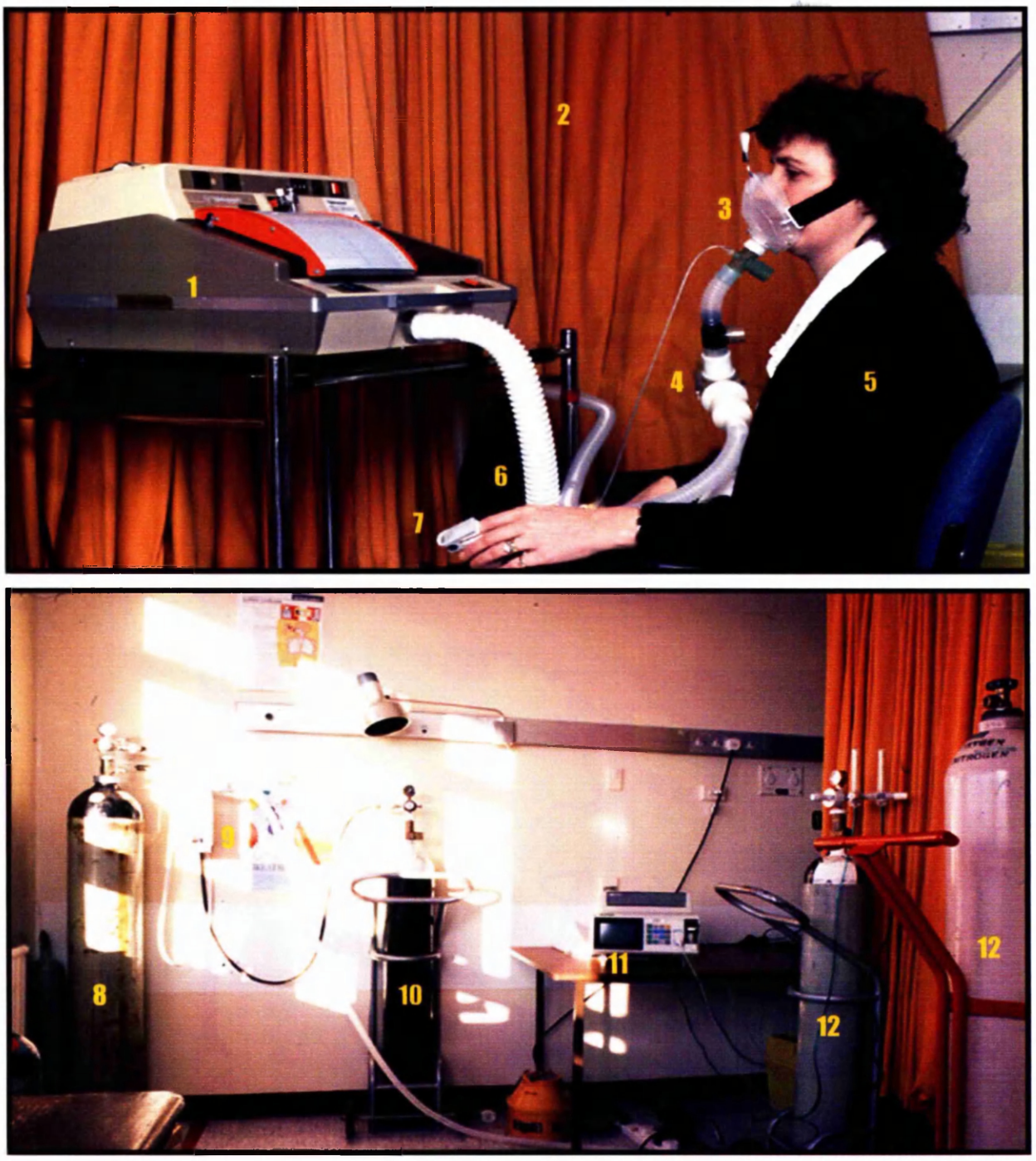

Illustration 1: Picture of asthma subject using the oxygen breathing circuit.

Key:

1. Dry Wedge Spirometer
2. Curtain for blinding
3. Aircraft Facemask
4. Two-way Breathing Valve
5. Subject
6. 5 litreRebreathing Bag
7. Pulse Oximetry Probe
8. Nitrogen Cylinder
9. Quantiflex air/oxygen Flowmixer
10. G Size Oxygen Cylinder
11. OSCARoxy TM Multigas Monitor
12. Oxygen Cylinders to Drive Nebulisers

CHAPTER 3

THE EFFECT OF ACUTE ALTERATIONS IN OXYGEN TENSION ON AIRWAY RESPONSES TO METHACHOLINE, HISTAMINE AND SALBUTAMOL IN HUMAN ISOLATED BRONCHIAL RINGS

3.1 Introduction

Our first study was stimulated by *in-vitro* observations we had made in our own laboratory which had suggested that acute alterations in ambient oxygen tension significantly affected the response of airway smooth muscle to both bronchoconstrictor and bronchodilator stimuli.[75,76,78] We had found that hypoxia (4% O_2) potentiated the constrictor effects of both methacholine[75] and endothelin-1[76] in bovine isolated bronchial rings, but attenuated the response to methacholine in rat isolated bronchial rings.[78] In bovine isolated bronchial rings the ability of salbutamol to relax airway smooth muscle preconstricted with methacholine was significantly attenuated by hypoxia (4% O_2) and potentiated by hyperoxia (95% O_2) when compared the normoxia (O_2 20%).[75] We were aware that if these observations could be demonstrated in man then they might have relevance in the management of patients with acute exacerbations of asthma admitted to hospital who are commonly hypoxaemic. As part of their routine management such patients receive high inspired oxygen tensions to inhale as well as nebulised and intravenous bronchodilators. This first study was designed therefore to examine the effect of acute alterations in ambient oxygen tension on histamine and methacholine induced constriction and salbutamol induced relaxation of human isolated bronchial rings.

3.2 Materials and methods

Tissue collection and preparation

Macroscopically normal human bronchi (2nd to 4th order, internal diameter 3-5mm) were obtained from non-asthmatic patients undergoing elective thoracic surgery. Tissues were dissected free of connective tissue and fat and stored overnight at 4°C in oxygenated Krebs-Henseleit solution of the following composition (mM), NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2 and glucose 11.1. Published data has shown that the overnight storage of this tissue does not alter its reactivity.[114]

Measurement of contractile responses

Bronchial rings were suspended in vertical organ baths (10ml) at 37(+/-0.5)^{0}C in oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution. Resting tension of 2g wt was applied via two stainless steel wires inserted into the lumen. One wire was anchored and the other attached to a force displacement transducer (Grass FT03T) to measure isometric changes in tension. Tissues were allowed to equilibrate for 45 minutes, during which time tension was reapplied where necessary. The concentration of O_2 in the gas mixture was reduced from 95% to 20% or 4% by substituting O_2 with nitrogen. The oxygen tension of the solution was measured directly using a Jencons oxygen probe placed in the organ bath. Concentrations of O_2 reached steady state in the organ baths within 2-3 minutes. Gas mixtures containing 95%, 20% and 4% O_2, produced O_2 tensions of 524, 147 and 26 mm Hg, respectively. The pH was buffered to 7.4 by the inclusion of 5% CO_2. A Mettler pH meter was used to measure the pH of the solution in the organ baths. The oxygen tension and pH of each organ bath was maintained at the appropriate value by altering the gas flow where necessary. Prior to the methacholine and histamine studies

the viability of each ring was ascertained by the addition of a single concentration (10^{-4} M) of methacholine to evoke a reference contractile response. Once the response had reached a plateau the vessels were then washed 3 times with Krebs-Henseleit solution and allowed to return to baseline tensions. Tensions were then adjusted if necessary and the vessels left for a further 45 minutes before the addition of any other drugs. In the histamine and methacholine studies cumulative concentration-response curves (CCRCs) to histamine (10^{-9}-$3x10^{-4}$M) and methacholine (10^{-9}-$3x10^{-4}$M) were constructed in each of the O_2 tensions. In the case of methacholine, three consecutive CCRCs each in a different O_2 tension were conducted on the same bronchial ring. To avoid tachyphylaxis, only one concentration-response curve was conducted to histamine in each tissue, therefore the three different oxygen tensions were imposed upon three separate bronchial rings. In the salbutamol study tissues were pre-incubated with $3x10^{-6}$mol/l methacholine (approximately the EC_{50} for methacholine in this tissue) and the contraction allowed to reach a plateau. Cumulative concentration-response curves were then constructed for salbutamol (10^{-9} - 10^{-4} mol/l). To avoid tachyphylaxis, only one concentration-response curve was conducted to salbutamol in each tissue, therefore once again three different oxygen tensions were imposed upon three separate bronchial rings. In each experiment one tissue acted as a time control to ensure that the methacholine-induced contraction was maintained. The orders in which the gas mixtures were used were randomised between study days.

Materials

Histamine, methacholine and salbutamol were all obtained from Sigma Chemical Co, United Kingdom. The concentration in the text refers to the salts, with the exception of

salbutamol which is expressed as the base. Stock solutions of drugs were prepared in distilled water and subsequent dilutions made in Krebs-Henseleit solution.

Statistical analysis

Number of observations (n) refers to the number of individual patients from whom tissue was obtained and in each case, n=8. Results for the methacholine and histamine studies are expressed as the mean maximum response (mg wt) in each of the three oxygen tensions. The salbutamol results are expressed as the mean (SEM) percentage of the control mean maximum inhibition in hyperoxia. Statistical analysis was performed using a Minitab package. Significance between data points (mean maximum responses) was calculated by Students t- test. A probability of $p<0.05$ was considered to be statistically significant.

3.3 Results

Methacholine *in-vitro*

Methacholine-evoked contractile responses in human isolated bronchial rings in a concentration-dependent manner. In both hyperoxia and normoxia responses to methacholine were initiated at between 10^{-9} and $3x10^{-9}$M and between $3x10^{-8}$M and 10^{-7} in hypoxia. Reducing the oxygen fraction in the gas mixture from 95% to 20% significantly attenuated the contractions evoked by methacholine. Lowering the oxygen tension further, from hyperoxic to hypoxic levels produced a further reduction in the contractile response to methacholine (responses in hyperoxia were significantly $p<0.001$ greater than responses in hypoxia). Responses to methacholine in normoxia were significantly $p<0.01$ greater than in hypoxia. The maximum response to methacholine in hyperoxia was not significantly different from the maximum response in normoxia (mean (SEM) maximum response to methacholine in hyperoxia at the 10^{-4}M level; 2015 ± 504 mg wt, in normoxia at the 10^{-4}M level; 1469 ± 609 mg wt). The mean (SEM) maximum response in hypoxia however was significantly less than in hyperoxia ($p<0.01$) and normoxia ($p<0.05$). The mean (SEM) maximum response in hypoxia at the 10^{-4} level was; 816 ± 321 mg wt. In the time control studies there was no significant differences between the three consecutive concentration-response curves to methacholine in hyperoxia indicating that the contractile responses to methacholine in this tissue were not altered by time (figure 3.1).

Histamine *in-vitro*

Histamine induced concentration-dependent contractions of the human isolated bronchial rings. In each of the oxygen tensions, responses to histamine were initiated at between 10^{-7} M and $3x10^{-7}$ M, indicating that changes in oxygen tension did not alter the sensitivity of the tissue to histamine. Throughout the whole concentration-response curve, contractions evoked by histamine were significantly ($p<0.01$ and $p<0.001$ for data sets, respectively) greater in hyperoxia than in normoxia or hypoxia, and responses in normoxia were significantly ($p<0.05$ for data sets) greater than in hypoxia (figure 3.2). There was however no significant difference in the mean maximum response mg wt (at a concentration of $3x10^{-4}$M) between hyperoxia: 1995 ± 509.7 mg wt and normoxia 1076.7 ± 347.4 mg wt, or between the maximum response in normoxia and hypoxia 683.3 ± 199.2 mg wt. The maximum response in hyperoxia, however, was significantly ($p<0.05$ for data points) greater than the maximum response in hypoxia (figure 3.2).

Salbutamol *in-vitro*

Salbutamol evoked a marked, concentration dependent reversal of the methacholine-induced contraction. Under hyperoxic and hypoxic conditions, responses to salbutamol were initiated at between 3×10^{-9} mol/l and 10^{-8} mol/l and between 10^{-9} mol/l and 3×10^{-9} mol/l in normoxia. Lowering the oxygen tension from 95% to 20% oxygen did not significantly alter the ability of salbutamol to reverse methacholine-induced tone (mean maximal inhibition at 10^{-4} mol/l: 103.02 ± 9.04 % in hyperoxia and 74.07 ± 11.65 % in normoxia, n=8). In contrast, responses evoked by salbutamol

were significantly ($p<0.001$ for data sets) attenuated in hypoxia when compared to hyperoxia (mean maximal inhibition at 10^{-4} mol/l: 103.02 ± 9.04 % in hyperoxia and 59.36 ± 7.85 % in hypoxia, $p<0.01$ for all data points). Responses to salbutamol were also significantly ($p<0.01$ for data sets) less in hypoxia compared with normoxia, although there was no significant difference between the maximum responses (mean maximal inhibition at 10^{-4} mol/l: 74.07 ± 11.65 % in normoxia and 59.36 ± 7.85 % in hypoxia) (figure 3.3). The absolute amount of isometric tension (mg wt) evoked by a single concentration of methacholine did not differ significant between the three oxygen tensions. The absolute amount of isometric tension generated by a single dose of methacholine in each oxygen tension prior to incubation with salbutamol was: hyperoxia 761.3 ± 178.9 mg wt, normoxia 613.8 ± 156.2 mg wt and hypoxia 483.5 ± 149.9 mg wt.

3.4 Discussion

Our initial *in-vitro* studies have shown that acute hypoxia (O_2 tension 4%) significantly attenuates the ability of both histamine and methacholine to constrict human isolated bronchial rings when compared to normoxia (O_2 20%) and hyperoxia (O_2 95%). In addition the ability of the bronchodilator salbutamol to relax human isolated bronchial rings is also significantly attenuated by hypoxia (O_2 4%) when compared to normoxia (O_2 20%) and hyperoxia (O_2 95%).

The effect of hypoxia on histamine and methacholine induced constriction of airway smooth muscle in human isolated bronchial rings that we have found, differs from our original observations in bovine isolated bronchial rings constricted with methacholine and endothelin-1[75,76] Such a fundamental difference in results is difficult to explain when we consider that the studies were undertaken in the same laboratory using identical materials, methods and oxygen tensions. This suggestion is perhaps supported by original observations in rat isolated bronchial rings which also suggested attenuation of methacholine induced contractions by hypoxia.[78] It would appear therefore that bovine airway smooth muscle behaves differently in hypoxia when compared to rat and human tissue.

Our results are however in keeping with several other groups who have reported hypoxic relaxation following non-specific challenge in airway smooth muscle from several other species.[72,73,80,82] While there is agreement that hypoxia causes impairment of airway smooth muscle constriction following non-specific challenge, considerable debate still rages over the mechanism of the effect and whether or not it is linear. Stephens et al[72,73] have suggested that hypoxia impairs mitochondrial

function which impairs intracellular energy release and reduces the ability of airway smooth muscle to generate contractile forces. Vannier et al[80] have suggested that the effect is linear with greatest relaxation at the lowest oxygen tensions. His group also demonstrated that hypoxic relaxation of porcine airway smooth muscle constricted with carbachol was abolished when the bronchial rings were incubated with BAY K 8644, a L-type Ca2+ channel agonist suggesting that hypoxia impairs calcium entry into cells. They however were unable to show that the effect was abolished by removing the epithelium from the bronchial rings as had been shown by Gao and Vanhoutte.[82] Gao and Vanhoutte have suggested that the bronchial epithelium released an unidentified relaxing factor in response to hypoxia.

The observation of hypoxic relaxation has not been observed *in-vivo*.[88,91,92] Several groups have suggested that hypoxia potentiates airway smooth muscle contraction following pharmacological challenge. Suggested mechanisms include local release of inflammatory mediators[89,92,93] and reflex bronchoconstriction as an indirect effect of hypoxia on carotid and peripheral chemoreceptors.[88,91] It is worth noting that our bronchial rings are deprived of their normal circulating humoral factors and neural innervation allowing us to observe the true direct effects of hypoxia on airway smooth muscle. It must however be remembered that the ambient oxygen tensions used are significantly lower *in-vitro* than *in-vivo* and that this may influence our results. Further *in-vitro* studies employing a range of oxygen tensions would allow us to form dose response curves to various constrictor stimuli at oxygen tensions which are similar to those we have used in our *in-vivo* studies.

Our *in-vitro* studies have also shown that hypoxia impairs the ability of salbutamol to relax airway smooth muscle in human isolated bronchial rings. This has not previously been reported in the literature. It is however in keeping with our own *in-vitro* observations in bovine isolated bronchial rings.[75,77]

In our study we have used a single concentration of methacholine (10^{-6}M) to preconstrict our bronchial rings prior to the addition of salbutamol. Although we have already suggested that hypoxia attenuates methacholine induced constriction in human isolated bronchial rings this effect is observed only when the full concentration-response curves are compared. No significant difference was observed in maximum isometric tension developed between each oxygen tension when a single concentration of methacholine was added to the organ baths. We have not therefore made any adjustment to baseline tension prior to the addition of salbutamol. If however our hypoxic bronchial rings were less constricted at baseline then this may have contributed to our observation that salbutamol induced relaxation of human isolated bronchial rings is attenuated by hypoxia.

Our result is however somewhat surprising as it might be anticipated, that if hypoxia causes impairment of airway smooth muscle contraction as we have previously suggested then the ability of salbutamol to relax airway smooth muscle should if anything be augmented by hypoxia. It would seem likely therefore that hypoxia has other effects which impair the ability of salbutamol to relax airway smooth muscle. Hypoxia has been reported to cause down regulation of cardiac ß-receptors[115] and uncoupling of ß-adrenoceptors from their regulatory G-proteins.[116] Both of these observations could explain our *in-vitro* findings. Hypoxia also induces opening of

membrane-bound potassium channels in isolated bronchial rings.[117] Salbutamol mediates smooth muscle relaxation by opening these channels.[118] If the majority of these channels were already open, as they almost certainly would be at an ambient oxygen tension of 4%, the effectiveness of salbutamol to relax the bronchial rings would be significantly impaired. Our *in-vitro* observations may have particular relevance to hypoxaemic patients with acute exacerbations of asthma who receive nebulised and intravenous bronchodilators. It however must be remembered that we have used very profound levels of hypoxia in our *in-vitro* studies, which could not be used to *in-vivo* in patients with asthma. However our *in-vitro* findings to date, suggest that it would be interesting to examine the effect of acute alterations in oxygen tension on both constrictor and dilator stimuli in patients with asthma.

In conclusion our initial *in-vitro* studies have suggested that hypoxia significantly attenuates methacholine and histamine-induced constriction and salbuatmol induced relaxation in human isolated bronchial rings.

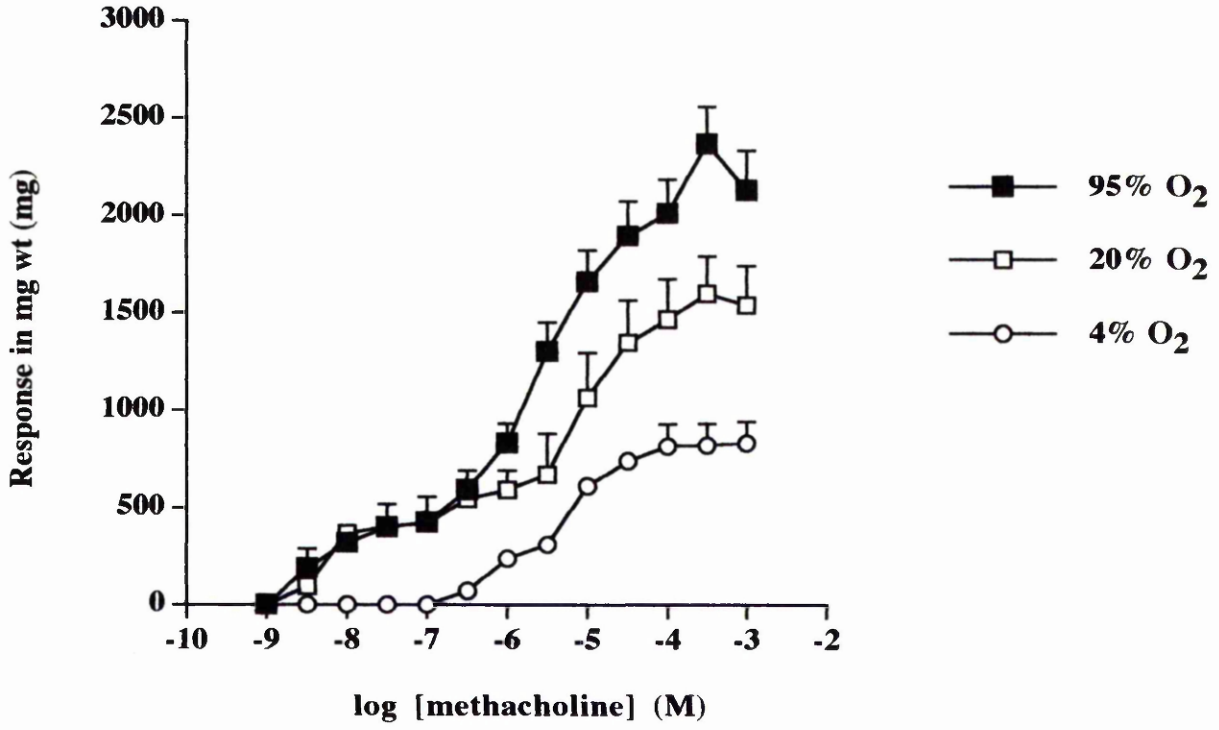

Fig 3.1 Contractions evoked by methacholine in human isolated bronchi in hyperoxia, normoxia and hypoxia. Methacholine was added cumulatively to give final bath concentrations of 10^{-9} x $3x10^{-4}$M. Responses are expressed in mg wt. Methacholine was significantly ($P<0.05$ and $P<0.001$ respectively) more effective in hyperoxia than in normoxia and hypoxia and significantly ($P<0.01$) more effective in normoxia than in hypoxia. Number of observations (n)=8 in each case.

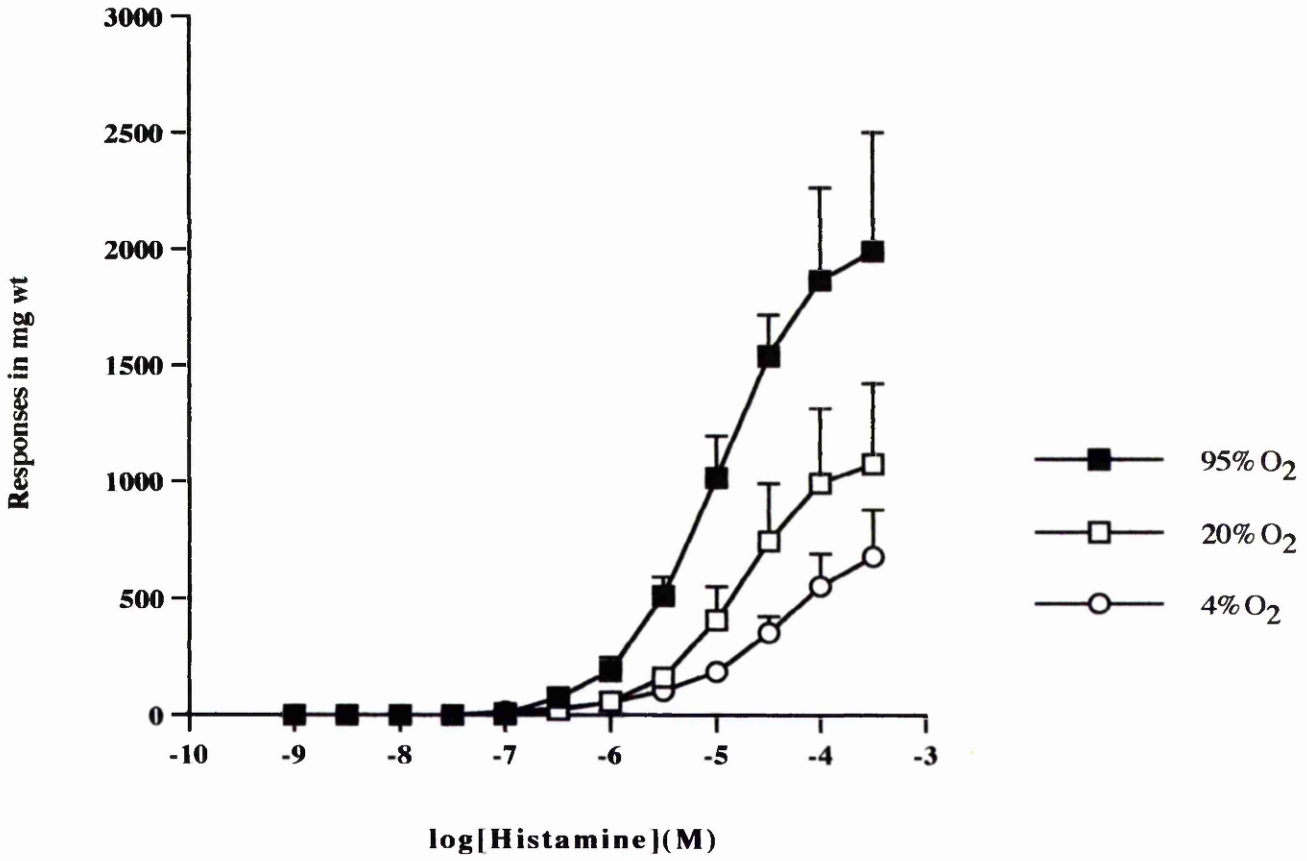

Fig 3.2 Contractions evoked by histamine in human isolated bronchi in hyperoxia, normoxia and hypoxia. Histamine was added cumulatively to give final bath concentrations of 10^{-9} x $3x10^{-4}$M. Responses are expressed in mg wt. Histamine was significantly ($P<0.01$ and $P<0.001$ respectively) more effective in hyperoxia than in normoxia and hypoxia and significantly ($P<0.05$) more effective in normoxia than in hypoxia. Number of observations (n)=8 in each case.

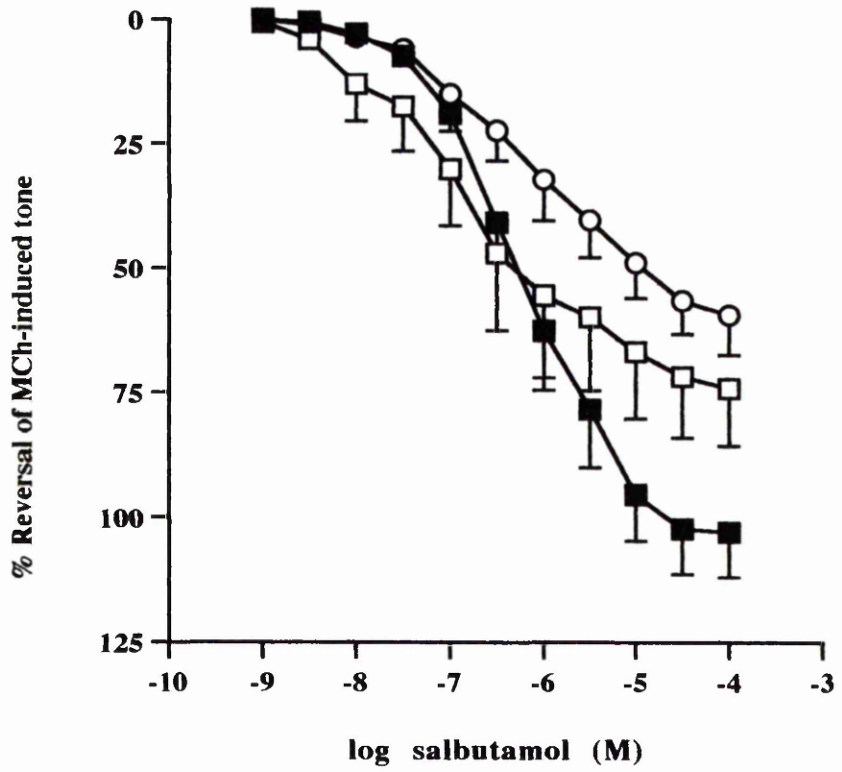

Fig 3.3 The ability of salbutamol to reverse methacholine-induced tone in human isolated bronchi in hyperoxia, normoxia and hypoxia. Salbutamol was added cumulatively to give final bath concentrations of 10^{-9} - 10^{-4}M. Responses are expressed as a percentage reversal of the methacholine contraction. Salbutamol was significantly ($P<0.001$) more effective in hyperoxia than in hypoxia and significantly ($P<0.01$) more effective in normoxia than in hypoxia. Number of observations (n)=8 in each case.

CHAPTER 4

THE EFFECT OF ACUTE CHANGES IN INSPIRED OXYGEN TENSION ON VENTILATORY AND CARDIOVASCULAR RESPONSES IN NORMAL SUBJECTS USING A NOVEL CLOSED BREATHING CIRCUIT

4.1 Introduction

The effects of acute alterations in inspired oxygen tension, on cardiovascular and respiratory responses, have been extensively studied in both animals and man for more than one hundred years.[86,119-122] Despite the considerable resources which have been employed in these investigations, controversy about the effects still exist in some areas, particularly the effects of hypoxia and hyperoxia on airway tone *in-vivo* in man.[95-99,101] For the purposes of this thesis our interest lies not in the direct effect of acute alterations in oxygen tension on airway smooth muscle but the effects of acute hypoxia and hyperoxia on airway smooth muscle tone following inhalation of constrictor and dilator stimuli in patients with asthma. This interest has been stimulated by *in-vitro* observations from our own laboratory that suggest that the ambient oxygen tension of organ baths can significantly alter the effects of constrictor and dilator stimuli in human (chapter3), bovine and rat isolated bronchial rings.[75-78] We have hypothesised that acute alterations in oxygen tension may have an important role *in-vivo* in modulating the action of bronchoconstrictor and bronchodilator stimuli on airway tone and that this may have particular relevance to patients suffering an acute exacerbation of asthma. We have therefore designed a closed breathing circuit which allows us to closely control the inspired oxygen tension we can deliver to patients with asthma. The aim of our initial studies was to ensure that our closed breathing circuit was capable of producing and maintaining either a hypoxic or hyperoxic environment in which to conduct our future *in-vivo* investigations. In addition we wished to determine a level of inspired oxygen that would produce significant hypoxaemia in our patients without the unwanted additional effects of increased sympathetic activity which can accompany hypoxaemia[86,119-121] and which may itself have indirect effects on airway tone which could influence the results of

our *in-vivo* studies.

4.2 Subjects and methods

Subjects

In the first study looking at the effect of hypoxia on cardiovascular and respiratory responses, seven non-smoking healthy subjects (all male) with a mean (SD) age of 31 (8.5) years were recruited from the staff of the respiratory unit in Gartnavel General Hospital. In a second study looking at the effects of hyperoxia on cardiovascular and respiratory responses, eight non-smoking healthy subjects (6 males) with a mean (SD) age 33 (3) years were also recruited from the staff of the respiratory unit. All subjects had an FEV_1 greater than 80% of predicted and no evidence of airflow obstruction. None of the subjects had a history of respiratory or cardiovascular disease and none were taking any routine medication. All of the subjects gave informed consent to the study which had previously been approved by the West Ethics Committee.

Study design

Study1: Hypoxia in normal subjects

Subjects attended the laboratory on two study days. On the first study day simple spirometry was performed and a full medical history obtained. On a subsequent study day after 30 minutes of supine rest subjects were commenced on our closed breathing circuit (chapter 2.6). After 10 minutes breathing air (FiO_2 21%) baseline measurements of oxygen saturation (SaO_2%), heart rate (HR), respiratory rate (RR), inspired oxygen and carbon dioxide levels (insp O_2%, insp CO_2%), end-tidal oxygen and carbon dioxide levels ($PETO_2$%, $PETCO_2$%) and blood pressure (BP) were made. Subjects then inhaled inspired oxygen tensions of 21%, 18%, 15% and 12% sequentially for 12 minutes at each level in a single blind fashion.

Study 2: Hyperoxia in normal subjects

Subjects attended the laboratory on two study days. On the first study day simple spirometry was performed and a full medical history obtained. In a second study following 30 minutes of supine rest patients breathed air (FiO_2 21%) for 10 minutes through the closed breathing circuit following which the baseline measurements already described were made. The subjects then inhaled oxygen (FiO_2 100%) or air (FiO_2 21%) for 15 minutes each in a randomised double blind fashion. The measurements made at baseline were repeated every 3 minutes until completion of the study day. The oxygen and nitrogen cylinders and mixing valve were obscured from the subjects vision and the inspired oxygen tension was controlled by a second operator. The measurements made at baseline were repeated every 3 minutes until completion of the study day.

Measurements

Systolic, diastolic and mean arterial blood pressure were measured using a semi-automatic sphygmomanometer (Dinamap®, 1846 FX vital signs monitor, Critikon, Berkshire, UK) – see chapter 2.4.

Heart rate and oxygen saturation were measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) – see chapter 2.3.

Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) – see chapter 2.3.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) – see chapter 2.2.

Statistical analysis

Statistical analysis for this study was performed using an Apple Mackintosh LC II computer. A Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabasas) was used. Analysis of Variance (ANOVA) corrected for multiple comparisons was used to compare differences between similar time points at each inspired oxygen tension and differences from baseline at each time point. A p value of < 0.05 was accepted as significant.

4.3 Results

Study 1 Hypoxia in normal subjects

Oxygen saturation: Oxygen saturation was significantly lower ($p<0.01$) at 3, 6, 9 and 12 minutes breathing FiO_2 15% and 12% compared to baseline and inspired oxygen tensions of 21% and 18%. The oxygen saturation breathing FiO_2 12% was also significantly lower at 3, 6, 9 and 12 minutes compared to the inspired oxygen tension 15%. There was no significant difference ($p>0.05$) in oxygen saturations breathing FiO_2 21% compared to 18% (figure 4.1).

End-tidal CO_2% levels: End-tidal CO_2% levels were significantly lower ($p<0.01$) at all time points breathing an inspired oxygen tension of 12% compared to 21%, 18% and 15% (figure 4.2).

Respiratory rate: There were no significant differences ($p>0.05$) in respiratory rates at any time point between any of the inspired oxygen tensions or baseline. (figure 4.2).

Heart rate: Heart rate was significantly greater ($p<0.01$) breathing an inspired oxygen tension of 12% compared to 21%, 18%, 15% and baseline. There was no significant differences ($p<0.05$) in heart rate between any of the other inspired oxygen tensions at any time point (figure 4.1).

Diastolic blood pressure: Diastolic blood pressure was significantly lower ($p<0.05$) breathing an inspired oxygen tension of 12% compared to baseline and breathing FiO_2 21%, 18% and 15% at all time points.

Systolic and mean arterial blood pressure: There were no significant differences ($p>0.05$) in systolic and mean arterial blood pressures when comparing each of the inspired oxygen tensions to each other and baseline at any time point.

Study 2 Hyperoxia in normal subjects

Oxygen saturation: Oxygen saturation was significantly higher ($p<0.001$) at all time points when subjects breathed oxygen (FiO_2 100%) compared to air (FiO_2 21%). Oxygen saturation breathing oxygen (FiO_2 100%) was also significantly greater ($p<0.001$) than the oxygen saturation recorded at baseline (figure 4.3).

End-tidal CO_2% levels: End-tidal CO_2% levels were significantly lower ($p<0.001$) at 6, 9, 12 and 15 minutes after breathing oxygen compared to breathing air. There was no significant difference ($p>0.05$) at 3 minutes. End-tidal CO_2% levels were also significantly lower at 6, 9, 12 and 15 minutes breathing oxygen compared to baseline (figure 4.4).

Respiratory rate: There were no significant differences ($p>0.05$) in respiratory rate at any time point when oxygen and air were compared (figure 4.4).

Heart rate: Heart rate was significantly lower ($p<0.001$) at all time points when breathing oxygen (FiO_2 100%) compared to breathing air (FiO_2 21%). Heart rate was also significantly lower ($p<0.001$) breathing oxygen compared to baseline (figure 4.3).

Systolic, diastolic and mean arterial blood pressure: There were no significant differences ($p>0.05$) in systolic, diastolic and mean arterial blood pressure when breathing oxygen was compared to breathing air at 3, 6, 9, 12 and 15 minutes.

Raw data for this chapter is included in the appendix (tables 2A – 15A)

4.3 Discussion

In normal subjects, oxygen (FiO_2 100%) inspired through our closed breathing circuit is associated with a rise in oxygen saturation, fall in end-tidal CO_2% levels and a reduction in heart rate compared to breathing air (FiO_2 21%). The effects of hypoxia on cardiovascular and respiratory responses in normal subjects are more variable and clearly dependent on the inspired oxygen tension used. Oxygen saturation only fell significantly when patients inspired oxygen tensions of 15% and 12% when compared to breathing air (FiO_2 21%). Inspiring an oxygen tension of 12% was also associated with a significant fall in end-tidal CO_2% levels, increases in heart rate and reduction in diastolic blood pressure compared to inspiring oxygen tensions of 21%, 18% and 15%.

Our findings in normal subjects breathing oxygen (FiO_2 100%) are similar to those of several other groups.[86,119,121] Jennet[119] in her thesis from 1964 suggested a biphasic ventilatory response to breathing 100% oxygen. An initial rise in end-tidal CO_2% levels in the first 10-20 seconds was followed several minutes later by a more sustained fall in end-tidal CO_2% levels which persisted throughout the period subjects inspired 100% oxygen. She proposed that an initial fall in minute ventilation, reflected in the rise in end-tidal CO_2% levels, was mediated as part of a reflex arc stimulated by the direct effect of the high vascular oxygen tension on carotid chemoreceptors. The subsequent fall in end-tidal CO_2% levels reflected an increase in minute ventilation which occurred as a consequence of increased cerebral CO_2% levels which acted on central chemoreceptors in the respiratory centre which provoked an increase in respiratory rate which persisted throughout the period that subjects breathed 100% oxygen. She postulated that raised cerebral CO_2% levels

occurred because of cerebral vasoconstriction as a consequence of a fall in cardiac output. The fall in cardiac output, detected in subjects breathing oxygen, was due to a fall in heart rate rather than any change in stroke volume. Jennet was however unable to show an increase in CO_2% levels in the internal jugular veins which one might have expected to see if this hypothesis was correct. We have also detected a significant fall in heart rate in our subjects breathing oxygen but no significant change in respiratory rate. While it is almost certain that the sustained fall in end-tidal CO_2% levels we have observed reflect an increase in minute ventilation, our findings would suggest that this is due to changes in tidal volume rather than respiratory rate. Green and Widdicombe[86] also observed a sustained fall in end-tidal CO_2% levels in anaesthetised dogs breathing oxygen (FiO_2 100%). They however suggested that the effect, which also took 4 minutes to fully develop in their models, was due to a reflex increase in vagal tone following stimulation of carotid chemoreceptors by 100% oxygen. This hypothesis was supported by the observation that the effect was abolished when the dogs were pre-treated with intravenous atropine. Like others[121] we have also found a significant fall in heart rate in our subjects which occurs immediately. It has been suggested that stimulation of carotid chemoreceptors by 100% oxygen results in increased vagal tone causing bradycardia and a subsequent fall in cardiac output. Once again this effect is blocked by the addition of intravenous atropine.[86]

We have shown that in our normal subjects inspiring oxygen tensions of 12% and 15% causes a significant fall in oxygen saturation in keeping with the presence of moderate hypoxaemia. However of more interest to us are the additional effects which occurred at an inspired oxygen tension of 12%. At this low inspired oxygen

tension we have observed, in our healthy volunteers, a significant increase in heart rate, fall in diastolic blood pressure and reduced end-tidal CO_2% levels. This would suggest significant stimulation of sympathetic nervous activity which is likely to be mediated through carotid and peripheral chemoreceptors.[119,121,123] The changes we have observed have been reported by many authors[123] and are acknowledged to be mediated via carotid and aortic body chemoreceptors. It is believed that normoxaemia causes resting tonic activity in carotid chemoreceptors[121,124] and that a rise in ambient oxygen tension results in a fall in tonic activity provoking increased vagal discharge.[119,121] Conversely a fall in ambient oxygen tension increases tonic activity and results in increased sympathetic activity raising heart rate and increasing ventilation.[119,121] Controversy however remains as to what level of hypoxaemia is required to provoke an increase in sympathetic activity in man. Comroe and Schmidt[125] have suggested a threshold arterial oxygen tension of 50 mm Hg is while others[123] have suggested that chemoreceptor activity is inversely related to the ambient oxygen tension of the chemoreceptor.[124] Using our novel closed breathing circuit we have found that an inspired oxygen tension between 15% and 12% provokes an increase in sympathetic activity in our normal subjects and the pattern is similar to that reported by other groups.[119,121,123] For this reason we plan to use an inspired oxygen tension of 15% in which to conduct our hypoxic *in-vivo* studies because at that level we have provoked a significant fall in oxygen saturation in keeping with moderate hypoxaemia without the unwanted effects of sympathetic activation. We therefore would not anticipate any indirect effects of hypoxaemia on airway tone mediated via carotid and arterial chemoreceptors in our hypoxic studies which could potentially influence our results.

In conclusion the changes in oxygen saturation, heart rate and end-tidal CO_2% levels that we have observed in our studies, would suggest that our novel closed breathing circuit has allowed us to deliver precisely controlled inspired oxygen tensions which have achieved significant changes in arterial and airway oxygen tensions. These studies were a necessary prerequisite prior to undertaking our planned *in-vivo* studies in patients with asthma. In addition it would appear that an inspired oxygen tension of 15% produces significant falls in oxygen saturation and arterial PaO_2 without stimulation of sympathetic activity, and will therefore be the inspired oxygen tension of choice for our hypoxic studies. We had already planned to use an inspired oxygen tension of 100% in our hyperoxic studies to mimic the oxygen tensions used in our *in-vitro* studies.

Subjects	Age (years)	Sex	FEV_1 Absolute (litres)	FEV_1 Predicted (%)	FVC Absolute (litres)	FVC Predicted (%)
1	27	M	4.83	97	6.07	99
2	49	M	3.48	88	4.30	92
3	46	M	3.62	103	4.38	98
4	28	M	3.96	82	5.01	86
5	28	M	4.50	100	5.51	104
6	23	M	4.78	112	5.34	106
7	24	M	4.18	85	5.18	91
Mean (SEM)	**32 (11)**		**4.19 (0.54)**		**5.11 (0.62)**	

Table 4.1 Characteristics of 7 normal subjects inhaling inspired oxygen tensions of 21%, 18%, 15% and 12% for 12 minutes each.

Subjects	Age (years)	Sex	FEV_1 Absolute (litres)	FEV_1 Predicted (%)	FVC Absolute (litres)	FVC Predicted (%)
1	28	M	3.96	82	5.01	86
2	44	F	3.60	118	4.00	136
3	46	M	3.62	103	4.38	98
4	27	M	4.83	97	6.07	99
5	25	M	4.42	93	5.30	97
6	49	M	3.48	88	4.30	92
7	29	F	3.17	103	3.81	107
8	28	M	4.60	90	5.48	96
Mean (SEM)	**33 (8.4)**		**3.85 (0.73)**		**4.79 (0.79)**	

Table 4.2 Characteristics of 8 normal subjects inhaling air (FiO_2 21%) and oxygen (FiO_2 100%) for 15 minutes each.

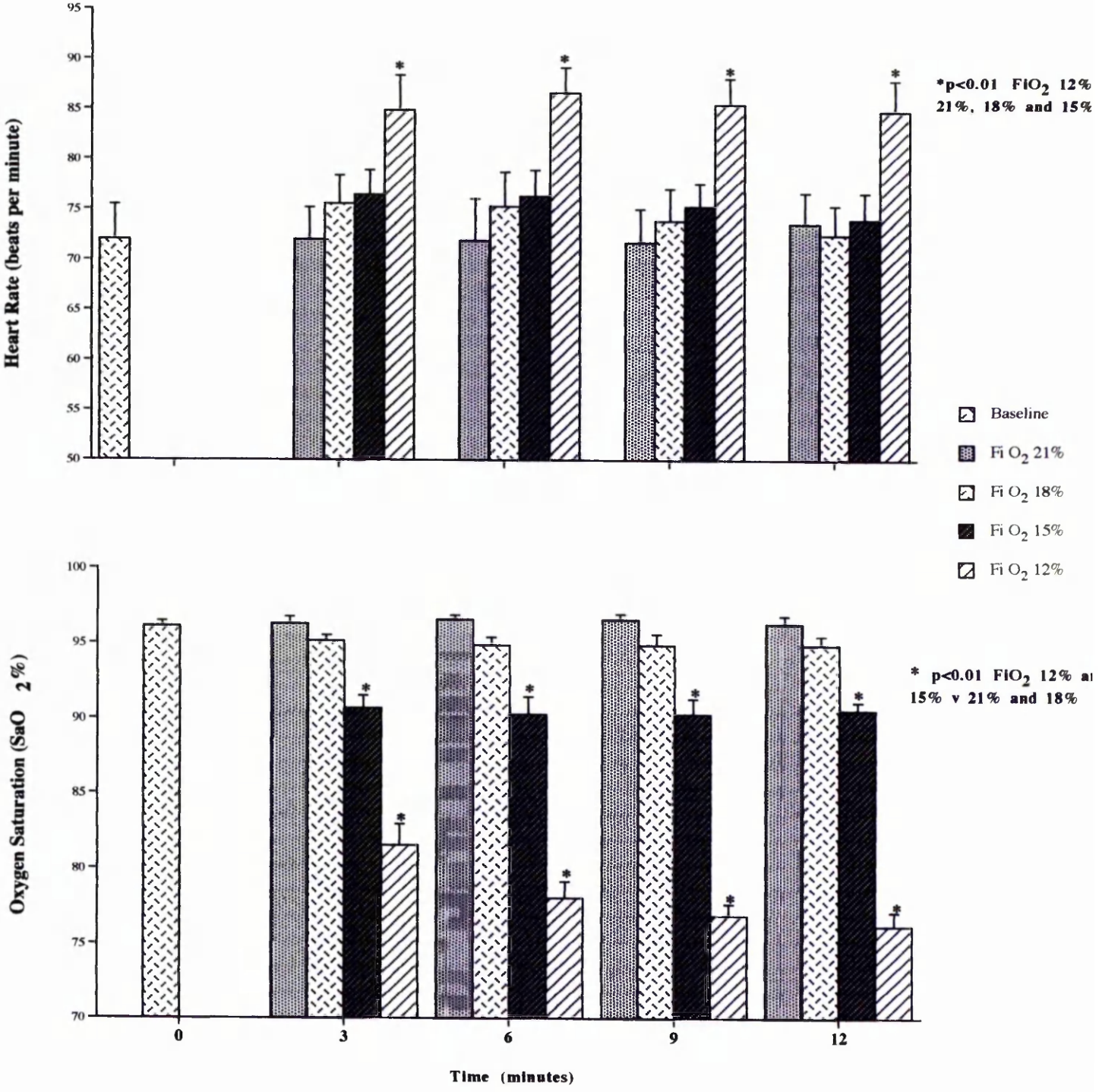

Fig 4.1 Heart rate (beats per minute) and oxygen saturation (SaO_2%) in normal subjects inhaling inspired oxygen tensions of 21%, 18%, 15% and 12% for 12 minutes each in a single blind fashion. (n=7)

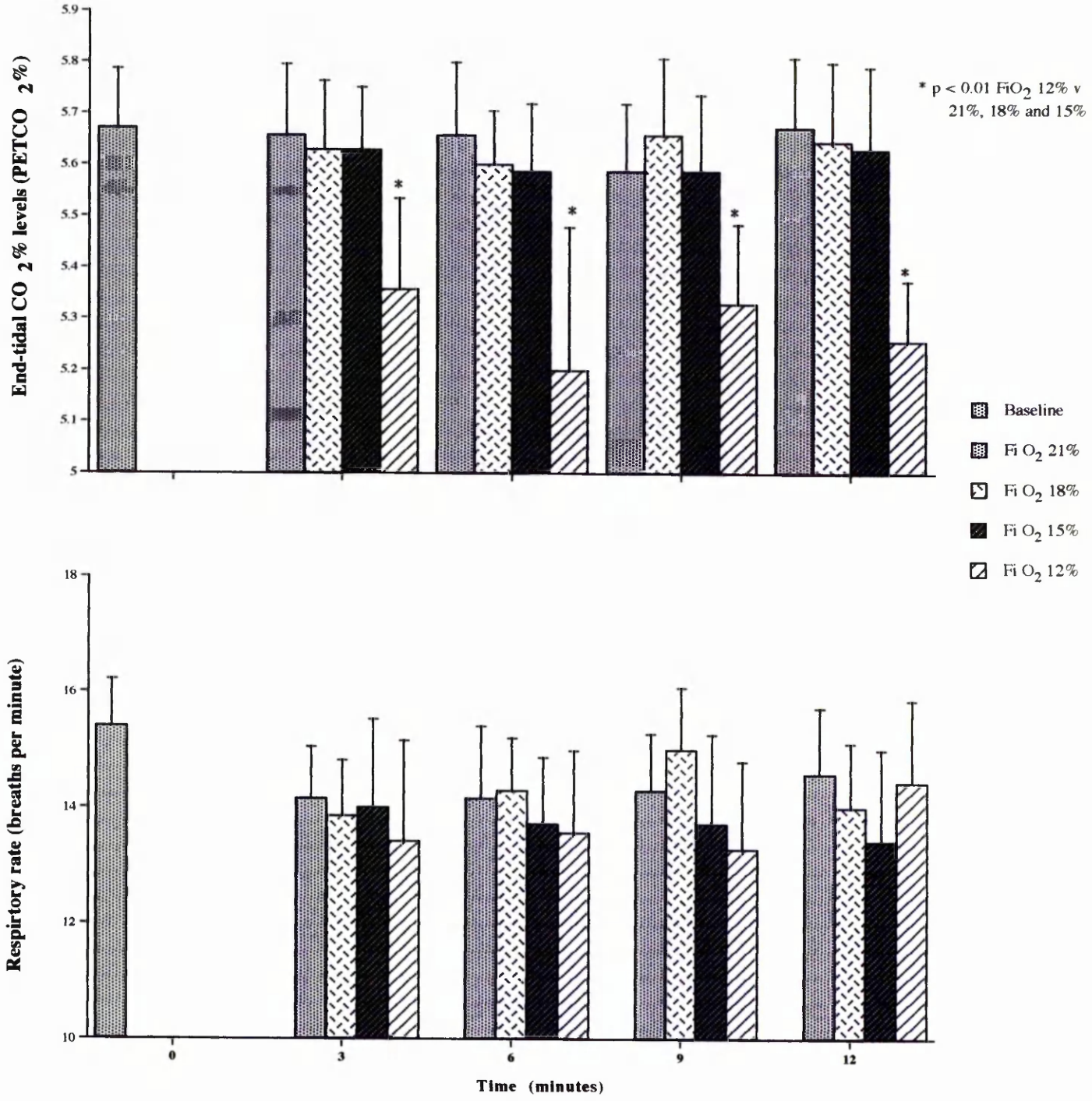

Fig 4.2 End-tidal CO_2% levels ($PETCO_2$%) and respiratory rate (breaths per minute) in normal subjects inhaling inspired oxygen tensions of 21%, 18%, 15% and 12% for 12 minutes each in a single blind fashion. (n=7)

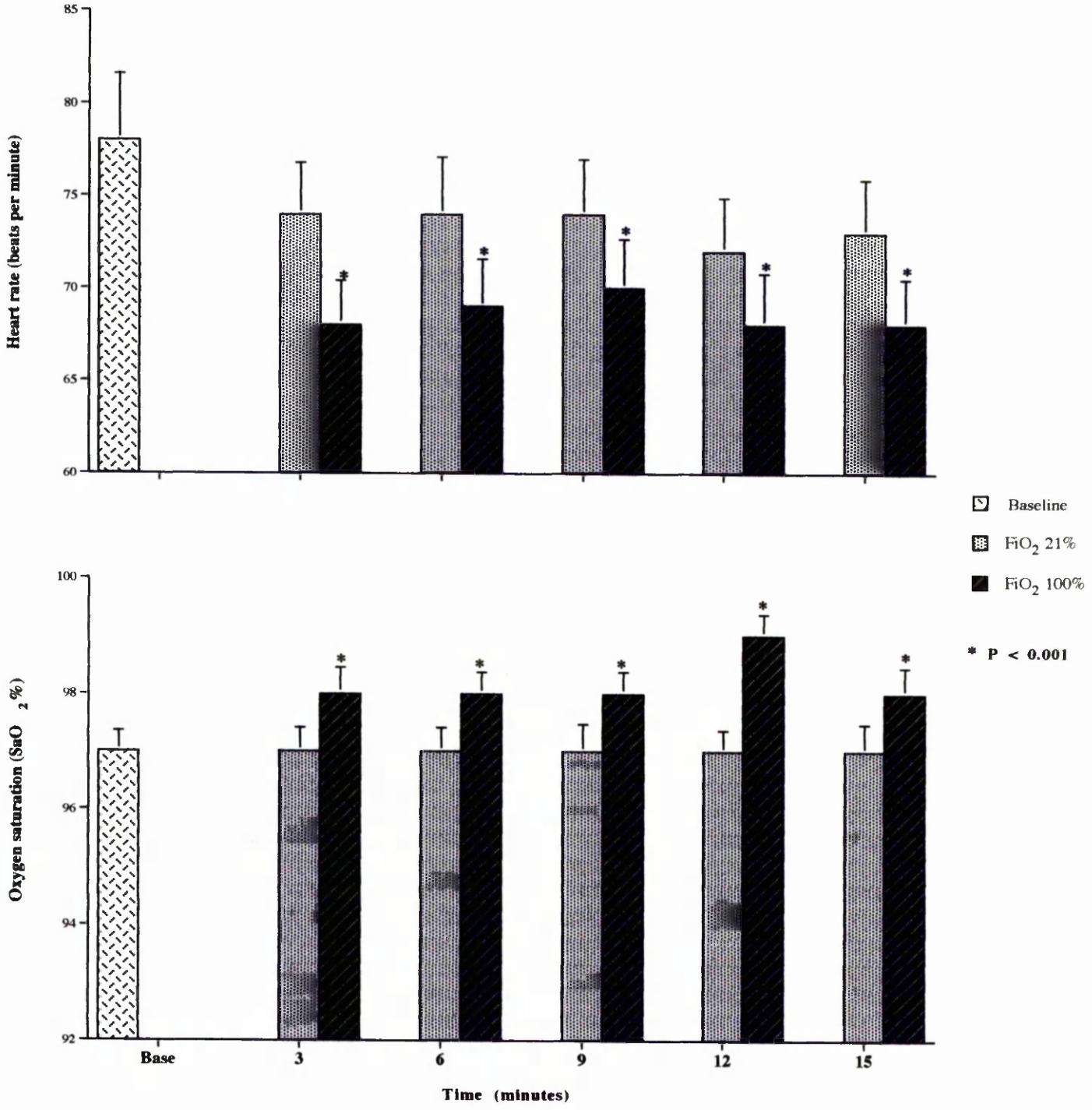

Fig 4.3 Heart rate (beats per minute) and oxygen saturation (SaO_2%) in normal subjects inhaling air (FiO_2 21%) and oxygen (FiO_2 100%) for 15 minutes each in a double blind and randomised fashion.(n=8)

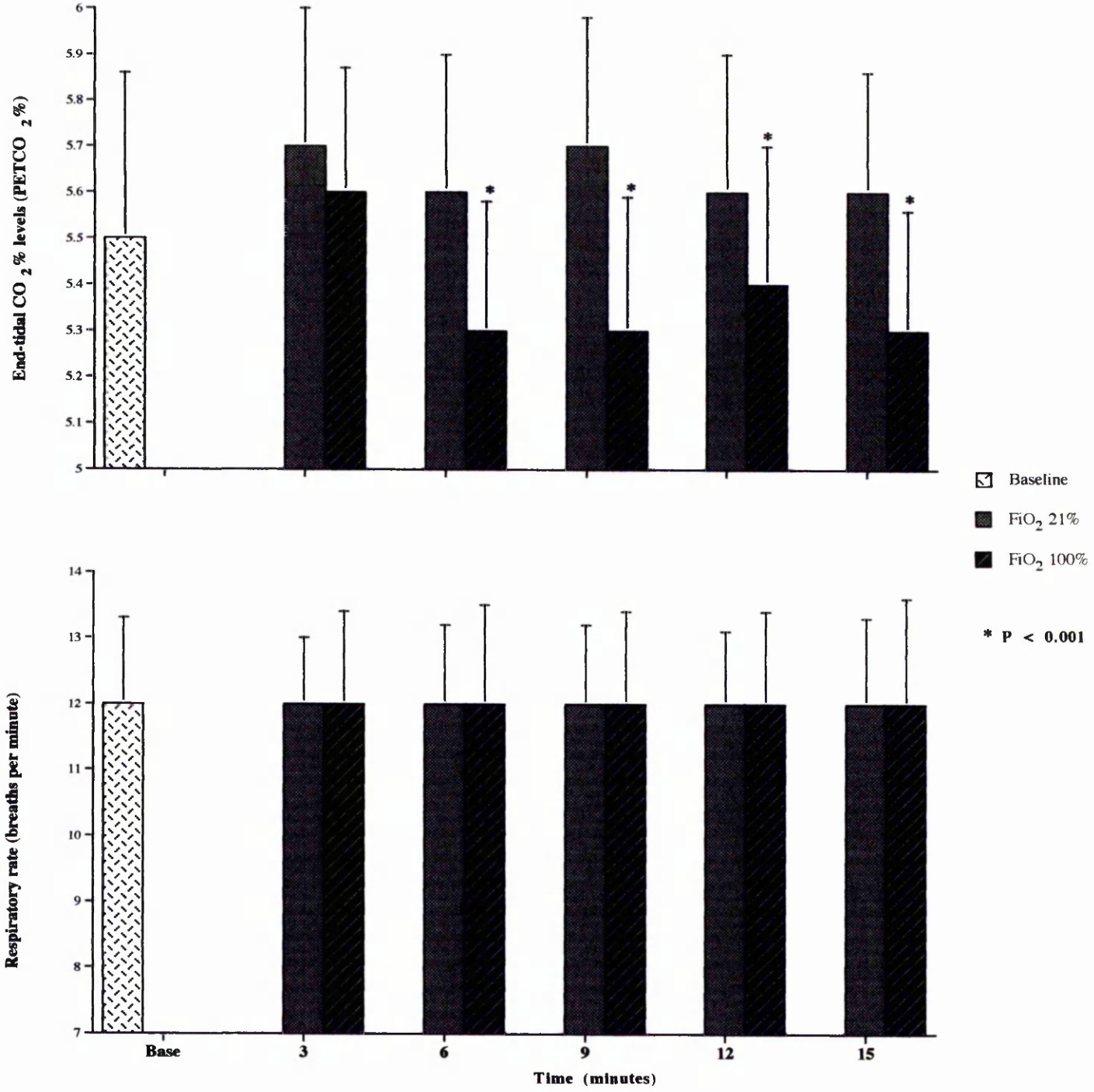

Fig 4.4 End-tidal CO_2% levels ($PETCO_2$%) and respiratory rate in normal subjects inhaling air (FiO_2 21%) and oxygen (FiO_2 100%) for 15 minutes each in a double blind and randomised fashion.(n=8)

CHAPTER 5

THE EFFECT OF ACUTE HYPEROXIA ON METHACHOLINE INDUCED BRONCHOCONSTRICTION IN PATIENTS WITH MILD ASTHMA

5.1 Introduction

The first of our *in-vivo* study was prompted by observations from our own laboratory that hyperoxia (O_2 tension 95%) significantly attenuated the constrictor response of both methacholine and endothelin-1 in bovine isolated bronchial rings when compared to normoxia (O_2 tension 20%) and hypoxia (O_2 tension 4%).[75,76] A review of the relevant literature revealed that similar findings had been made *in-vivo* in dogs where histamine induced tracheal constriction was reported to be inhibited by oxygen (FiO_2 100%).[88] The effects of hyperoxia on airway responsiveness in asthma however are more uncertain. Previous studies had suggested that exercise induced bronchoconstriction was significantly attenuated by inhaling high inspired oxygen tensions although controversy persists about the potential mechanism of this effect.[107,108] In patients with asthma methacholine inhalation challenge has been reported to be both attenuated[103] or unaffected[102] by hyperoxia. If the administration of high concentrations of inspired oxygen can reduce the responsiveness of the airways to bronchoconstrictor stimuli in patients with asthma then this effect of oxygen may be of relevance in the treatment of acute exacerbations of asthma. Our first *in-vivo* study was designed to examine the effect of breathing oxygen (FiO_2 100%) on methacholine-induced bronchoconstriction in patients with mild asthma.

5.2 Patients and methods

Patients

Fourteen adult mild asthmatic patients (five male) mean (SD) age 36 (9.2) years were recruited into the study (table 5.1). All had a history of asthma and an FEV_1 > 80% of predicted at a screening visit. None had any other significant illnesses. All patients were taking inhaled $ß_2$-agonists on an as required basis. A further ten were on regular inhaled corticosteroids and of these two were taking regular oral theophyllines and one patient inhaled salmeterol. The research was carried out in accordance with the Declaration of Helsinki (1989) and all of the patients gave written informed consent to the study protocol which had the approval of the West Ethics Committee.

Study design

Patients attended the laboratory on three separate study days at approximately the same time each day. During the initial screening visit each patient underwent a methacholine inhalation challenge test to determine a PC_{20} value ie: that concentration of methacholine causing a 20% fall in FEV_1. On the subsequent two days, after thirty minutes of supine rest, the patients were commenced on our closed breathing circuit. Following a ten minute run in period breathing air (FiO_2 21%) baseline measurements of FEV_1, respiratory rate (RR), heart rate (HR), oxygen saturation (SaO_2%) inspired oxygen and carbon dioxide levels (insp O_2%, insp CO_2%) and expired oxygen and carbon dioxide levels ($PETO_2$%, $PETCO_2$%) were made. Venous blood was also taken for assay of plasma catecholamines. Patients were then administered either air (FiO_2 21%) or oxygen (FiO_2 100%) in a randomised double

blind fashion for the remainder of the study day. A methacholine inhalation challenge was commenced ten minutes after starting the study gas. The study day was terminated when a PC_{20} value was reached and the measurements made at baseline had been repeated.

Measurements

Heart rate and oxygen saturation was measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) - see chapter 2.3.

Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) - see chapter 2.3.

Methacholine PC_{20} value (mg/ml) was measured according to the technique described by Cockcroft et al and Hargreaves et al[111,112] – see chapter 2.5.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) - see chapter 2.2.

Plasma catecholamines were measured using reverse phase high performance liquid chromatography - see chapter 2.8.

Statistical analysis

Statistical analysis was performed on an Apple Mackintosh LC II computer using a Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabasas). Paired T-tests were used to compare measurements between study days. The PC_{20} values were logarithmically transformed before analysis. A p-value below 0.05 was accepted as significant.

5.3 Results

Baseline measurements: There were no significant differences between baseline measurements of FEV_1, heart rate, respiratory rate, oxygen saturation, end-tidal CO_2%, adrenaline and noradrenaline levels on the two study days.

Methacholine PC_{20} values: There were no significant differences in the geometric mean (range) PC_{20} values mg/ml between the normoxic and hyperoxic study days. The geometric mean (range) PC_{20} value mg/ml on the normoxic day was 0.96 (0.02 – 10.6) mg/ml compared to 1.38 (0.13 – 15.9) mg/ml on the hyperoxic study day (figure 5.1).

Oxygen saturation: Oxygen saturation was significantly higher ($p<0.01$) following hyperoxia [mean (SEM) SaO_2%: baseline 96.7 (0.35)%, pre-methacholine 98.1 (0.23)%, post-methacholine 98.1 (0.20)%] than during the normoxic study day [mean (SEM) SaO_2%: baseline 96.5 (0.33)%, pre-methacholine 96.7 (0.37)%, post-methacholine 96.0 (0.52)%] (figure 5.2).

Heart rate: Heart rate was significantly lower ($p<0.05$) on the hyperoxic study day both before and after the methacholine inhalation test when compared to the normoxic study day. The mean (SEM) HR (bpm) on the hyperoxic study day was: baseline 75 (4) bpm, pre-methacholine 71 (4) bpm, post-methacholine 71 (4) bpm and on the normoxic study day: baseline 77 (4) bpm, pre-methacholine 75 (5) bpm, post-methacholine 77 (4) bpm (figure 5.2).

End-tidal CO_2%: There was a significant ($p<0.05$) fall in $PETCO_2$% on both study days, before and after methacholine inhalation when compared to baseline but no significant difference in $PETCO_2$% between the study days . The mean maximum

(SEM) reduction in $PETCO_2$% during the normoxic day was 0.56 (0.16)% and 0.60 (0.13)% on the hyperoxic day (figure 5.2).

Catecholamine levels: The circulating plasma noradrenaline levels (n=12) on the normoxic study day were [mean (SEM) noradrenaline nmol/l: baseline 2.14 (0.58) nmol/l, post-methacholine 1.66 (0.28) nmol/l] were not statistically different from the hyperoxic study day [mean (SEM) noradrenaline nmol/l: baseline 1.80 (0.26) nmol/l, post-methacholine 1.63 (0.28) nmol/l]. Plasma adrenaline levels (n=12) on the normoxic study day were [mean (SEM) adrenaline nmol/l: baseline 0.11 (0.02) nmol/l, post-methacholine 0.07 (0.02) nmol/l] were also no different from the hyperoxic study day [mean (SEM) adrenaline nmol/l: baseline 0.07 (0.02) nmol/l, post-methacholine 0.07 (0.02) nmol/l].

Respiratory rate: There were no significant differences in respiratory rate on either study day at any time point (data not shown).

Raw data for this chapter is included in the Appendix (tables 16A - 17A)

5.4 Discussion

These results demonstrate that acute hyperoxia does not attenuate methacholine-induced bronchoconstriction *in-vivo* in mild asthmatic patients. This result clearly differs from both the *in-vitro* work from our own laboratory[75,76] and the *in-vivo* animal studies we have discussed earlier.[88]

It is in the first instance worth reconsidering our own study design to ensure that our hypothesis has been appropriately tested before postulating on more profound reasons for the differences in the results we have observed. The rise in oxygen saturation and fall in heart rate[119,121] we have noted in our patients would suggest that significant increases in airway and arterial oxygen tensions have been obtained from our closed breathing circuit. The absence of any difference in end-tidal CO_2% between study days at any time point indicates that hypocapnic bronchoconstriction,[126-129] as a consequence of hyperoxia,[119] was not a confounding factor in our results. Nebuliser output, which may be affected by the molecular weight of the gas used to drive the nebuliser, was prior to the commencement of the study confirmed to be 0.12 mls/min at a flow rate of 6 litres per minute for both study gases (FiO_2 100% and FiO_2 21%) – chapter 2.7. This would suggest that patients received the same quantity of methacholine from each nebulisation. Circulating adrenaline and noradrenaline have effects on airway smooth muscle tone.[94] In the present study we observed no difference in circulating catecholamine levels on either study day both before and after methacholine challenge.

There are several possible reasons why the results of our study are in contrast to the findings of both the *in-vitro* and *in-vivo* animal studies which reported that hyperoxia attenuates airway reactivity.[75,76,88] While hyperoxia attenuates the bronchoconstrictor effect of methacholine and endothelin-1 in isolated bovine bronchial rings, hyperoxia *in-vivo* may have effects on bronchomotor tone via neural or humoral pathways which could offset a direct effect of oxygen alone on airway smooth muscle responsiveness. The fall in heart rate associated with hyperoxia is indicative of carotid chemoreceptor stimulation. Airway tone may be influenced by carotid chemoreceptor activity and this influence may offset any protective effects that hyperoxia has on airway reactivity. It is also possible that the differences are explained quite simply by species variation. Our other studies have examined bovine bronchial rings and the trachea of dogs.

Our results support and extend those of Wollner et al.[102] In addition to examining the effects of breathing 100% oxygen on methacholine airway responsiveness we have also studied the potential influences of hypocapnia and circulating catecholamines on airway reactivity during hyperoxia. It is difficult to explain the difference in findings between our study and that of Inoue et al[103] who found that breathing oxygen attenuated methacholine-induced bronchoconstriction in asthmatic subjects. They however used an inspired oxygen concentration of 30% whereas we used 100% and it is possible that the higher inspired oxygen concentration itself causes paradoxical bronchoconstriction through neural or other pathways. Review of the patients in the study of Inoue et al[103] shows differences from our own study population. None of their patients were receiving inhaled corticosteroids and seventeen of the thirty

patients were on no therapy at all. More importantly however six patients had arterial oxygen tensions below 10 Kpa in keeping with chronic hypoxaemia. Studies have suggested that in patients with chronic hypoxaemia inspiring oxygen (FiO_2 30%) causes bronchodilation[104,106] and this effect may have significantly biased their results.

It is worth noting that in our study a small subgroup of patients with methacholine PC_{20} values of less than 1.0 mg/ml on the normoxic study day derive a significant protective effect from breathing oxygen (FiO_2 100%) against methacholine induced bronchoconstriction. It is interesting to speculate that attenuation of methacholine induced bronchoconstriction by hyperoxia in asthmatic patients may be relative to the degree of airway hyperresponsiveness present. In the present study we have examined the effect of hyperoxia on airway reactivity in a group of normoxic stable asthmatic patients. It is worth considering however, that in acute severe asthma patients are often hypoxaemic and have increased airway reactivity and it may be that administration of high concentrations of inspired oxygen in this group may attenuate airway responsiveness to bronchoconstrictor stimuli.

In conclusion these results demonstrate that breathing oxygen (FiO_2 100%) in mild asthmatic patients does not attenuate methacholine-induced bronchoconstriction.

Patient No	Age (years)	Sex	FEV_1 absolute value (L)	predicted %	Therapy	Met PC_{20} mg/ml
1	36	F	2.18	81	Sprn, B, TH, S	2.89
2	26	M	3.70	95	Sprn, B	0.18
3	21	F	2.90	103	Sprn, B	1.05
4	35	F	2.28	86	Tprn, B	0.04
5	36	F	2.20	83	Sprn, B	0.77
6	30	F	2.62	94	Sprn	0.63
7	29	F	3.12	104	Sprn, B	1.23
8	46	M	3.90	96	Sprn	3.71
9	35	F	2.95	100	Sprn, B	2.00
10	34	F	2.40	81	Tprn, B, TH	3.9
11	45	M	2.60	87	Sprn, B	7.90
12	56	F	2.13	80	Sprn	0.34
13	29	F	2.84	93	Sprn, B	0.41
14	43	M	3.45	81	Sprn	0.19
Mean (SD)	**36 (9.2)**		**2.80 (0.60)**	**90 (8.6)**		***0.84**

Table 5.1: Patient characteristics. Met PC_{20} = methacholine concentration causing a 20% fall in FEV_1 at an initial screening visit (* geometric mean), Sprn = inhaled salbutamol as reqd, Tprn = inhaled terbutaline as reqd, B = inhaled steroid (budesonide or beclomethasone dipropionate), TH = oral theophylline, S = inhaled salmeterol twice daily.

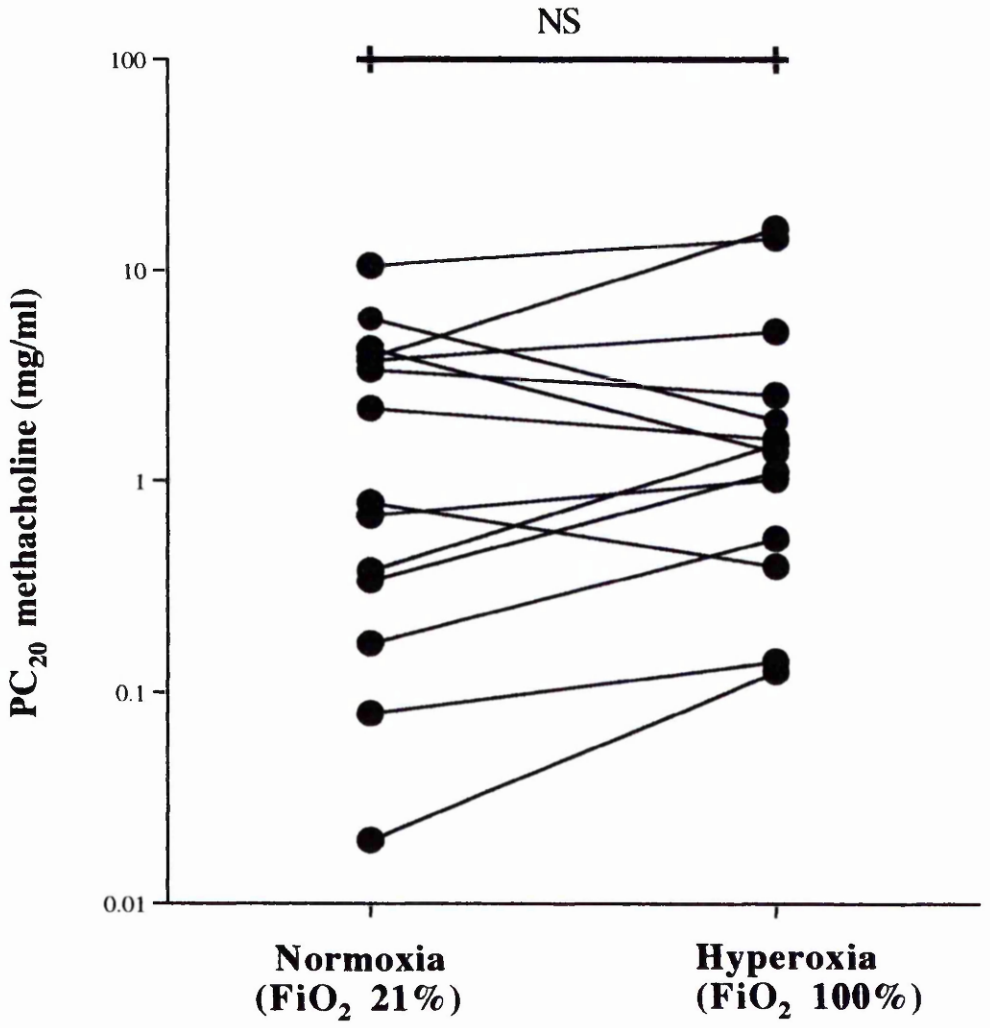

Fig 5.1 The effect of inhaling oxygen (FiO_2 100%) and air (FiO_2 21%) on methacholine PC_{20} values mg/ml in 12 mild asthmatics. ($P>0.05$)

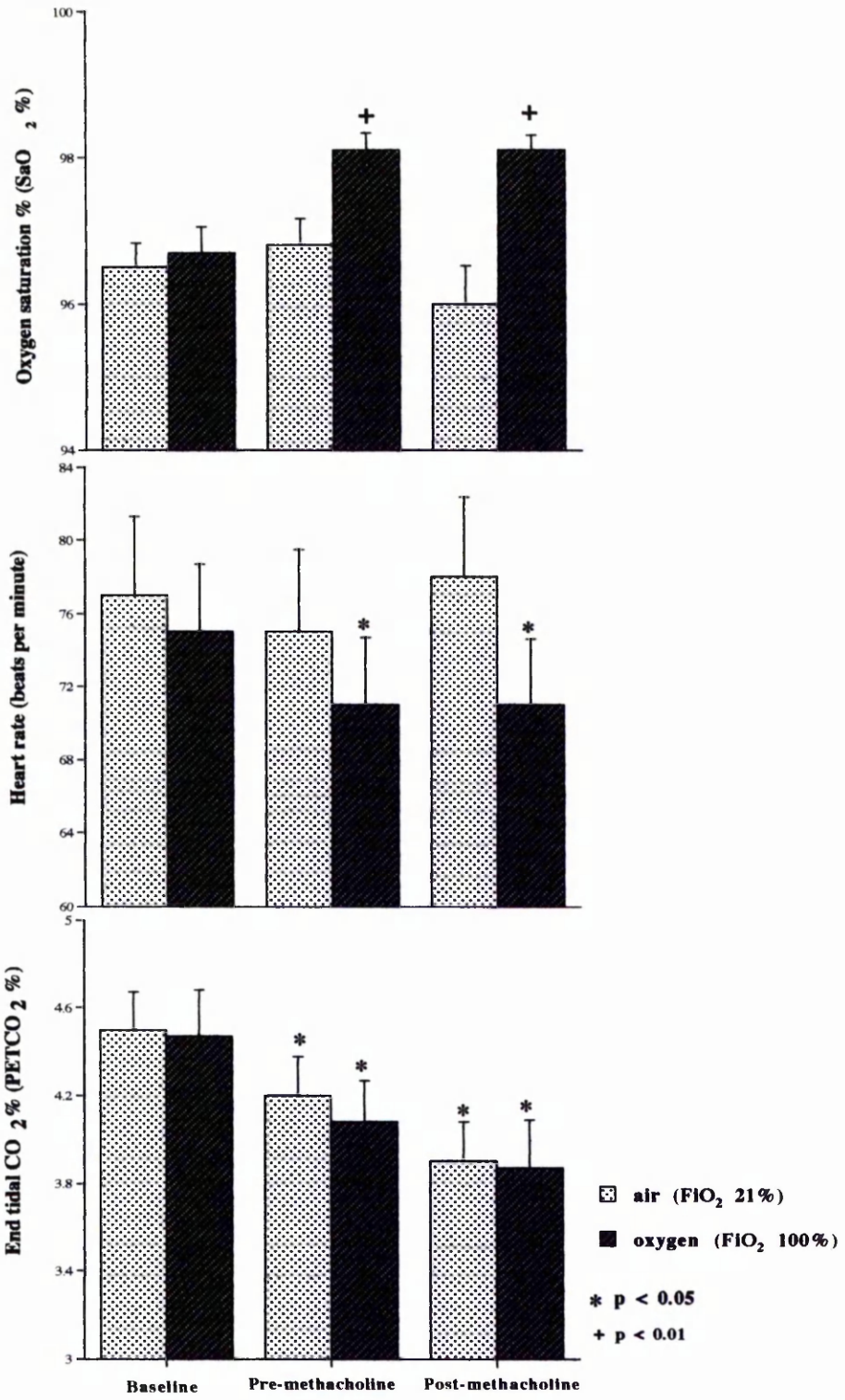

Fig 5.2 The effect of inhaling oxygen (FiO_2 100%) and air (FiO_2 21%) on oxygen saturation (SaO2%), heart rate (beats per minute) and end-tidal CO_2% levels ($PETCO_2$%) before, during and after a methacholine inhalation challenge in 12 mild asthmatics.

CHAPTER 6

THE EFFECT OF ACUTE HYPOXIA ON METHACHOLINE INDUCED BRONCHOCONSTRICTION IN PATIENTS WITH MILD ASTHMA

6.1 Introduction

We have heard in the previous chapter how methacholine induced bronchoconstriction is unaffected by breathing oxygen (FiO_2 100%) in patients with asthma despite our *in-vitro* findings (chapter 5). The same *in-vitro* studies had also suggested that acute hypoxia (FiO_2 4%) potentiated the constrictor effect of both methacholine[75] and endothelin-1[76] in bovine isolated bronchial rings. Several *in-vivo* studies have also suggested that acute hypoxia may potentiate histamine induced airway constriction in both dogs[88] and sheep.[91,92] Few studies have examined this effect in patients with asthma. Denjean et al[97] suggested that in patients with asthma airway hyperresponsiveness to methacholine was increased by hypoxia whereas Tam et al[109] showed acute hypoxia had no effect on dry air challenge in patients with asthma. As hypoxaemia is common in patients admitted to hospital with acute exacerbations of asthma[61,67,68] such an observation may have relevance in their emergency management. The aim of this study was to examine the effect of acute hypoxia (FiO_2 15%) on methacholine induced-bronchoconstriction in patients with asthma.

6.2 Patients and methods

Patients

Eleven mild adult asthmatic patients mean (SD) age 42 (12) years were recruited into the study (Table 6.1). All of the patients gave a history of asthma and had an FEV_1 > 80% of predicted at an initial screening visit. None of the patients had any other significant illnesses. All eleven patients were receiving inhaled $ß_2$-agonists as required and ten patients regular inhaled corticosteroids. Two were taking inhaled salmeterol and a further one a long acting oral theophylline. $ß_2$-agonists were discontinued for 8 hours, salmeterol 24 hours and oral theophylline for 48 hours prior to attending for each visit. All patients gave informed consent to the study which had previously been approved by the West Ethics Committee.

Study design

Patients were asked to visit the laboratory on three separate study days each separated by one week. During the initial visit following a physical examination patients performed a methacholine inhalation test to determine a PC_{20} value for methacholine. Following thirty minutes of supine rest patients were commenced on our closed breathing circuit. After 10 minutes breathing air (FiO_2 21%) baseline measurements of FEV_1, heart rate (HR), respiratory rate (RR), oxygen saturation (SaO_2%), inspired oxygen and carbon dioxide levels (insp O_2%, insp CO_2%) and expired oxygen and carbon dioxide levels ($PETO_2$%, $PETCO_2$%) were made. A blood sample was taken for assay of plasma catecholamines. Patients then breathed either air (FiO_2 21%) or a nitrogen and oxygen mixture (FiO_2 15%) for the remainder of the study day. The measurements made at baseline were repeated 10 minutes after

commencing the study gas. All gases were administered in a randomised double blind fashion by a second investigator obscured from the patients vision. After breathing the study gas for 10 minutes patients performed a methacholine inhalation challenge. The study day was terminated when a PC_{20} value for methacholine was obtained and a third set of measurements had been made.

Measurements

Heart rate and oxygen saturation was measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) - see chapter 2.3.

Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) - see chapter 2.3.

Methacholine PC_{20} value (mg/ml) were measured according to the technique described by Cockcroft et al[111] and Hargreaves et al[112] – see chapter 2.5.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) - see chapter 2.2.

Plasma catecholamines were measured using reverse phase high performance liquid chromatography - see chapter 2.8.

Statistical analysis

Statistical analysis was performed using a Statview software package (Brainpower Inc, 24009 Ventura boulevard, Suite 250, Calabassas) on an Apple Mackintosh LC II computer. Paired t-tests were used to compare measurements made at each time

point between study days. The PC_{20} values for methacholine were logarithmically transformed before analysis. A p value of less than 0.05 was accepted as significant.

6.3 Results

Baseline measurements: There were no significant differences in baseline measurements of FEV_1, heart rate, respiratory rate, oxygen saturation, end-tidal CO_2%, plasma adrenaline and noradrenaline levels between the two study days.

PC_{20} value for methacholine: The geometric mean (range) PC_{20} value mg/ml was significantly lower ($p<0.05$) on the hypoxic day 0.89 (0.04-15.5) mg/ml than the normoxic day 2.45 (0.26-23.02) mg/ml (figure 6.1).

Oxygen saturation: Oxygen saturation was significantly lower on the hypoxic day ($p<0.01$) both before and after methacholine challenge when compared to the normoxic day. The mean (SEM) oxygen saturation % on the normoxic day was: baseline 96.5 (0.16) %, pre-methacholine 96.3 (0.27) %, post-methacholine 96.0 (0.43) %, and on the hypoxic day: baseline 96.3 (0.24) %, pre-methacholine 91.0 (0.56) %, post-methacholine 90.5 (1.0) % (figure 6.2).

End-tidal carbon dioxide level %: End-tidal CO_2% levels were not significantly different between study days. However on both study days end-tidal CO_2% levels were significantly lower following methacholine challenge ($p<0.05$) when compared to baseline on both study days. The mean (SEM) end-tidal CO_2% on the normoxic day was: baseline 4.7 (0.11), pre-methacholine 4.16 (0.12), post-methacholine 3.7 (0.19) and on the hypoxic day: baseline 4.6 (0.14), pre-methacholine 4.4 (0.18), post-methacholine 3.9 (0.17) (figure 6.2).

Forced expiratory volume in one second: The mean FEV_1 (litres) after 10 minutes of hypoxia alone, prior to methacholine inhalation was not significantly different ($p>0.05$) compared to the normoxic study day prior to methacholine inhalation. The

mean FEV_1 (L) following 10 minutes of hypoxia alone was 2.40 (0.15) litres compared to 2.38 (0.14) litres on the normoxic study day.

Plasma catecholamine levels: Circulating plasma noradrenaline levels (n=11) on the normoxic study day [mean (SEM) noradrenaline nmol/l: baseline 1.70 (0.14) nmol/l, post-methacholine 1.60 (0.16) nmol/l] were not statistically different from the hypoxic study day [mean (SEM) noradrenaline nmol/l: baseline 1.61 (0.29) nmol/l, post-methacholine 1.40 (0.25) nmol/l]. Plasma adrenaline levels (n=11) on the normoxic study day [mean (SEM) adrenaline nmol/l: baseline 0.10 (0.02) nmol/l, post-methacholine 0.08 (0.02) nmol/l] were also no different following hypoxia [mean (SEM) adrenaline nmol/l: baseline 0.11 (0.02) nmol/l, post-methacholine 0.06 (0.02) nmol/l].

Heart rate and respiratory rate: There were no significant differences in either heart rate (figure 6.2) or respiratory rate (data not shown) between either of the study days at any time point.

Raw data for this chapter is included in the Appendix (tables 18A – 19A)

6.4 Discussion

The results from this study would suggest that in patients with mild stable asthma, acute hypoxia potentiates methacholine induced airway bronchoconstriction.

The oxygen saturations that we have observed on the hypoxic study, day both before and after methacholine challenge were approximately 90%. This is in keeping with a moderate degree of hypoxaemia.[130] Most patients admitted to hospital with acute exacerbations of asthma are hypoxaemic[61] and approximately one third with severe asthma have a PaO_2 of less than 8 kPa on air.[67,68] It would appear that our closed breathing circuit has managed to produce and maintain a significant level of hypoxaemia in our patients. We have already considered how the development of airway tone in man is complex and has both direct and indirect influences. During the design of this study, and the selection of an inspired oxygen tension of 15% we were acutely aware of fact that hypoxia itself might influence airway tone via a number of different mechanisms (chapter 4). Hypoxia has been noted to stimulate ventilation by an unknown mechanism.[119,121] The level of hypoxia required to stimulate ventilation in man is however controversial.[122] An increase in minute ventilation will cause a fall in end-tidal CO_2%. Hypocapnia is associated with bronchoconstriction *in-vivo*.[126-129] In our study we have not observed any difference in end-tidal CO_2% between the study days suggesting that hypocapnia has not been a confounding factor in our results. Airway smooth muscle tone may be influenced directly by the action of hypoxia on smooth muscle cells and indirectly by altered carotid chemoreceptor activity during hypoxaemia.[98,99] In our study the FEV_1 on both study days, after breathing the study gases for 10 minutes but prior to commencement of the methacholine challenge, was not significantly different suggesting that hypoxia

has not influenced resting airway tone in our patients. The absence of significant heart rate changes in our patients between the study days would suggest that the level of hypoxaemia we have induced has not resulted in sympathetic nervous stimulation which might itself have influenced resting airway tone. In our study circulating catecholamine levels are no different on the hypoxic and normoxic study days suggesting that changes in adrenaline and noradrenaline levels have not influenced our results.

It would appear therefore that hypoxia itself potentiates methacholine induced bronchoconstriction. The mechanism of this effect is unknown. Studies in sheep[89,92] have suggested that alveolar hypoxia (FiO_2 13%) increases non-specific bronchial reactivity to both histamine and carbachol. This effect is abolished by infusing cromolyn sodium, an agent which stabilises mast cell membranes[89]. The authors concluded that mast cells might play an important role in increasing non-specific bronchial reactivity in sheep exposed to alveolar hypoxia. In a similar series of studies D'Brot and Ahmed[92] observed that inhaled FPL 57231, a leukotriene receptor antagonist, also attenuated increases in non-specific bronchial reactivity observed during alveolar hypoxia. They concluded that increased bronchial reactivity during alveolar hypoxia might be due to priming of airway smooth muscle by leukotrienes. If acute hypoxia causes release of inflammatory mediators within the airways, this could explain why hypoxia potentiates methacholine induced bronchoconstriction in our patients with mild asthma. Other groups have suggested that prior surgical chemodenervation[88,91] in animals models abolishes potentiation of methacholine and histamine induced bronchoconstriction by hypoxia. If this is the case and the effect is mediated by peripheral nerves it does not explain our own *in-vitro* findings where

endothelin-1 and methacholine induced smooth muscle contractions were potentiated by hypoxia in bovine isolated bronchial rings deprived of their normal neural and humoral influences.[75,76] Hypoxia may act directly on peripheral nerves to stimulate neurotransmitter release or directly on smooth muscle to cause an increase in smooth muscle tone. These possibilities seem less likely in light of the difficulties researchers have had in establishing that hypoxia does indeed cause increased airway tone in man.

These findings may well have clinical relevance in acute exacerbations of asthma as hypoxia may not only occur as a consequence of airway constriction but also act to potentiate the airway constriction itself. In conclusion our study has suggested that acute hypoxia (FiO_2 15%) potentiates methacholine induced bronchoconstriction in patients with mild stable asthma.

Patient No	Age (years)	Sex	FEV_1 absolute Value (L)	FEV_1 predicted %	Therapy	Met PC_{20} mg/ml
1	35	F	2.83	97	Sprn, B	0.77
2	33	M	3.87	81	Sprn, B	5.60
3	57	F	2.30	85	Sprn, B	0.34
4	35	F	2.45	80	Sprn	0.63
5	69	M	2.58	82	Sprn, B, S	1.71
6	45	M	2.72	86	Sprn, B	7.90
7	30	F	3.12	104	Sprn, B	1.23
8	48	M	3.17	82	Sprn, B	0.35
9	42	F	2.08	80	Tprn, B,S	0.04
10	35	F	2.53	85	Sprn, B, TH	3.96
11	33	M	2.78	83	Sprn, B	4.42
Mean (SD)	**42 (12)**		**2.76 (0.49)**	**86 (7.7)**		*** 1.13**

Table 6.1: Patient characteristics. Met PC_{20} = methacholine concentration causing a 20% fall in FEV_1 at an initial screening visit (* geometric mean), Sprn = inhaled salbutamol as reqd, Tprn = inhaled terbutaline as reqd, B = inhaled steroid (budesonide or beclomethasone dipropionate), TH = oral theophylline, S = inhaled salmeterol twice daily.

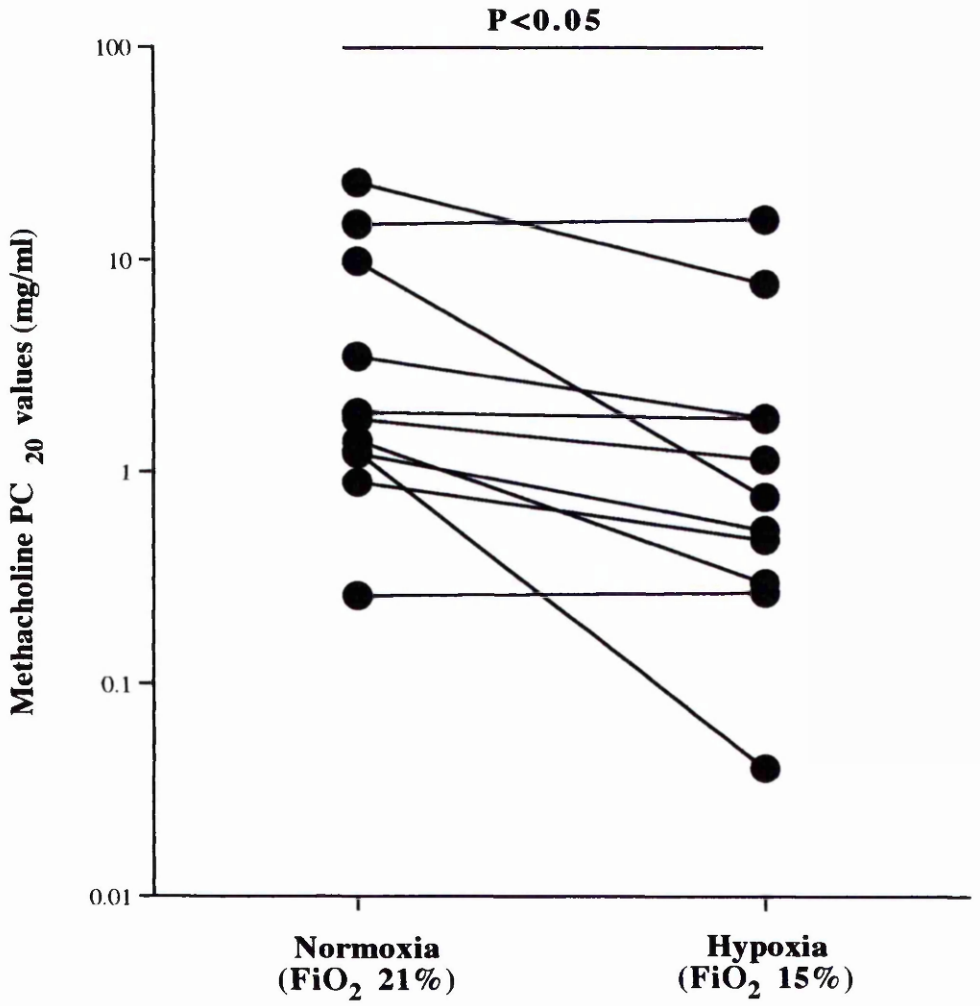

Fig 6.1 The effect of inhaling oxygen (FiO_2 15%) and air (FiO_2 21%) on methacholine PC_{20} values mg/ml in 11 mild asthmatics. ($P<0.05$)

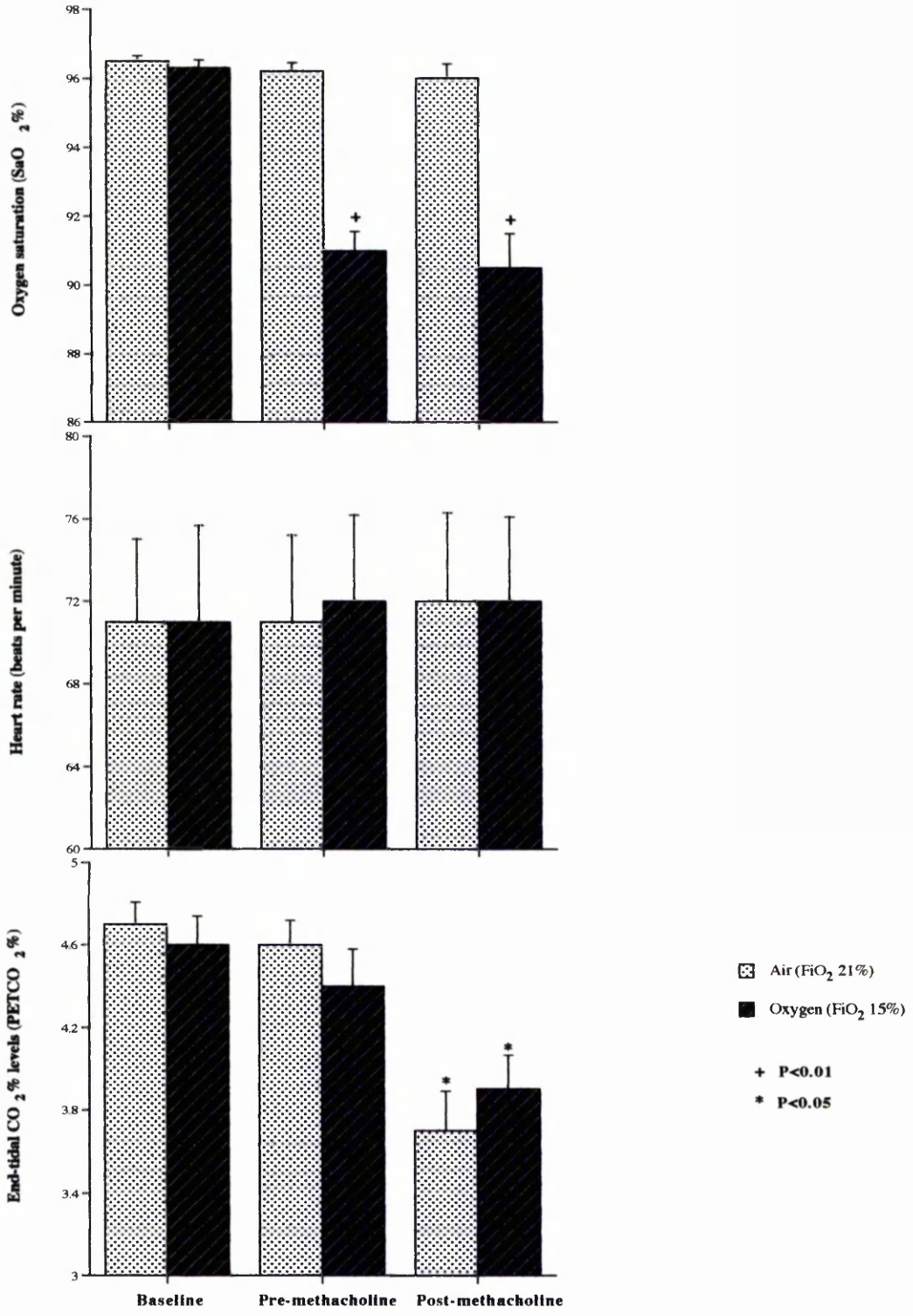

Fig 6.2 The effect of inhaling oxygen (FiO_2 15%) and air (FiO_2 21%) on oxygen saturation (SaO2%), heart rate (beats per minute) and end-tidal CO_2% levels ($PETCO_2$%) before, during and after methacholine inhalation challenge in 11 mild asthmatics.

CHAPTER 7

THE EFFECT OF ACUTE ALTERATIONS IN INSPIRED OXYGEN TENSION ON HISTAMINE INDUCED BRONCHOCONSTRICTION IN PATIENTS WITH MILD ASTHMA

7.1 Introduction

We already know that *in-vitro* results from our own laboratory have suggested that acute alterations in ambient oxygen tension may have significant effects on airway smooth muscle responses to constrictor stimuli.[75-77] In bovine isolated bronchial rings hypoxia potentiates and hyperoxia attenuates airway smooth muscle constriction to methacholine.[75] Paradoxically however in human isolated bronchial rings hypoxia attenuates the contractile response to both methacholine and histamine (chapter 3). *In-vivo* studies in sheep have also suggested that hypoxia potentiates histamine induced bronchoconstriction.[91,92] From our own studies described in chapters 5 and 6 airway hyperresponsiveness to methacholine, in patients with asthma, is potentiated by hypoxia and unaffected by hyperoxia. It is not however known if the effects we have described are specific to methacholine or if changes in airway oxygen tension alter airway responsiveness *in-vivo* to other constrictor stimuli. As we have previously suggested such observations may have relevance to the management of patients with acute exacerbations of asthma. The present study was designed to examine the effect of acute alterations in inspired oxygen tension on airway responsiveness to histamine *in-vivo* in patients with mild stable asthma.

7.2 Subjects and methods

Subjects

Fourteen mild asthmatic patients (8 female) with a mean SD age of 39 (13) years were recruited into the study (table 7.1). All patients had a history of asthma and a FEV_1 > 80% of predicted at an initial screening visit. None of the patients gave a history of any other significant cardiac or respiratory illness. The patients were all receiving inhaled $ß_2$-agonists as required. 12 of the patients were also taking regular inhaled corticosteroids and of these 2 were also receiving the long acting $ß_2$-agonist salmeterol. All of the patients gave written informed consent to the study and the protocol had the approval of the West Ethical Committee.

Study design

Patients were asked to attend the laboratory on four separate study days at approximately the same time each day. Inhaled $ß_2$-agonists were discontinued for 8 hours and salmeterol for 24 hours prior to attendance at the laboratory. Patients were asked to continue their inhaled corticosteroids as normal. At an initial screening visit patients underwent a full physical examination, performed baseline spirometry and then undertook a histamine inhalation challenge test to determine a PC_{20} value for histamine: ie that concentration of histamine causing a 20% fall in FEV_1. Those with a PC_{20} value of less than 8 mg/ml were deemed eligible for the remaining 3 study days.

On three subsequent study days on arrival patients were asked to rest supine for 30 minutes. They were then commenced on the closed breathing circuit already described – chapter 2.6. Following a ten minute run in period breathing air (FiO_2

21%) baseline measurements of FEV_1, heart rate (HR), respiratory rate (RR), oxygen saturation (SaO_2%), end-tidal carbon dioxide and oxygen levels ($PETCO_2$%, $PETO_2$%) and inspired carbon dioxide and oxygen levels (insp CO_2%, insp O_2%) were made. Patients then received the study gas, air (FiO_2 21%), hyperoxia (FiO_2 100%) or a hypoxic gas mixture (FiO_2 15%) for the remainder of that study day. All of the study gases were administered in a randomised double blind fashion by a second operator obscured from the vision of both the patient and the doctor performing the histamine inhalation challenge. 10 minutes after commencing the study the measurements made at baseline were repeated and the histamine test was performed. The study day was terminated when a PC_{20} value had been obtained and the measurements made at baseline had been repeated.

Measurements

Heart rate and oxygen saturation was measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) - see chapter 2.3.

Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) - see chapter 2.3.

Histamine PC_{20} value (mg/ml) was measured according to the technique described by Cockcroft et al[111] and Hargreaves et al[112] – see chapter 2.5.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) - see chapter 2.2.

Statistical analysis

Statistical analysis was performed using a Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabassas) on an Apple Mackintosh LC II computer. Analysis of variance (ANOVA) corrected for multiple comparisons were used to compare measurements between study days. The PC_{20} values were logarithmically transformed before analysis using paired t-tests. A p-value below 0.05 was accepted as significant.

7.3 Results

Baseline measurements: There were no significant differences in baseline measurements of FEV_1, heart rate, respiratory rate, oxygen saturation and end-tidal CO_2% between the three study days.

Histamine PC_{20} values: There was no significant difference ($p>0.05$) in the mean histamine PC_{20} values mg/ml between the three study days. The mean (range) PC_{20} values mg/ml on each study day were: hypoxia study day 1.91 (0.27-27.4) mg/ml, normoxia study day 2.19 (0.3-15.4) mg/ml and hyperoxia study day 2.32 (0.29-19.0) mg/ml (figure 7.1).

FEV_1: There were no significant differences in FEV_1 values ($p>0.05$) between the study days 10 minutes after commencing the study gas prior to inhalation of histamine. The mean (SEM) FEV_1 value litres 10 minutes after commencing the study gas on each day was: hypoxia study day 2.65 (0.18) litres, normoxia study day 2.73 (0.16) litres and hyperoxia study day 2.65 (0.18) litres.

Oxygen saturation: Oxygen saturation was significantly higher ($p<0.01$) on the hyperoxic study day when compared to the normoxic and hypoxic study days both before and after histamine inhalation. Oxygen saturation was significantly lower ($p<0.01$) on the hypoxic study day when compared to the normoxic study day both before and after histamine inhalation. The mean (SEM) oxygen saturation % on completion of each study day was: hypoxia study day 89 (0.9)%, normoxia study day 96 (0.3)% and hyperoxia study day 98 (0.2)% (figure 7.2).

Heart rate: Heart rate was significantly lower ($p<0.01$) on the hyperoxic study day when compared to both the normoxic and hypoxic study days both before and after inhalation of histamine. The mean (SEM) heart rate (bpm) on each study day at baseline, prior to histamine inhalation and after histamine inhalation was: hypoxia

study day 71 (3) bpm, 74 (3) bpm and 72 (3) bpm: normoxia study day 71 (4) bpm, 71 (4) bpm and 74 (4) bpm: hyperoxia study day 71 (3) bpm, 66 (3) bpm and 68 (3) bpm. There were no significant differences in heart rate ($p>0.05$) at any time point between the hypoxic and normoxic study days (figure 7.2).

End-tidal CO_2%: There were no significant differences in the end-tidal CO_2% levels between the three study days at any time point. The mean (SEM) end-tidal CO_2% levels on each study day following inhalation of histamine were: hypoxia study day 3.8 (0.2)%, normoxia study day 4.2 (0.2)% and hyperoxia study day 4.0 (0.1)% (figure 7.2).

Respiratory rate: There were no significant differences in the respiratory rates at any time point between any of the three study days (data not shown).

Raw data for this chapter is included in the Appendix (tables 20A – 21A)

7.4 Discussion

In the two previous chapters we have demonstrated that hypoxia potentiates and hyperoxia has no effect on methacholine induced bronchoconstriction in patients with stable asthma. In the current study both hypoxia and hyperoxia had no effect on histamine induced bronchoconstriction in a similar group of patients.

The rise in oxygen saturation and fall in heart rate seen on the hyperoxic study day and the fall in saturation on the hypoxic study day indicate that our closed breathing circuit has once again achieved the required changes in airway and arterial oxygen tension we have anticipated.[119,131] The absence of a significant rise in heart rate on the hypoxic study day suggests that the level of hypoxia we have produced has not resulted in an increase in resting sympathetic tone. An increase in sympathetic activity may have indirect effects on airway tone which could offset the effect of hypoxia alone.[94] However once again hyperoxia has induced a fall in heart rate suggesting reduced tonic activity of carotid chemoreceptors which may have indirect effects on airway tone hence influencing our results. In this study we have not observed any significant difference in end-tidal carbon dioxide levels between study days either before or after inhalation of histamine suggesting that hypocapnic bronchoconstriction has not influenced our results.[126-129] The nebuliser output which had been checked prior to the study for all of the study gases used and was found to be 0.13 mls/min at a flow rate of 7 litres/min.

These results differ from our own *in-vitro* findings in human isolated bronchial rings where we found that hypoxia attenuated histamine induced smooth muscle constriction (chapter 3). This finding is in keeping with the results of several other

similar studies.[72,73,80,82] In the absence of circulating humoral factors and neural innervation it seems likely that the profoundly low oxygen tension (4%) which was used *in-vitro* has acted directly on smooth muscle causing impairment of the contractile processes. It is not possible to use such low inspired oxygen tensions in our own *in-vivo* studies. Previous studies have shown that hypoxia impairs the contractile strength of airway smooth muscle,[72,73] possibly by impairing the cells ability to generate ATP or by inhibiting the entry of extracellular calcium into smooth muscle cells.[80] It seems likely that this effect does not occur at higher ambient oxygen tensions similar to those used in our *in-vivo* studies. It is also worth noting that our human bronchial rings are largely from elderly smokers undergoing elective thoracotomy for pulmonary malignancies rather than young fit asthmatic patients. This observation may also account for some of the differences between our *in-vitro* and *in-vivo* studies.

Our study has shown no change in airway hyperresponsiveness to histamine at various inspired oxygen tensions in patients with asthma. This contrasts with the findings of Vidruk and Sorkness[88] who found hypoxia potentiated and hyperoxia attenuated histamine induced tracheal constriction in mongrel dogs. It is worth noting that they used an inspired oxygen tension of 12% and that the animals in his studies had been anaesthetised. The anaesthetic drugs in combination with the altered oxygen tensions used may have effects on circulating humoral factors or chemoreceptor activity which have influenced their results. Our study results also differ from those of D'Brot and Ahmed[92] who using conscious sheep also demonstrated potentiation of histamine induced bronchoconstriction by hypoxia. The prevailing pattern of histamine receptors determines the effect of histamine on airway smooth muscle. Since it varies

considerably between species and even between different sites in the same airway,[132] extrapolation of results from animal models to man has created considerable confusion. It is possible therefore that differences between the animal studies and our own can be explained by species variation alone.

Our findings also differ from the results of our own previous study in which we demonstrated that hypoxia potentiates methacholine induced bronchoconstriction in patients with mild asthma. This difference has occurred despite using the same inspired oxygen tension, study design and breathing circuit in both studies. This is an intriguing observation that is difficult to account for. Both methacholine and histamine interact with specific receptors on airway smooth muscle cells. This interaction results in the production of inositol phospholipid metabolites via a complex biochemical pathway. Inositol 1,4,5 triphosphate, an intracellular second messenger, causes release of intracellular calcium ions which promotes smooth muscle contraction.[133] One could speculate that hypoxia has a specific effect on acetylcholine receptors which when methacholine binds to them augments smooth muscle contraction, perhaps via an effect on regulatory G-proteins. Hypoxia (FiO_2 15%) does not seem to have the same effect on histamine receptors *in-vivo* in patients with mild asthma.

In conclusion we have shown that acute alterations in inspired oxygen tension do not alter histamine induced bronchoconstriction in patients with stable asthma.

Patient No	Age (years)	Sex	FEV_1 absolute	FEV_1 predicted	Therapy	PC_{20} histamine (mg/ml)
1	21	F	2.94	97	Sprn, B	1.18
2	28	F	2.84	93	Sprn, B	0.41
3	69	M	2.61	88	Sprn, B	1.31
4	41	F	2.90	116	Sprn, B	2.00
5	42	F	2.27	88	Tprn, B, S	5.86
6	31	F	2.27	80	Sprn	2.98
7	57	F	2.19	83	Sprn, B	1.81
8	48	M	3.24	83	Sprn	1.27
9	29	M	4.20	87	Sprn, B	2.65
10	33	M	4.28	81	Sprn, B	3.03
11	35	F	2.59	92	Sprn, B, S	3.46
12	32	M	3.84	85	Sprn, B	0.29
13	34	M	4.14	91	Sprn, B	6.80
14	46	F	2.30	97	Sprn, B	1.27
Mean (SD)	**39 (12.8)**		**3.04 (0.77)**	**91 (9.2)**		*** 1.79**

Table 7.1: Patient characteristics. Histamine PC_{20} = histamine concentration causing a 20% fall in FEV_1 at an initial screening visit (* geometric mean) Sprn = inhaled salbutamol as reqd, Tprn = inhaled terbutaline as reqd, B = inhaled steroid (budesonide or beclomethasone dipropionate), S = inhaled salmeterol twice daily.

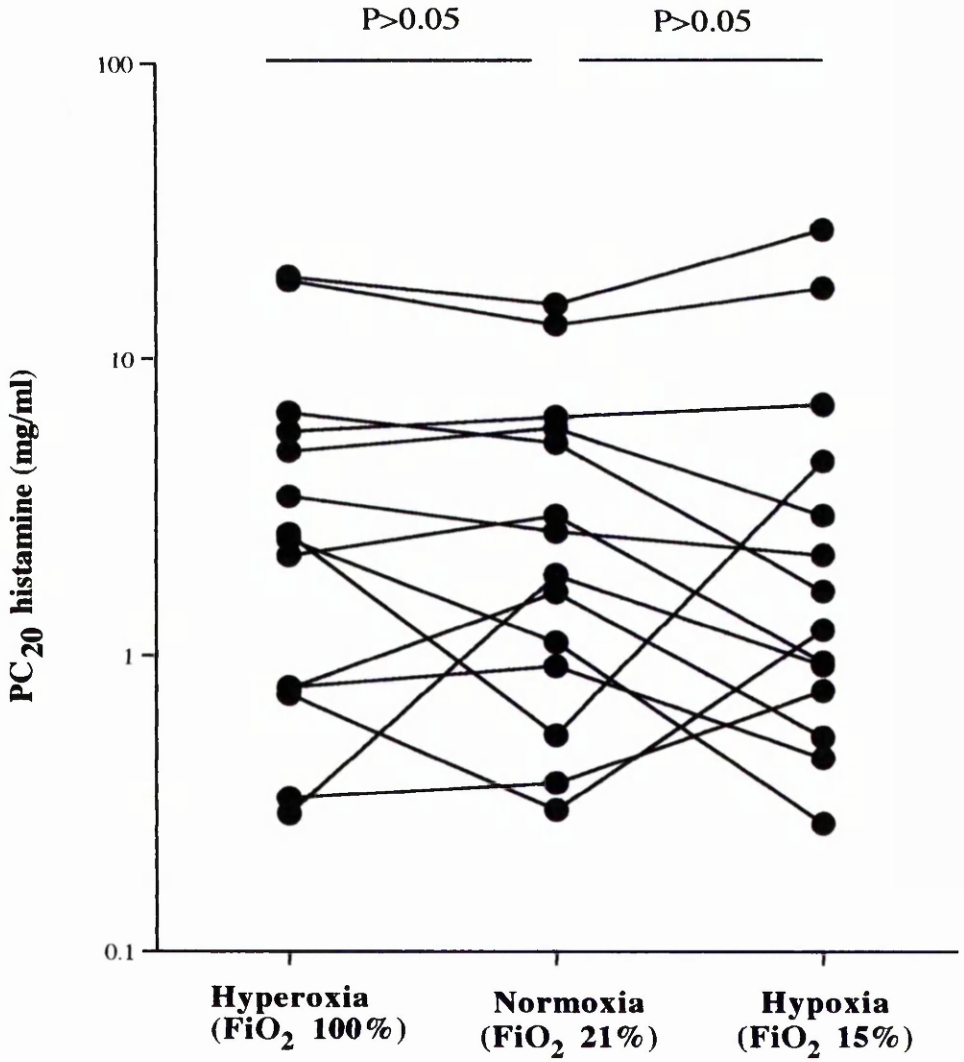

Fig 7.1 The effect of inhaling oxygen (FiO_2 15%, 21% and 100%) histamine PC_{20} values mg/ml in 14 mild asthmatics. (P>0.05)

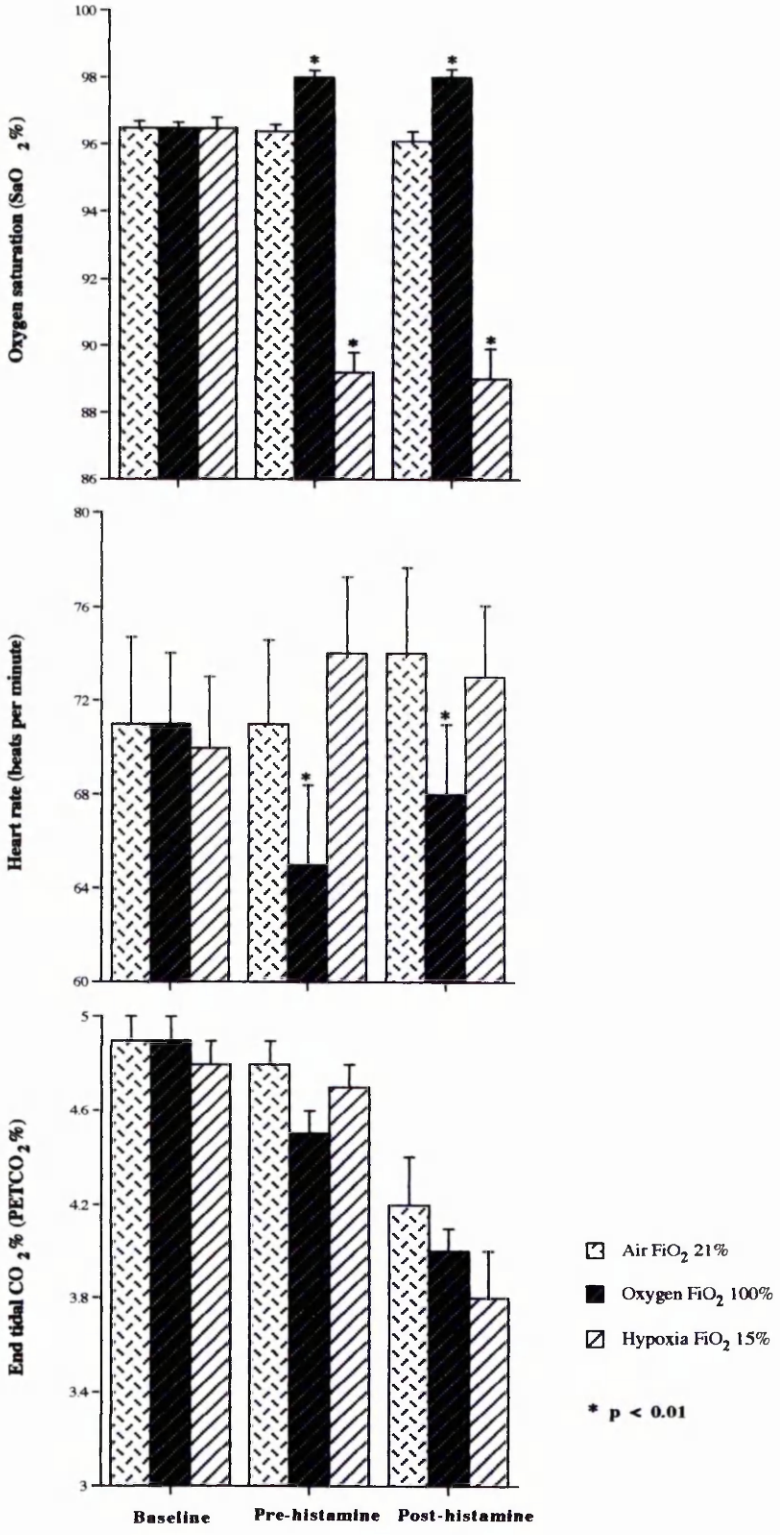

Fig 7.2 The effect of inhaling oxygen (FiO_2 15%, 21% and 100%) on oxygen saturation (SaO_2%), heart rate (beats per minute) and end-tidal CO_2% levels ($PETCO_2$%) before, during and after a histamine inhalation challenge in 14 mild asthmatics.

CHAPTER 8

THE EFFECT OF ACUTE HYPEROXIA ON THE BRONCHODILATOR RESPONSE TO SALBUTAMOL IN PATIENTS WITH ASTHMA

8.1 Introduction

We have focussed, in the last three chapters, on the effect of acute alterations in inspired oxygen tension on bronchoconstrictor responses in patients with asthma. Our own *in-vitro* studies on isolated bronchial rings had also suggested that acute alterations in ambient oxygen tension had effects on airway responses to bronchodilator stimuli.[75,76] In bovine isolated bronchial rings hyperoxia (O_2 95%) potentiates salbutamol induced smooth muscle relaxation in rings preconstricted with methacholine.[75] In human isolated bronchial rings hypoxia attenuates the bronchodilator response to salbutamol whereas hyperoxia has no effect on salbutamol induced smooth muscle relaxation when compared to normoxia (chapter 3). It has previously been shown that *in-vivo* hyperoxia attenuates exercise induced bronchoconstriction in patients with asthma.[107,108] No previous studies, to our knowledge, have investigated the effect of acute hyperoxia on bronchodilator responses in patients with asthma. If acute hyperoxia can alter the airway response to bronchodilators such as salbutamol then the administration of high inspired oxygen concentrations during acute exacerbations of asthma may not only improve gas exchange but could also enhance the effects of both nebulised and intravenous bronchodilators which are administered. The present study was designed to examine the effect of breathing 100% oxygen on the bronchodilator response to salbutamol in patients with stable asthma.

8.2 Subjects and methods

Subjects

Twelve adult asthmatic patients (three female) with a mean (SD) age of 47 (14) were recruited into the study (table 8.1). All of the patients had a diagnosis of asthma and no patient had a history of any other significant cardiac or respiratory illness. All of the patients were taking inhaled $ß_2$-agonists as required and 11 regular inhaled corticosteroids. Two were also receiving the long acting $ß_2$-agonist salmeterol and 2 had been provided with a home nebuliser to take salbutamol on an as required basis. One patient was receiving a short acting oral theophylline. The research was carried out in accordance with the Declaration of Helsinki (1989) and all of the patients gave written informed consent to the study protocol which had the approval of the West Ethics Committee.

Study design

Patients were asked to attend the laboratory on five separate days at approximately the same time on each day. Prior to each day patients were required to withhold all inhaled $ß_2$-agonists for 8 hours, long acting $ß_2$-agonists such as salmeterol for 24 hours and oral theophyllines for 48 hours. They were asked to continue their normal inhaled corticosteroids as usual. At an initial screening visit patients had their FEV_1 measured before and after inhaling 5mg of nebulised salbutamol. Those patients who demonstrated a 15% rise in FEV_1 following nebulised salbutamol were deemed eligible for the remaining four study days.

On four subsequent study days after 30 minutes of supine rest patients were commenced on the closed breathing circuit already described – chapter 2.6.

Following a ten minute run-in period during which patients breathed air (FiO_2 21%) baseline measurements of FEV_1, oxygen saturation (SaO_2%), respiratory rate (RR), heart rate (HR), inspired oxygen and carbon dioxide levels (insp O_2%, insp CO_2%) and expired oxygen and carbon dioxide levels (PET O_2%, PET CO_2%) were made. Venous blood was also taken for assay of plasma catecholamines. Patients were then randomised to inhale either air (FiO_2 21%) on two study days or oxygen (FiO_2 100%) on the remaining two study days. The study gases were administered in a randomised double blind fashion by an observer hidden from the view of both the subject and the operator. After 10 minutes breathing the study gas through the closed breathing circuit the patients received either three incremental concentrations of nebulised salbutamol (0.05 mg/ml, 0.17 mg/ml, 5 mg/ml) or placebo (normal saline) at 15 minute intervals again in a double blind fashion. Nebulised salbutamol was given through a micro cirrus nebuliser (Intersurgical Ltd, Crane House, Wokingham, Berkshire) for 2 minutes driven by either the hyperoxic or normoxic study gas at a flow rate of 7 L/min to produce a nebuliser output of 0.13 mls/min. Baseline measurements were repeated thirteen minutes after each nebulisation until completion of the study day.

Measurements

Heart rate and oxygen saturation were measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) - see chapter 2.3.

Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) - see chapter 2.3.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) - see chapter 2.2.

Plasma catecholamines were measured using reverse phase high performance liquid chromatography - see chapter 2.8.

Statistical analysis

Statistical analysis was performed using a Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabassas) on an Apple Mackintosh LC II computer. Analysis of variance (ANOVA) corrected for multiple comparisons was used to compare measurements made at baseline and following each dose of nebulised saline or salbutamol between study days. A p value of <0.05 was considered significant.

8.3 Results

Baseline measurements: There were no significant differences in baseline measurements of heart rate, respiratory rate, oxygen saturation, FEV_1 and end-tidal CO_2% between the four study days.

Absolute change in FEV_1 from baseline: There was no significant difference ($p>0.05$) in the mean change in FEV_1 (litres) from baseline after breathing the study gas for 10 minutes, but before administration of nebulised salbutamol or placebo on any study day. The mean (SEM) change in FEV_1 (litres) from baseline on each study day was: hyperoxia/salbutamol study day –0.08 (0.05) litres, normoxia/salbutamol study day – 0.06 (0.09) litres, hyperoxia/placebo study day 0.01 (0.05) litres and normoxia/placebo study day 0.05 (0.07) litres. There was no significant difference in the mean maximum change in FEV_1 (Litres) from baseline after nebulised salbutamol on the normoxic and hyperoxic study days. The mean maximum change in FEV_1 from baseline was significantly greater ($p<0.01$) on the days on which nebulised salbutamol was administered when compared to placebo (nebulised saline). The mean maximum (SEM) change in FEV_1 (litres) from baseline was on the: hyperoxia/salbutamol study day 0.37 (0.08) litres, normoxia/salbutamol study day 0.36 (0.05) litres, hyperoxia/placebo study day –0.04 (0.08) litres and normoxia/placebo study day –0.08 (0.10) litres (figure 8.1).

Oxygen saturation: Oxygen saturation was significantly greater ($p < 0.01$) on both hyperoxic study days at 15, 30 and 45 minutes when compared to the normoxic study days. The mean (SEM) oxygen saturation % at 45 minutes on each study day was hyperoxia/salbutamol study day 98 (0.5) %, normoxia/salbutamol study day 96 (0.5) %, hyperoxia/placebo study day 98 (0.5) % and normoxia/placebo study day 96 (0.5) % (figure 8.2).

Heart rate: The mean maximum fall in heart rate from baseline was significantly greater ($p < 0.05$) at 15, 30 and 45 minutes on the study days on which the high inspired oxygen tension was administered to the subjects. The mean (SEM) fall in heart rate (bpm) from baseline following completion of each study day was: hyperoxia/salbutamol study day -5.2 (1.3) beats per minute, normoxia/salbutamol study day -2.1 (1.3) beats per minute, hyperoxia/placebo study day –4.6 (1.9) beats per minute and normoxia/placebo study day –3.1 (0.7) beats per minute (figure 8.1).

End-tidal CO_2%: The mean (SEM) end-tidal CO_2% was significantly lower ($p < 0.05$) on the hyperoxia/placebo study day when compared to the normoxia/placebo day at 45 minutes only. There were no other time points between study days that end-tidal CO_2% levels were significantly different. End-tidal CO_2% levels were similar at all time points on the study days on which salbutamol was administered. The mean (SEM) end-tidal CO_2% levels at 45 minutes on each study day were: hyperoxia/salbutamol study day 4.2 (0.19)%, normoxia/salbutamol study day 4.1 (0.25)%, hyperoxia/placebo study day 3.9 (0.2)% and normoxia/placebo study day 4.2 (0.2)% (figure 8.2).

Plasma catecholamine levels: There were no significant differences in circulating catecholamine levels at 15, 30 and 45 minutes between the study days ($p>0.05$). The mean (SEM) circulating adrenaline levels at 45 minutes on each study day were: hyperoxia/salbutamol study day 0.11 (0.02) nmol/l, normoxia/salbutamol study day 0.19 (0.03) nmol/l, hyperoxia/placebo study day 0.07 (0.02) nmol/l and normoxia/placebo study day 0.09 (0.02) nmol/l. The mean (SEM) circulating noradrenaline levels at 45 minutes on each study day were: hyperoxia/salbutamol study day 2.11 (0.39) nmol/l, normoxia/salbutamol study day 2.81 (0.77) nmol/l,

hyperoxia/placebo study day 2.22 (0.37) nmol/l and normoxia/placebo study day 1.91 (0.41) nmol/l.

Respiratory rate: There was no significant differences in respiratory rate (breaths per minute) at 10, 15, 30 and 45 minutes between any of the study days (data not shown).

Raw data for this chapter is included in the Appendix (tables 22A – 26A)

8.4 Discussion

The results from our study have confirmed that acute hyperoxia (FiO_2 100%) has no effect on the bronchodilator response to salbutamol in patients with stable asthma.

We have observed in our patients a significant rise in oxygen saturation and fall in heart rate on the hyperoxic study days when compared to the normoxic study days.[119,121] These observations would suggest that once again our closed breathing circuit has achieved a high arterial oxygen tension. Airway oxygen tension will be similar to that of the inspired gas, in this case 100%. These changes in arterial and airway oxygen tensions should have allowed appropriate testing of our hypothesis. The possibility of hyperoxia causing an increase in minute ventilation by an indirect action on either the brainstem or peripheral chemoreceptors was raised as a possible confounding variable in our study.[119] An increase in minute ventilation causes hypocapnia which is known to cause increased airway tone.[126-129] A fall in end-tidal CO_2% would be expected to accompany any increase in minute ventilation. In this study we have observed a significant fall in end-tidal CO_2% at 45 minutes on the hyperoxia/placebo day compared with the other days. This observation raises the possibility that in our study breathing 100% oxygen has caused mild hypocapnia which has increased airway tone and opposed any beneficial effects of hyperoxia alone on salbutamol induced bronchodilation in our patients. It is worth noting however that in our study no difference was observed, in end tidal CO_2% levels, between the hyperoxia/salbutamol and normoxia/salbutamol study days suggesting that this is unlikely. We have in our study used the fall in heart rate from baseline as a surrogate marker of raised arterial oxygen tension in our patients.[119,121,131] The fall in heart rate that accompanies hyperoxia has been attributed to a reduction in tonic

activity in carotid chemoreceptors.[121] The influence of carotid chemoreceptors on airway tone remains controversial and poorly understood.[85,98,99] It is possible that alterations in carotid and peripheral chemoreceptors tonic activity has had influences on airway tone in our patients which have obscured any direct effect of hyperoxia on salbutamol induced airway relaxation. Circulating catecholamines have influences on airway tone *in-vivo* in patients with asthma.[94] There was however no significant difference in plasma adrenaline and noradrenaline levels between any of our study days at any time point suggesting that their levels have not influenced our results in this study. As in the other studies the nebuliser output was found to be 0.13 mls/min at a flow rate of 7 litres/min for oxygen (FiO_2 100%) and air (FiO_2 21%).

Once again we are left with significant differences between our *in-vitro* findings in bovine[75,76] and human isolated bronchial rings and our *in-vivo* clinical findings in patients with asthma (chapter 3). While species variation may explain the differences in findings from our *in-vitro* studies on bovine bronchial rings it certainly does not account for the differences in human rings. It is worth noting that our human bronchial rings came mainly from patients undergoing elective thoracotomy for resection of lung cancer. These are predominantly elderly patients with a smoking related lung condition. As such their airways may well be significantly different from those of a younger patient with asthma. It is more likely that hyperoxia has an indirect effect on airway tone via unrecognised neural or humoral pathways. The most striking difference between our *in-vitro* and *in-vivo* studies, using high oxygen tensions, is the absence of neural innervation and circulating humoral factors in our bronchial rings. Against this hypothesis is the absence of change in FEV_1 that we have found in our patients breathing 100% oxygen for 10 minutes before receiving

nebulised salbutamol or placebo. This would suggest that breathing oxygen has not had a direct effect on resting airway tone in our patients.

It has been suggested from *in-vitro* studies that the density of $ß_2$-receptors on cell membranes may be influenced by the ambient oxygen tension.[115] A reduction in ambient oxygen tension may reduce β_2-receptor density and conversely hyperoxia may increase receptor numbers. It is not clear however how long alterations in receptor density take following a change in ambient oxygen tension. Our study has only examined the effect of hyperoxia on salbutamol induced relaxation over 60 minutes. It is possible that any change β_2 receptor density takes longer than this to develop. Such an observation may have influenced our results.

We conclude from our study that breathing 100% oxygen for a period of 60 minutes has no effect on salbutamol induced bronchodilation in patients with stable asthma.

Patient No	Age (years)	Sex	FEV_1 absolute value (L) pre-sal	FEV_1 absolute value (L) post-sal	FEV_1 post-sal % predicted	Current treatment
1	46	F	1.55	2.01	84	sal prn, B
2	48	M	2.54	3.38	94	sal prn, B, S, TH
3	55	M	1.84	3.08	75	sal prn, B
4	69	M	2.18	2.69	81	sal prn, B
5	41	M	2.50	2.97	76	sal prn, B
6	42	M	2.18	3.14	90	sal prn,B
7	61	M	1.04	1.37	40	sal prn, B, P, Neb
8	34	F	2.28	2.87	104	T prn, B
9	40	M	1.45	2.45	77	sal prn, B, P, Neb
10	26	F	1.89	2.31	72	sal prn, B, S
11	70	M	1.81	2.21	64	sal prn, B
12	39	M	3.33	3.87	96	sal prn
Mean (SD)	**47 (14)**		**2.05 (0.60)**	**2.70 (0.67)**	**79 (17)**	

Table 8.1: Patient characteristics. sal prn = inhaled salbutamol as required, T prn = inhaled terbutaline as required, B = inhaled steroid (beclomethasone or budesonide), P = oral prednisilone, S = inhaled salmeterol twice daily, Th = oral theophylline, Neb = nebulised salbutamol and ipratropium bromide as required, pre-sal = pre-salbutamol FEV_1, post-sal = post-salbutamol FEV_1.

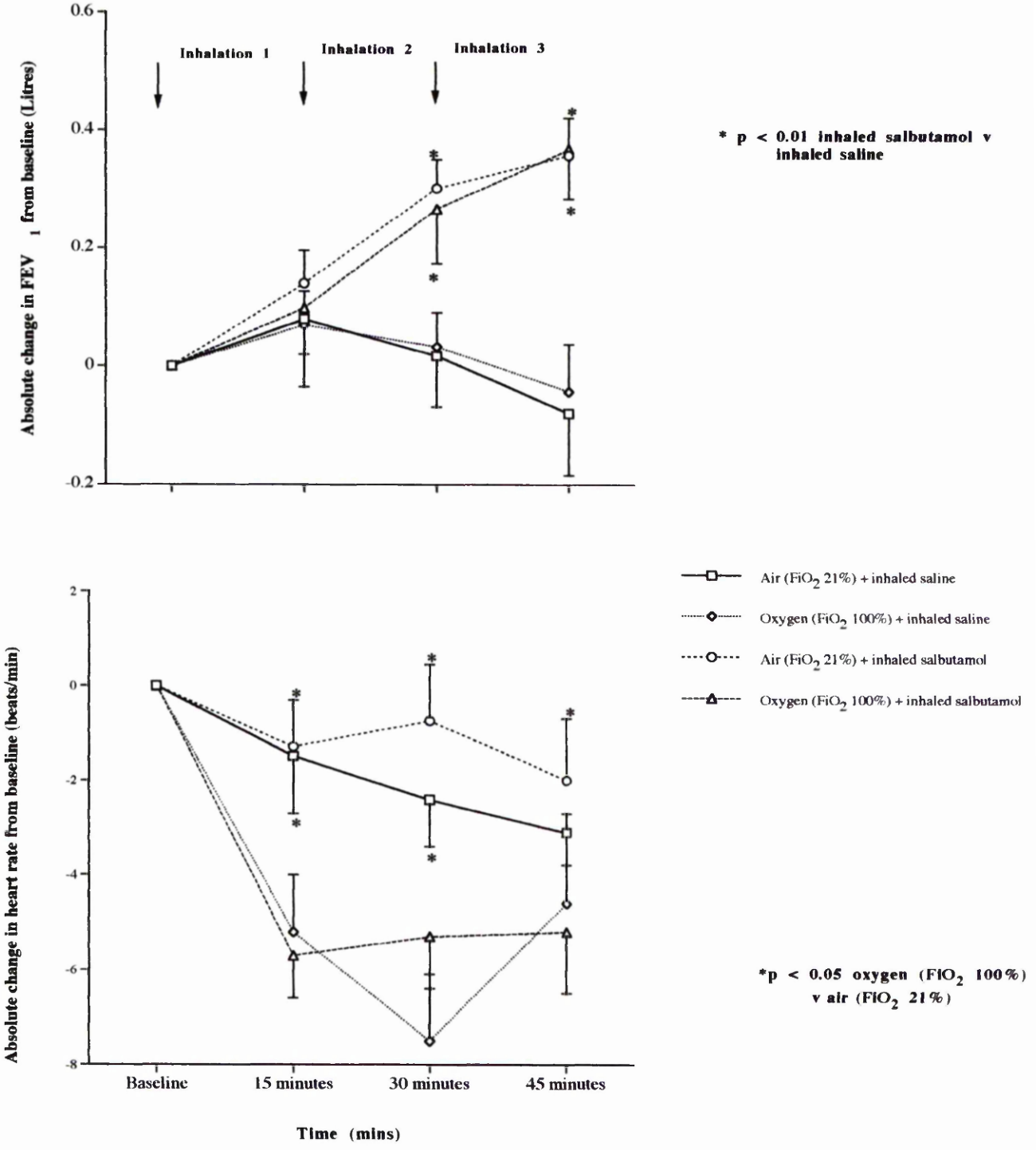

Fig 8.1 The effect of 3 incremental doses of nebulised salbutamol or placebo on absolute change in FEV_1 and heart rate from baseline whilst breathing air (FiO_2 21%) or oxygen (FiO_2 100%) in twelve asthmatic patients.

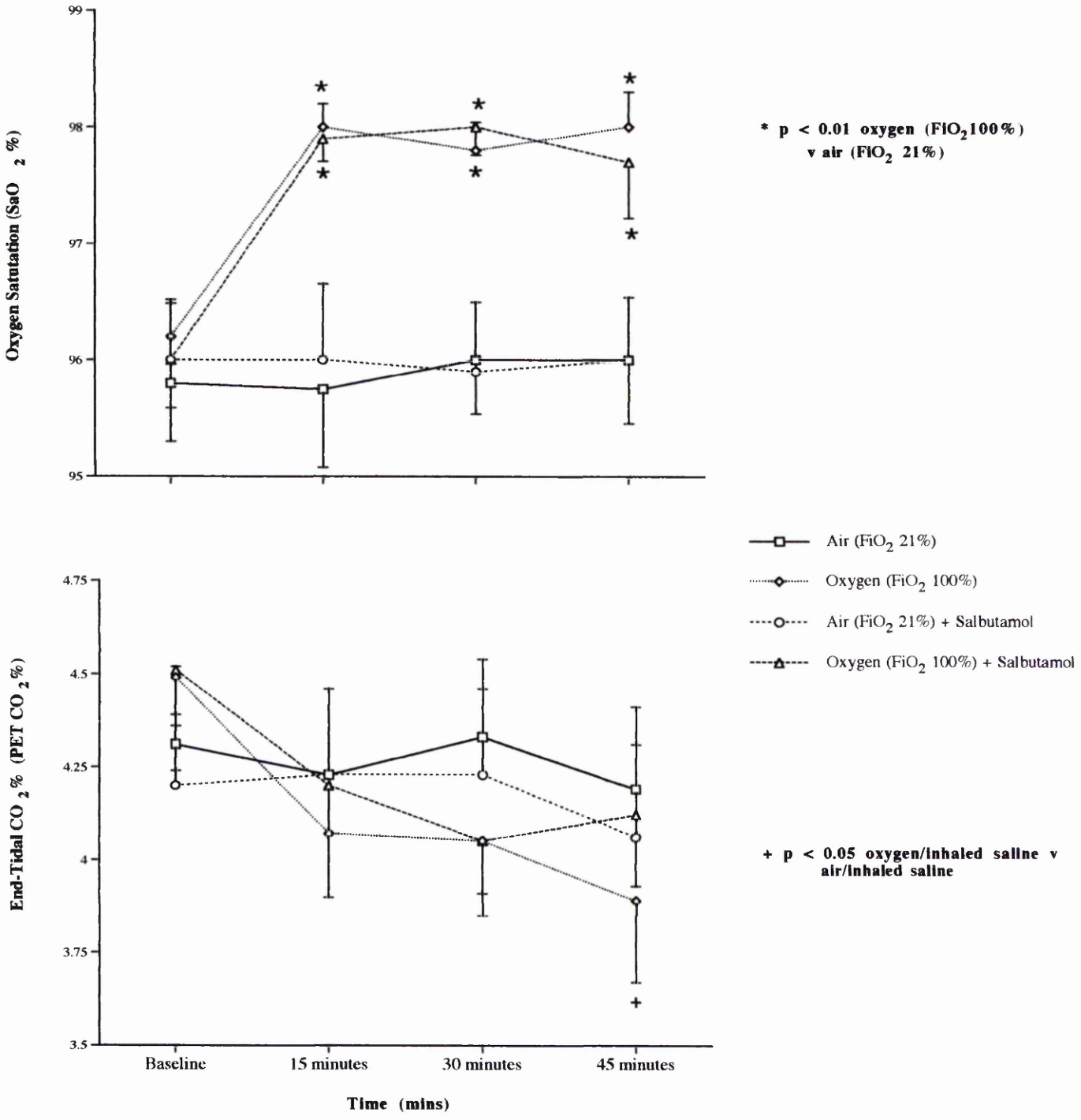

Fig 8.2 The effect of 3 incremental doses of nebulised salbutamol or placebo on oxygen saturation (SaO_2%) and end-tidal CO_2% levels ($PETCO_2$%) breathing air (FiO_2 21%) or oxygen (FiO_2 100%) in twelve asthmatic patients.

CHAPTER 9

THE EFFECT OF ACUTE ALTERATIONS IN INSPIRED OXYGEN TENSION ON THE BRONCHODILATOR RESPONSE TO SALBUTAMOL IN PATIENTS WITH ASTHMA

9.1 Introduction

In-vitro studies have suggested that acute alterations in ambient oxygen tension may significantly alter the effect of bronchodilator stimuli on airway smooth muscle tone.[75-78] We have found in our own laboratory that in bovine isolated bronchial rings, reversal of methacholine-induced tone by the bronchodilator salbutamol is significantly enhanced by hyperoxia and attenuated by hypoxia.[75] *In-vitro* studies described in chapter 3, also from our own laboratory have suggested that in human isolated bronchial rings acute hypoxia significantly reduces the ability of the bronchodilator salbutamol to reverse methacholine induced airway tone. Such observations may have particular relevance to the management of acute exacerbations of asthma where patients admitted to hospital are often hypoxaemic.[61,67,68] In the previous chapter we have suggested that breathing 100% oxygen has no effect on salbutamol induced relaxation of airway smooth muscle in patients with stable asthma. The effect of acute hypoxia on airway smooth muscle relaxation in patients with asthma has not to our knowledge been previously examined. The present study was designed to examine the effect of acute hypoxia on salbutamol induced bronchodilation in patients with asthma.

9.2 Methods

Subjects

Twelve adult asthmatic patients (2 female) with a mean (SD) age 39 (8) years agreed to participate in the study (table 9.1). All of the patients gave a history of asthma and none had any other significant cardiac or respiratory disease. All of the patients were taking inhaled $ß_2$-agonists as required and eleven regular inhaled corticosteroids. Three were also receiving the long acting $ß_2$-agonist salmeterol and one had been provided with a home nebuliser to take salbutamol on an as required basis. One patient was receiving a short acting oral theophylline. The research was carried out in accordance with the Declaration of Helsinki (1989) and all of the patients gave written informed consent to the study protocol which had the approval of the West Ethics Committee.

Study design

Patients were asked to attend the study laboratory on five separate days at approximately the same time each day. Prior to each study day patients were asked to withhold their inhaled and nebulised $ß_2$-agonists for 8 hours and inhaled salmeterol for 24 hours. Oral theophyllines were discontinued 48 hours prior to each study day. Patients were asked to continue taking their inhaled corticosteroids as usual. Patients who at an initial visit, following 30 minutes of supine rest, demonstrated an improvement in FEV_1 of more than 15% following the administration of 5mg of nebulised salbutamol were deemed eligible for the remaining four study days.

On subsequent study days after arrival at the laboratory patients were asked to rest in a supine position for 30 minutes following which they were connected to a closed breathing circuit for the delivery of all study gases. After breathing air (FiO_2 21%) for 10 minutes

through the closed breathing circuit, baseline measurements of oxygen saturation (SaO_2%), heart rate (HR), respiratory rate (RR), inspired oxygen and carbon dioxide levels (insp O_2%, insp CO_2%) and end-tidal oxygen and carbon dioxide levels ($PETO_2$%, $PETCO_2$%) were made.

After the baseline measurements had been recorded patients were then randomised, in a double blind fashion, to breathe either a hypoxic gas mixture (FiO_2 15%) on two days or oxygen (FiO_2 100%) on two further study days for the remainder of that day. Ten minutes after commencing the study gas for that day the measurements made at baseline were repeated. Subsequently at fifteen minute intervals in a randomised double blind fashion patients received either three incremental doses of nebulised salbutamol (0.05 mg/ml, 0.17 mg/ml, 5 mg/ml) on two days or placebo (nebulised saline) on two further days. Measurements were repeated thirteen minutes after each nebulisation until completion of the study day. Each dose of salbutamol or saline was given through a micro cirrus nebuliser (Intersurgical Ltd, Crane House, Wokingham, Berkshire) for two minutes driven by a hypoxic gas mixture (British Oxygen Corporation, Special Gases Division, Manchester) or oxygen (FiO_2 100%) at a rate of 6 litres/min to produce a nebuliser output of 0.11 mls/min.

Measurements

Heart rate and oxygen saturation were measured using a pulse oximetry probe (Datex Division of Instrumentarium Corp, Helsinki, Finland) - see chapter 2.3.
Respiratory rate, inspired and expired oxygen and carbon dioxide levels were measured using an OSCARoxy TM multigas monitor (Datex of Instrumentarium Corp, Finland, Helsinki) - see chapter 2.3.

FEV_1 was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckinghamshire, United Kingdom) - see chapter 2.2.

Statistical analysis

Statistical analysis was performed using a Statview software package (Brainpower Inc, 24009 Ventura Boulevard, Suite 250, Calabassas) on an Apple Mackintosh LC II computer. Analysis of variance (ANOVA) corrected for multiple comparisons was used to compare measurements made at baseline and following each dose of nebulised saline or salbutamol between study days. A p value of <0.05 was considered significant.

9.3 Results

Baseline measurements: There were no significant differences in the baseline measurements of FEV_1, heart rate, respiratory rate, oxygen saturation, end-tidal CO_2% made between any of the study days.

Absolute change in FEV_1 from baseline: The mean (SEM) maximum change in FEV_1 from baseline was significantly greater ($p<0.05$) on the days on which nebulised salbutamol was administered: hyperoxia/salbutamol 0.49 (0.11) litres, hypoxia/salbutamol 0.40 (0.09) litres when compared to the study days on which nebulised saline was administered: hyperoxia/placebo –0.013 (0.05) litres, hypoxia/placebo –0.074 (0.079) litres (figure 9.1). There was however no significant difference in the mean maximum change in FEV_1 (litres) from baseline at any time point between the study days on which nebulised salbutamol was given. There was no significant difference in the mean % change in FEV_1 from baseline on any study day 10 minutes after being commenced on the study gas for that day but prior to inhalation of nebulised salbutamol or saline. The mean (SEM) % change in FEV_1 from baseline 10 minutes after commencing each study gas was: hyperoxia/salbutamol –0.03 (3.5) %, hypoxia/salbutamol –0.02 (3.6) %, hyperoxia/placebo 0.04 (2.1) % and hypoxia/placebo 0.03 (2.5) %.

Oxygen saturation: Oxygen saturation was significantly higher ($p<0.01$) on the days on which the hyperoxic gas mixture was administered when compared to the days on which the hypoxic gas mixture was inhaled at all time points. The mean (SEM) oxygen saturation % at the completion of each study day was: hyperoxia/salbutamol study day 98 (0.24)%, hypoxia/salbutamol study day 87 (0.92)%, hyperoxia/placebo study day 98 (0.26)% and hypoxia/placebo study day 88 (0.84)% (figure 9.2).

Heart rate: The fall in heart rate from baseline was significantly greater ($p<0.01$) on the days that patients inhaled the hyperoxic gas mixture when compared to the days on which they inhaled the hypoxic gas mixture at all time points. The mean (SEM) fall in heart rate from baseline (beats per minute) at 15, 30 and 45 minutes on each study day was: hyperoxia/salbutamol study day –4 (2) bpm, -4 (2) bpm, -6 (1) bpm: hypoxia/salbutamol study day 2 (1) bpm, -1 (1) bpm, 1 (2) bpm: hyperoxia/placebo study day –6 (1) bpm, -8 (1) bpm, -10 (2) bpm: hypoxia/placebo study day 3 (2) bpm, 2 (1) bpm, 1 (2) bpm (figure 9.1).

End-tidal carbon dioxide: There were no significant differences in end-tidal carbon dioxide % and levels between any of the study days at any time point. The mean (SEM) end-tidal CO_2% levels on completion of each study day were: hyperoxia/salbutamol study day 4.6 (0.08)%, hypoxia/salbutamol study day 4.5 (0.11)%, hyperoxia/placebo study day 4.5 (0.14)% and hypoxia/placebo study day 4.6 (0.10)%.

Respiratory rate: There were no significant differences in respiratory rate between study days at any time point (data not shown)

Raw data for this chapter is included in the Appendix (tables 27A – 30A)

9.4 Discussion

Acute hypoxia *in-vivo* in patients with stable asthma has not in our study altered the ability of salbutamol to relax airway smooth muscle. This is in contrast to our *in-vitro* study (chapter 3) in human isolated bronchial rings where acute hypoxia significantly attenuated the ability of salbutamol to relax human isolated bronchial rings preconstricted with methacholine.

The changes in oxygen saturation that we have observed in our patients would suggest that our closed breathing circuit has achieved significant changes in arterial and airway oxygen tensions.[119,121] The presence of these changes is also supported by the fall in heart rate which we have observed on the study days that our patients inhaled oxygen (FiO_2 100%).[119] Reduced tonic activity in carotid chemoreceptors in response to hyperoxia, suggested by the fall in heart rate we have observed in our study, may have led indirectly to alterations in airway tone in our subjects which could potentially have influenced our results. An increase in vagal tone while breathing 100% oxygen may have indirectly caused an increase in airway smooth muscle tone significantly affecting the results of our study. We have not observed any differences in end-tidal carbon dioxide levels between the study days suggesting that alterations in minute ventilation following inspiration of our gas mixtures has not caused hypocapnic bronchoconstriction.[126-129] Nebuliser output for both the hypoxic and hyperoxic gas mixtures was found to be 0.11 mls/min at a flow rate of 6 l/min. This had been checked prior to the commencement of the study.

There are several possible explanations for the differences we have observed between our *in-vitro* and *in-vivo* studies. The human isolated bronchial rings we have used

were from stable patients undergoing elective thoracic surgery who did not have asthma. The lowest inspired oxygen tension used in the *in-vivo* study was 15% in contrast to 4% in the *in-vitro* study. Previous *in-vitro* studies have suggested that the contractile strength of airway smooth muscle is significantly impaired by profound hypoxia.[72,73] If hypoxic relaxation had occurred in our *in-vitro* study, one might have expected that hypoxia would potentiate the ability of salbutamol to relax smooth muscle rather than attenuate it as we have previously observed (chapter 3). That this has not occurred suggests that hypoxia has other effects *in-vitro*, which may include down regulation of ß-receptors[115] or uncoupling of ß-receptors from their regulatory G-proteins,[116] which have offset the direct action of hypoxia on smooth muscle. The presence of circulating humoral factors and intact neural innervation in our subjects *in-vivo*, which hypoxia and hyperoxia may directly affect, further complicates the interpretation of our results in this study. Circulating catecholamine levels may affect airway tone *in-vivo*.[94] In previous studies using the same closed breathing circuit and inspired oxygen tension, we have however shown no significant changes in circulating catecholamine levels. It is also worth considering that the concentrations of salbutamol used in the organ baths are significantly greater than the cumulative doses administered to our patients and this may also explain the differences in our findings.

We conclude that acute hypoxia has no effect on salbutamol induced bronchodilation in patients with stable asthma when compared to normoxia and hyperoxia.

Patient No	Age (years)	Sex	FEV1 absolute value (L) pre-sal	FEV1 absolute value (L) post-sal	FEV1 post-sal % predicted	Current treatment
1	44	M	2.14	2.82	83	sal prn, B
2	52	M	2.70	3.15	91	sal prn, B
3	39	M	3.12	3.60	90	sal prn
4	52	M	2.31	2.72	66	sal prn, B
5	34	F	3.02	3.52	106	sal prn, B
6	35	M	3.15	3.80	88	sal prn,B
7	49	M	2.08	2.75	77	sal prn, B, S, Neb
8	36	M	2.85	3.29	98	sal prn
9	29	M	2.94	3.59	79	sal prn, B
10	32	F	2.33	2.73	107	sal prn, B, S
11	29	M	3.34	3.93	87	sal prn, B
12	36	M	2.52	2.97	75	sal prn, B, S, Th
Mean	**39**		**2.71**	**3.24**	**87**	
(SD)	**(8)**		**(0.42)**	**(0.44)**	**(11)**	

Table 9.1: Patient characteristics. sal prn = inhaled salbutamol as required, B = inhaled steroid (beclomethasone or budesonide), S = inhaled salmeterol twice daily, Th = oral theophylline, Neb = nebulised salbutamol and ipratropium bromide as required, pre-sal = pre-salbutamol FEV_1, post-sal = post-salbutamol FEV_1.

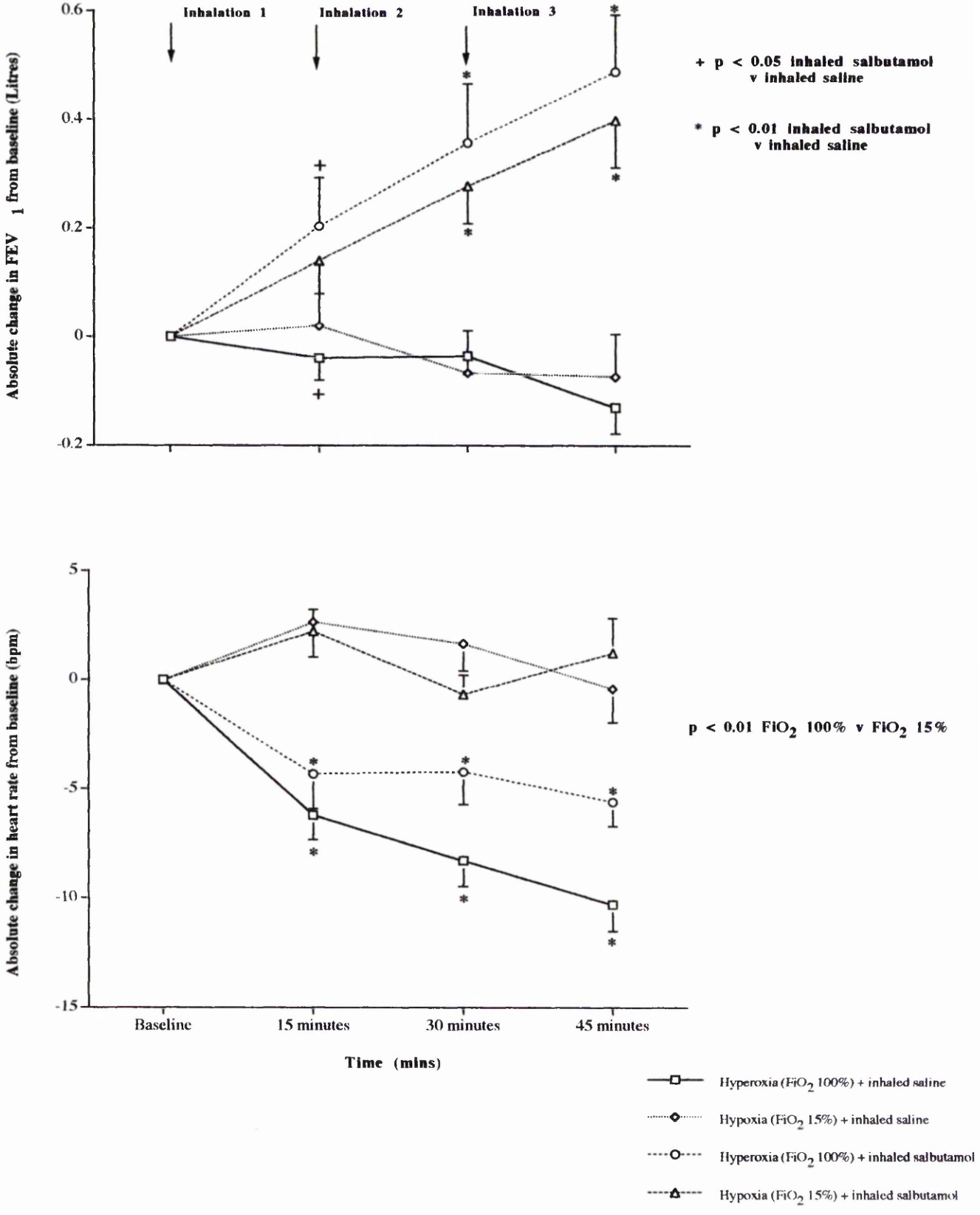

Fig 9.1 The effect of 3 incremental doses of nebulised salbutamol or placebo on absolute change in FEV_1 (litres) and heart rate (beats per minute) from baseline in 12 asthmatic patients breathing oxygen tensions of 100% and 15%.

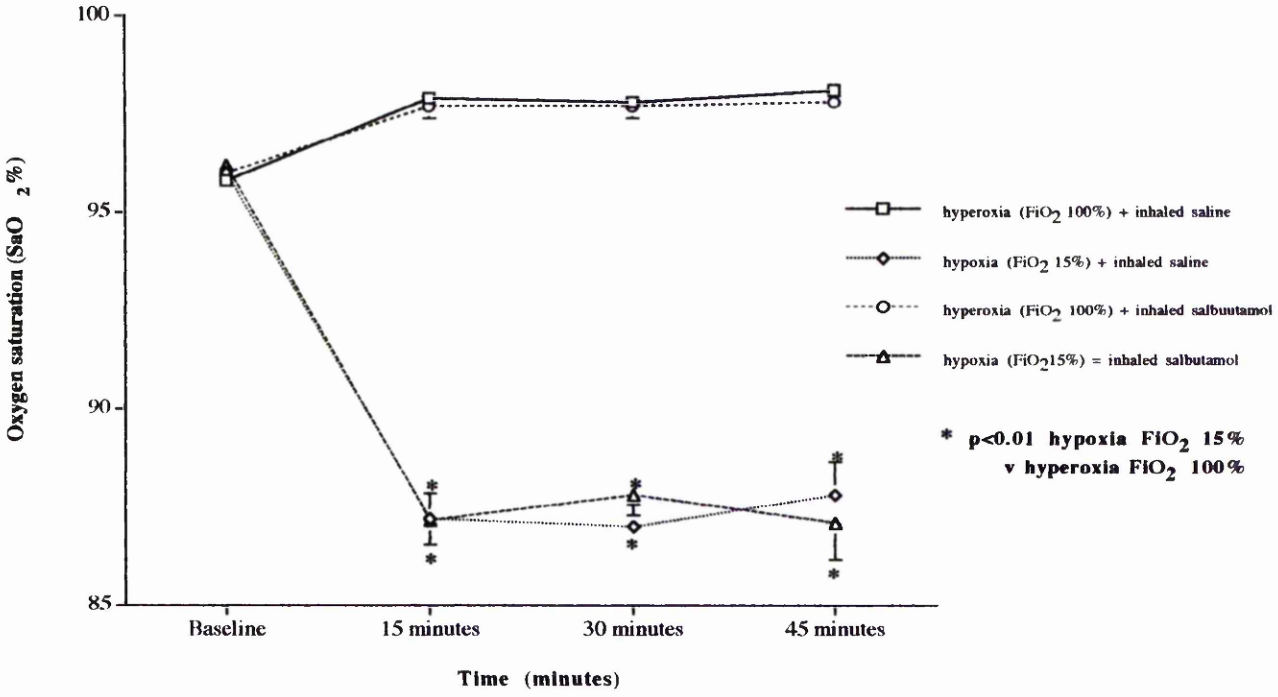

Fig 9.2 The effect of 3 incremental doses of nebulised salbutamol or placebo on oxygen saturation (SaO_2%) in 12 asthmatic patients breathing oxygen tensions of 100% and 15%.

CHAPTER 10

CONCLUSIONS

The studies outlined in this thesis have provided some insight into the effects of acute alterations in oxygen tension on airway tone following exposure to bronchodilator and bronchoconstrictor stimuli both *in-vitro* in man and *in-vivo* in patients with asthma. Such observations may be of importance in patients with acute exacerbations of asthma who as part of their initial management these patients receive high inspired oxygen tensions to breathe and both nebulised and intravenous bronchodilators. Our studies are the first to have suggested that *in-vitro* hypoxia significantly attenuates the effects of salbutamol, histamine and methacholine in human isolated bronchial rings. In patients with asthma hypoxia apparently potentiates the constrictor effect of methacholine but has no effect on histamine induced constriction and salbutamol induced relaxation of airway tone. These observations might suggest that hypoxia not only occurs as a consequence of acute exacerbations of asthma but may itself potentiate the constrictor effects of inflammatory mediators which are released during such exacerbations, and reduce the effectiveness of bronchodilating drugs such as salbutamol to relieve bronchoconstriction.

In-vitro studies

Our initial *in-vitro* studies were designed to examine the effects of acute alterations in oxygen tension on constrictor and dilator stimuli in bronchial rings from man, therefore expanding observations we had already made in bovine and rat isolated bronchial rings.[75-78] We have shown that the ability of histamine and methacholine to constrict airway smooth muscle declines as the ambient oxygen tension falls and that this occurs in a linear fashion (chapter3). This observation is in keeping with our original findings in rat isolated bronchial rings[78] pre-constricted with methacholine, and also in agreement with several other groups who have reported hypoxic relaxation

following pharmacological challenge *in-vitro* in porcine, canine and guinea pig bronchial rings.[79-82] It is now widely accepted that *in-vitro* the ability of airway smooth muscle to contract following electrical and pharmacological challenge in a hypoxic environment is impaired. The precise mechanism of this effect remains unclear.[72,73,78,80,82] Possible mechanisms, which have been postulated to promote this effect include, failure of mitochondrial function[72,73], secretion of an epithelial derived relaxing factor,[82] an increase in the influx of Ca^{++} ions into smooth muscle cells[80] and opening of membrane-bound K^+ channels.[117] All of these postulated mechanisms result from a direct effect of hypoxia on airway smooth muscle and are independent of the spasmogen which is used. It would therefore, be interesting to repeat our studies in human bronchial rings both with and without intact epithelium and following pre-incubation of the airway smooth muscle rings with both Ca^{++} and K^+ channel blockers in an attempt to determine the mechanism of hypoxic relaxation in human isolated bronchial rings constricted with histamine and methacholine. It would also be interesting to examine the effect of hypoxia on a number of other spasmogens including carbachol, adenosine and leukotrienes which cause airway smooth muscle contraction by other mechanisms. The data from such studies in human isolated bronchial rings might provide important evidence to support the hypothesis that hypoxic relaxation occurs as a direct effect of hypoxia on airway smooth muscle, as seems likely, and is not specific to the spasmogen used.

If hypoxia attenuates the effects of spasmogens on airway smooth muscle tone, one would speculate that during acute exacerbations of asthma this action would be beneficial rather than harmful. Observations from our own *in-vivo* studies have in fact suggested that if anything hypoxia potentiates methacholine induced

bronchoconstriction (chapters 6 and 7). This difference is almost certainly due to the profoundly different oxygen tensions that have been used in our studies. It will never be possible to undertake *in-vivo* studies using an FiO_2 of 4% but as we have suggested we could repeat our *in-vitro* studies using an ambient oxygen tension of 15%. This observation would also support our hypothesis that *in-vitro* profound hypoxia has a direct effect on the ability of airway smooth muscle to generate tone, and that this effect is independent of the action of any specific spasmogen. We would speculate that using a higher O_2 tension *in-vitro* would, like our *in-vivo* study, show potentiation of methacholine induced constriction possibly through release of inflammatory mediators.[89,92] We think that hypoxic relaxation *in-vitro* is not a linear effect but occurs below a certain threshold level of hypoxia. We believe that while functional *in-vitro* studies are traditionally carried out in oxygen tensions of 95%, it would be interesting to construct cumulative concentration-response curves for histamine and methacholine in human bronchial rings at various oxygen tensions between 0% and 100%. As we have stated it would be particularly interesting to see the effect of an oxygen tension of 15% *in-vitro*.

We have for the purposes of our *in-vitro* studies used human bronchial rings obtained from patients undergoing elective thoracotomy at our own hospital. These patients tend to be elderly, current or ex-cigarette smokers and have an underlying pulmonary malignancy. The majority of such patients do not have asthma. There is *in-vitro* evidence to suggest that the airways of patients with asthma are hyperresponsive to some agonists when compared to airway smooth muscle from healthy controls.[134,135] If there exists an intrinsic defect of asthmatic airway smooth muscle then the results from our *in-vitro* studies may not truly reflect what happens in asthmatic airways. It

is interesting to speculate what results we would obtain if we repeated our *in-vitro* studies using bronchial rings obtained from patients with asthma.

The findings from our *in-vitro* studies in human bronchial rings are however the converse of our earlier observations in bovine bronchial rings whereby hypoxia potentiated the constrictor effects of both methacholine and endothelin-1[75-77] when compared to hyperoxia. These studies were undertaken using the same protocols, employed the same oxygen tensions and were performed in the same laboratories making this difference difficult to explain. It may simply represent species difference although numerous other studies have shown no difference between species and the finding of hypoxic relaxation following pharmacological challenge.[72,73,78,80,82] It would be interesting to examine the effect of endothelin-1, a putative inflammatory mediator in asthma,[136] on human bronchial rings and to determine whether in the presence of varying degrees of hypoxia it potentiated airway smooth muscle constriction or relaxation. It is possible to speculate that hypoxia as well as having direct effects on airway smooth muscle may have effects on specific receptor sites[115] and their expression. Any alteration in receptor characteristics might overcome the direct effects of hypoxia on intracellular metabolic pathways as a possible explanation for the effect of endothelin-1 in bovine isolated bronchial rings. The activity or density of endothelin-1 receptors may therefore be enhanced by hypoxia. This could be further examined using functional antagonists of endothelin-1 receptors or looking directly at endothelin-1 receptor densities histologically using specific staining techniques.

In our second series of *in-vitro* studies we have suggested that the ability of salbutamol to relax airway smooth muscle is significantly attenuated by hypoxia when compared to hyperoxia. This observation may have important implications in the management of patients with acute severe asthma who are often hypoxaemic and receive both nebulised and intravenous salbutamol in an attempt to relieve bronchoconstriction.[70] We have already suggested that hypoxia has direct effects on airway smooth muscle significantly impairing the ability of cells to generate contractile strength. If this were the whole story one might anticipate that hypoxia in an additive fashion would potentiate, or at worst have no effect on the ability of salbutamol to relax hypoxic airway smooth muscle when compared to normoxia and hyperoxia. It seems likely therefore that hypoxia has additional effects on isolated airway smooth muscle cells which explain our *in-vitro* observations. There is evidence to suggest that hypoxia, induced by altitude, reduces the density of ß-adrenergic receptors and reduces intracellular adenylate cyclase levels in rat myocardium.[115] As salbutamol causes smooth muscle relaxation by increasing intracellular cyclic adenosine monophosphate (cAMP) via activation of adenylate cyclase a similar observation in man could explain our *in-vitro* findings. Interestingly the observations by Voelkel et al[115] occur at levels of hypoxia that a patient suffering an exacerbation of asthma might experience. It would therefore be interesting to measure ß-adrenergic receptor densities and intracellular adenylate cyclase activity in airway smooth muscle cells, exposed to varying levels of hypoxia and hyperoxia for differing amounts of time, using the techniques described by Voelkel et al.[115] Further support for our suggestion that hypoxia attenuates the dilator effect of salbutamol by down regulation of ß-receptors could be provided by examining the effects of other bronchodilators *in-vitro* which cause smooth muscle relaxation through mechanisms

which are independent of ß-adrenergic receptors. It is clear from our earlier studies in bovine isolated bronchial rings that the pattern of smooth muscle relaxation varies quite considerably depending on the oxygen tension and bronchodilator which is used *in-vitro*.[75,77] It has been postulated that this observation reflects differences in second-messenger pathways used by the bronchodilators to induce smooth muscle relaxation and the effects that hypoxia has on these pathways.[77] It would therefore be worthwhile exploring the effects of hypoxia on airway smooth muscle relaxation in bronchial rings incubated with other bronchodilators such as atrial natriuretic peptide which causes a rise in cyclic guanosine monophosphate (cGMP) via stimulation of particulate guanylate cyclase and isosorbide dinitrate, which also causes a rise cGMP but via stimulation of soluble guanylate cyclase.[75]

Previous investigators have postulated that salbutamol induced relaxation of airway smooth muscle cells is mediated partly by opening of membrane-bound K^+ channels.[118] There exists in the literature some evidence to suggest that in second and third order porcine isolated bronchial rings hypoxia increases the number of membrane-bound K^+ channels which are held open which could potentially reduce the ability of the bronchodilator salbutamol to relax airway smooth muscle cells.[117] This observation if applicable in human isolated bronchial rings could explain our own *in-vitro* observations whereby hypoxia attenuated the ability of salbutamol to relax human bronchial rings (chapter 3). If in human bronchial rings glibenclamide, a potent K^+ATP channel blocker, abolished or reduced the effect of hypoxia on salbutamol induced airway smooth muscle relaxation this might provide evidence for hypoxic opening of membrane-bound K^+ channels as a mechanism for our own *in-vitro* findings.[117]

To complete this series of studies it would be worthwhile considering the effect of hypoxia and hyperoxia on airway smooth muscle relaxation following the administration of other commonly used bronchodilators. Such observations may have important implication in the management of acute severe asthma. Other drugs which relax airway smooth muscle and are used in the management of acute severe and life threatening asthma, include nebulised ipratropium bromide and intravenous theophylline and occasionally intravenous magnesium and ketamine. As we have already suggested from our *in-vitro* findings for histamine, repeating these studies at various oxygen tension between 0% and 100% in bronchial rings from patients with asthma might provide valuable additional information about the nature of the relationship between hypoxia, airway smooth muscle tone and bronchodilators and whether or not this relationship is linear.

In conclusion, our first *in-vitro* studies have suggested that acute hypoxia attenuates histamine and methacholine induced constriction of human airway smooth muscle. We would suggest that this is due to a direct effect of hypoxia on airway smooth muscle and is independent of the spasmogen used. The cause of hypoxic relaxation however remains controversial and further studies are needed to determine the underlying mechanism. We have also concluded that the profound levels of hypoxia we have used in our *in-vitro* studies make it difficult to compare our *in-vitro* findings with those of our *in-vivo* studies. We have speculated as to the effects, on our results, of using both lesser degrees of hypoxia and bronchial rings from patients with asthma. We have also suggested that hypoxia inhibits salbutamol induced airway smooth muscle relaxation and conclude that this observation may have important implications

for patients with severe exacerbations of asthma who are admitted to hospital. Possible mechanisms for this important finding include reduced density of ß-adrenergic receptors and alterations in the resting state of membrane-bound K^+ channels. Further investigation as outlined above may provide important information about the mechanism of this effect and the interaction of hypoxia with other bronchodilators that have a role in the management of acute severe asthma.

Following review of our initial *in-vitro* studies we proceeded to investigate the effects of acute alterations in inspired oxygen tension on bronchoconstrictor and bronchodilator stimuli in patients with asthma. We have used a novel closed breathing circuit (chapter 2) to undertake these investigations. Our studies have suggested that acute hypoxia *in-vivo* potentiates the bronchoconstrictor effect of methacholine (chapter 6) but not histamine (chapter 7). Hyperoxia does not appear to have any effect on methacholine or histamine induced bronchial hyperresponsiveness (chapters 5 and 7). The ability of the bronchodilator salbutamol to attenuate airway tone in patients with asthma appears to be unaffected by hypoxia or hyperoxia (chapters 8 and 9).

Hypoxia *in-vivo* studies

In our hypoxic *in-vivo* studies we have examined the effect of breathing low (FiO_2 15%) inspired oxygen tensions on airway responsiveness to methacholine, histamine and salbutamol in patients with stable asthma. In our *in-vivo* studies we have used a novel closed breathing circuit (chapter 2) to induce a moderate level of hypoxaemia in our stable asthmatic patients. In our patients all of the airway mucosa and cells lining the alveolar air spaces will be exposed to a low oxygen tension (O_2 15%). The

alveolar air spaces are surrounded by a rich plexus of blood vessels derived from the pulmonary arterial circulation. As alveolar oxygen levels fall the oxygen tension in the pulmonary capilliaries surrounding the alveolar air sacs also falls. Blood from the pulmonary capilliaries drains back to the left atrium via the pulmonary veins. From the left atrium blood with a significantly reduced oxygen tension enters into the systemic circulation. The cells which constitute the airway walls of our patients, using the closed breathing circuit, will be hypoxaemic reflecting the low systemic arterial oxygen tension which we have induced.[137]

Previous studies, using multiple inert gas techniques, have shown that in patients with asthma ventilation throughout the lungs is uneven and reflects underlying differences in small airway calibre.[138] These abnormalities are found in all patients with asthma including those who are asymptomatic.[139,140] In severe asthma, hypoxaemia occurs almost exclusively as a consequence of ventilation perfusion mismatch.[138,140-142] The degree of ventilation perfusion mismatch appears related to asthma severity and is greatest in those admitted to hospital with hypoxaemia.[140,143-145] There is almost no contribution to hypoxaemia from intrapulmonary shunting or impairment of oxygen diffusion across the alveolar capillary membrane.[139] It is well recognised that the degree of hypoxaemia in asthma relates poorly to the severity of underlying airflow obstruction.[139] Following nebulised and intravenous bronchodilators, measurements of airflow obstruction will normalise but hypoxaemia often persists.[139] This would suggest that the uneven ventilation seen in patients with asthma occurs as a consequence of occlusion of smaller airways rather than larger airways.[144] Occlusion of smaller airways with mucus, inflammatory debris and odema fluid results in critically reduced ventilation to areas of lung parenchyma which remain perfused.

This results in significant ventilation perfusion mismatch and subsequent hypoxaemia in patients during acute exacerbations of asthma.[139] The mucosa cells lining the bronchial tree in these areas will be exposed to a low oxygen tension. In other areas, including larger airways and some alveolar air spaces, hyperventilation allied to the maintenance of normal airway calibre results in areas with elevated oxygen and reduced carbon dioxide tensions.[146] This would suggest that during acute exacerbations of asthma airway oxygen tension varies throughout the bronchial tree. Alveolar hypoxia occurs in areas with low ventilation. The degree of alveolar hypoxia will be variable and relate to the severity of the underlying asthma exacerbation and the treatment that a patient has received. In severe exacerbations of asthma cardiac output increases and may inadvertently raise perfusion to low ventilation areas paradoxically worsening existing hypoxaemia by increasing ventilation perfusion mismatch.[146] Hypoxic pulmonary vasoconstriction is a reflex which occurs around areas of low ventilation where alveolar hypoxia is greatest.[146] Hypoxic pulmonary vasoconstriction acts to reverse ventilation perfusion mismatch and improve hypoxaemia. The presence of hypoxic pulmonary vasoconstriction further complicates direct comparisons between hypoxaemia induced by our closed breathing circuit and hypoxaemia that occurs in patients suffering an acute exacerbation of asthma.

It is important to recognise that patients using our closed breathing circuit are therefore both hypoxic (low airway oxygen tension) and hypoxaemic (low systemic arterial oxygen tension). This should be contrasted with patients suffering an acute exacerbation of asthma where although alveolar hypoxia occurs in some areas much of the bronchial tree is exposed to normal or even increased oxygen tensions due to

hyperventilation. The mechanism of hypoxaemia in patients with acute exacerbations of asthma is therefore different when compared to patients using our closed breathing circuit where the entire bronchial epithelium is exposed to a low oxygen tension. Airway smooth muscle, which derives its blood supply from the systemic circulation, is exposed to hypoxaemia in both groups of patients and is therefore not significantly different. Our closed breathing circuit therefore compares favourably with our *in-vitro* studies where bronchial rings are exposed to a low oxygen tension but we must however be cautious in extending the results of our *in-vivo* studies to patients with acute exacerbations of asthma complicated by hypoxaemia.

The most significant finding from our *in-vivo* studies is the observation that methacholine induced bronchoconstriction is potentiated and histamine induced bronchoconstriction unaffected, by hypoxia (FiO_2 15%) in patients with mild asthma (chapters 5, 6 and 7). Hypoxia does not appear to impair the ability of salbutamol to relax airway smooth muscle. Potentiation of the constrictor effects of methacholine by hypoxia and hypoxaemia may have relevance to patients admitted to hospital with acute exacerbations of asthma and might suggest that hypoxaemia occurs not only as a consequence of acute asthma but may also act to amplify the constrictor effects of inflammatory mediators released during such exacerbations.

This observation differs from our *in-vitro* finding of attenuation of methacholine induced constriction by hypoxia in human isolated bronchial rings. It seems likely that hypoxia has diverse actions which occur at different ambient oxygen tensions. These actions may lead to elevated inflammatory mediator release,[80] impairment of mitochondrial function,[72,73] reduced density of cell membrane receptors[115] and

inhibition of Ca^{++} entry into cells.[80] We believe that differences between *our in-vitro* and *in-vivo* studies reflect different actions of oxygen at different ambient oxygen tensions. *In-vivo* it seems likely that mediator release potentiates methacholine induced bronchoconstriction. We believe that *in-vivo* as inspired oxygen tension falls hypoxaemia causes increased sympathetic activity and reduced airway tone via neural innervation. This manifests itself as an elevated heart rate. At profound levels of hypoxia, as found in our *in-vitro* studies, there may be impairment of mitochondrial function,[72,73] release of an epithelial relaxing factor,[82] reduction in receptor densities[115] or impairment of second messenger pathways to explain attenuation of methacholine and histamine induced constriction and salbutamol induced relaxation of airway tone.

Our observation of potentiation of methacholine induced bronchoconstriction in patients with asthma is not new but contributes significantly to the literature where there have previously been a number of conflicting reports. Our observations are in agreement with Denjean et al[97] whose group suggested that hypoxia potentiates methacholine induced bronchoconstriction in patients with asthma but disagrees with Tam et al[109] who were unable to show a similar effect in patients inspiring dry gases as a non-specific bronchial challenge. There is perhaps more agreement in animal studies where several authors have reported[88,91,92] that hypoxia potentiates non-specific bronchial reactivity *in-vivo* in animals. What perhaps remains more controversial are the potential mechanisms of this effect. Several different mechanisms have been suggested for hypoxia having indirect influences on airway tone including reflex neural constriction following stimulation of carotid chemoreceptors,[88] a direct action of hypoxia on smooth muscle cells causing

constriction,[91] local release of inflammatory mediators[92] and release of circulating humoral factors which may then have indirect influences on airway tone.

One of the more likely possibilities is the release of local inflammatory mediators from cells by hypoxia. D'Brot and Ahmed[92] were able to demonstrate that hypoxic potentiation of carbachol and histamine induced bronchoconstriction in sheep was abolished using cromolyn sodium,[89] a mast cell stabiliser and FPL 57231,[92] a novel leukotriene receptor antagonist. To investigate the potential mechanism of hypoxic constriction in patients with asthma it would be interesting to examine samples from the airways of the lower respiratory tract both before and after methacholine challenge in patients breathing a hypoxic gas mixture when compared to a control group breathing air. Samples can now readily be obtained from the lower airways using induced sputum techniques. This would allow direct measurement of a number of inflammatory mediators including histamine, leukotrienes and some cytokines which may be released in response to airway hypoxia and arterial hypoxaemia. If the release of local inflammatory mediators were the cause of hypoxic potentiation of bronchial reactivity then such observations might provide support for the use of novel asthma therapies in patients with hypoxaemia complicating acute exacerbations of asthma. For example the new leukotriene receptor antagonists now available may find a role in the management of acute exacerbations of asthma complicated by hypoxaemia.[36]

We were acutely aware during the design of our hypoxic studies that hypoxaemia might have effects on circulating humoral factors which could influence airway tone and that such an effect might confound our results.[94] We have for this reason

measured serum catecholamine levels in our studies and found them to be no different following inhalation of a hypoxic gas mixture suggesting that changes in circulating adrenaline and noradrenaline levels have not influenced our results. However it remains possible that other humoral factors released in response to hypoxia may have influenced our results. Millar[147] suggested that the renin angiotensin system may be activated by hypoxia[148] leading to a rise in serum angiotensin levels. She also observed in her studies that angiotensin potentiates methacholine induced bronchoconstriction in patients with mild asthma.[149] A rise in angiotensin levels in our studies in response to hypoxia could explain our results. It may be appropriate in future hypoxic studies to measure plasma levels of other peptides and hormones which might have influences on airway tone including renin, angiotensin, natriuretic peptides, histamine and endothelin.[94] Once again the detection of a circulating humoral factor which acts synergistically with hypoxia to increase airway tone during an acute exacerbation of asthma raises the possibility of developing novel treatments in patients with acute exacerbations of asthma associated with hypoxaemia.

It has been suggested by some authors that acute hypoxaemia causes stimulation of carotid and peripheral chemoreceptors which indirectly increases airway tone by stimulation of airway vagal nerves.[88,91] Vidruk and Sorkness[88] demonstrated that combined cervical vagotomy and bilateral transection of the superior laryngeal nerve abolished increased airway reactivity caused by hypoxia in tracheal segments of intact dogs that had inhaled histamine. In a similar study Denjean et al[91] suggested that surgical chemodenervation abolished the increased methacholine bronchial reactivity they had observed in sheep inhaling a hypoxic gas mixture. We would suggest that stimulation of carotid and arterial chemoreceptors is unlikely to have influenced the

results of our hypoxic studies as we have not observed any changes in heart rate or end-tidal CO_2 levels in our patients exposed to hypoxia which one might anticipate would accompany any chemoreceptor stimulation.[86,119,121,123] It is interesting to note that in the study by Tam et al[109] they were unable to show an increase in bronchial reactivity in their patients inhaling eucapnic dry air with an inspired oxygen tension of 8%. They however found a dramatic increase in the heart rate of their patients suggesting a significant increase in sympathetic output. This increase in sympathetic output almost certainly had indirect effects on airway tone in their patients which influenced their results. Vidruk and Sorkness[88] in their study on dogs lowered arterial oxygen tension to 45 mm Hg It is likely that at this level of hypoxaemia they had also caused stimulation of carotid chemoreceptors which may indirectly have influenced their results. There is no account in their paper of heart rate responses in the mongrel dogs that they studied.

Hypoxia may also have direct effects on airway smooth muscle tone. Studies in man have given rather conflicting results. Some authors have suggested that hypoxia causes bronchoconstriction,[98,99] others have shown no change in airway tone[96,97] and more recently some have even suggested bronchodilation occurs.[101] In the presence of such conflicting observations it seems unlikely that hypoxia induces any clinically significant change in airway tone and certainly not of a magnitude which is likely to have influenced our results. We have not shown any change in FEV_1 measured 10 minutes after inhaling an inspired oxygen tension of 15% in any of our *in-vivo* studies, an observation that lends further support to our conclusion that hypoxia has not had any significant direct effect on airway tone in our patients.

If as we are suggesting hypoxia increases airway reactivity by indirect means, then our observation that hypoxia does not potentiate histamine induced bronchoconstriction must be regarded as surprising. This difference in observations has occurred despite using the same inspired oxygen tension, study design and breathing circuit in both studies. This is an intriguing observation that is difficult to account for. Specific receptors on airway smooth muscle cells bind methacholine and histamine. This interaction results in the production of inositol phospholipid metabolites via a complex biochemical pathway. Inositol 1,4,5 triphosphate, an intracellular second messenger, causes release of intracellular calcium ions which promotes smooth muscle contraction.[133] One could speculate that hypoxia has a specific effect on acetylcholine receptors which when methacholine binds to them augments smooth muscle contraction, perhaps via an effect on regulatory G-proteins. It would seem that this effect does not occur with histamine receptors *in-vivo*. The results from our study examining the effect of hypoxia on histamine induced bronchoconstriction have shown a trend towards increased bronchial reactivity with hypoxaemia. It may well be that a larger study would have converted this trend to statistical significance. However this observation raises the importance of examining the effect of hypoxaemia on a number of different spasmogens in order to determine whether the effect is specific to methacholine or occurs with constrictor agents that work via other mechanisms. It would be interesting to see the effect of hypoxaemia on inhaled endothelin-1, leukotrienes and adenosine.

We have also examined the effect of hypoxia on salbutamol induced relaxation of airway smooth muscle in patients with asthma. Following observations from our *in-vitro* studies we had hypothesised that hypoxia might attenuate the ability of

salbutamol to relax airway smooth muscle. We were however acutely aware that our *in-vitro* studies had used much more profound levels of hypoxaemia and much higher concentrations of salbutamol than we would expect to find in patients with asthma, and that this might directly affect the results of our *in-vivo* studies. We had suggested as possible mechanisms for this effect, down regulation of ß-receptors,[115] opening of membrane bound K^+ channels,[118] and as we have already suggested release of local inflammatory mediators. Results from our study have not shown attenuation of airway relaxation following inhaled salbutamol by hypoxia. It is possible that a larger study would have achieved a statistically significant result. However it is also possible that the duration of our study was inadequate to allow sufficient down regulation of ß-receptors or opening of membrane bound K^+ channels for the effect to have become apparent. It would be worthwhile repeating the study with a longer period of hypoxaemia prior to the administration of salbutamol. The effect of hypoxia on other commonly used bronchodilators, with various mechanisms of action, in acute exacerbations of asthma should also be considered. This would include studies examining the effect of hypoxia on anti-cholinergic drugs, intravenous theophyllines, natriuretic peptides and magnesium.

We would conclude therefore that acute hypoxia potentiates methacholine induced bronchoconstriction *in-vivo* and that this may be relevant in patients with acute exacerbations of asthma admitted to hospital. We conclude that this effect is likely to be mediated by release of local inflammatory mediators, or release of circulating humoral factors in response to hypoxia and that further studies attempting to determine the mechanism of this effect may lead to the development of novel treatments for patients admitted to hospital with acute exacerbation of asthma

complicated by hypoxaemia. We have been unable to demonstrate any effect of hypoxia on salbutamol induced relaxation of airway tone in patients with asthma.

Hyperoxia *in-vivo* studies

We have been unable to show any significant effect of hyperoxia (FiO_2 100%) on methacholine (chapter 5) and histamine (chapter 7) induced bronchoconstriction or salbutamol induced relaxation (chapter 8) of airway smooth muscle in patients with asthma. These studies were prompted by *in-vitro* observations in our own laboratory that hyperoxia protected against methacholine and endothelin-1 induced constriction in bovine isolated bronchial rings.[75,76]

The rise in oxygen saturation, fall in heart rate and end-tidal CO_2% levels which we had observed our normal subjects (chapter 4) suggested to us that our novel closed breathing circuit was capable of producing and maintaining a high airway and arterial oxygen tension.[86,119,121] We had decided to use an inspired oxygen tension of 100% in our *in-vivo* studies to mimic the oxygen tensions which had been used *in-vitro*. It is likely that the fall in heart rate that we have observed on our hyperoxic study days has occurred due to reduced tone in carotid chemoreceptors which has caused increased vagal stimulation.[121] This increase in vagal stimulation may have had indirect effects on airway tone and confounded the results of our hyperoxic studies. Electrical stimulation of vagal motor efferents causes bronchoconstriction via muscarinic receptors on airway smooth muscle.[150,151] If hyperoxia has indirectly caused bronchoconstriction in our patients this could certainly have confounded the results in our studies examining the effect of hyperoxia on salbutamol induced relaxation and both methacholine and histamine induced constriction of airway smooth muscle in

patients with asthma (chapters 5, 7 and 8). This effect would also explain the differences between our *in-vitro* and *in-vivo* findings. Bronchial rings deprived of their normal neural innervation and circulating humoral factors can derive a protective effect from hyperoxia that does not translate into our *in-vivo* studies. This observation suggests that an indirect effect of hyperoxia on airway smooth muscle has occurred in our *in-vivo* studies.

Against this argument is the observation that FEV_1 after inhaling oxygen (FiO_2 100%) for 10 minutes in our studies was not significantly different from the measurements made on the normoxic and hypoxic study days suggesting that any effect was minimal. There is very little literature concerning the effect of acute hyperoxia on airway tone in patients with asthma, and what little there is has suggested that hyperoxia has no effect on airway tone.[102,103] In studies on normal subjects, periods of hyperoxia of about 12 hours can cause a fall in FVC.[105] Clearly the duration that this takes to develop would suggest that it is not mediated by vagal nerves. We must conclude therefore that hyperoxia may have had indirect effects on airway tone in our patients, and that further investigation of this area would require detailed examination of the neural and humoral effects of hyperoxia on airway tone in patients with asthma with detailed measurements of lung function using different inspired oxygen tensions prior to the administration of any challenge tests.

In summary this thesis has started to explore the effects of acute alterations in ambient oxygen tension on the interaction between constrictor and dilator stimuli and airway smooth muscle *in-vitro* in man and *in-vivo* in patients with asthma. This is clearly a complex interaction and we have suggested that that oxygen has different

effects depending on the ambient tension. We have suggested that hypoxia may attenuate the ability of salbutamol to relax airway smooth muscle *in-vitro* and potentiate methacholine induced bronchoconstriction *in-vivo*. These observations may be of particular relevance to patients with acute exacerbations of asthma complicated by hypoxaemia who as part of their management inhale high inspired oxygen tensions and receive both nebulised and intravenous bronchodilators. Further investigation as outlined in this conclusion may determine the mechanisms of these effects and lead to the development of novel asthma therapies for patients suffering acute exacerbations of asthma complicated by hypoxaemia.

REFERENCES

Bibliography

1. Davies RJ: Respiratory Disease, in Kumar PJ, Clark ML (eds): *Clinical Medicine.* London, Balliere Tindall, 1990, pp 627-704

2. Partridge MR, Stewart R, Mcloughlin I, Letts M: Purchasing and providing asthma care. *National Asthma Campaign* 1995;1-33

3. American Thoracic Society: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-244

4. Burr ML, Butland BK, King S, Vaughan-Williams E: Changes in asthma prevalence: two surveys 15 years apart. *Archives of Disease in Childhood* 1989;64:1452-1456

5. Mosmann TR, Hudson JB: Structural and functional heterogeneity of the murine cytomegalovirus genome. *Virology* 1974;62:175-183

6. Death from asthma in two regions of England. British Thoracic Association. *British Medical Journal Clinical Research Ed* 1982; 285:1251-1255

7. Bucknell CE, Slack R, Godley CC, Mackay TW, Wright SC: Scottish Confidential Inquiry into Asthma Deaths (SCIAD), 1994-96. *Thorax* 1999;54:978-984

8. Lamb D, Corrin B: Pathology, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine.* London, Saunders, 2000, pp 1154-1160

9. Dunnill MS: The pathology of asthma with special reference to the changes in bronchial mucosa. *J Clin Path* 1960;13:27-33

10. Dunnill MS, Massarella GR, Anderson JA: A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis, and in emphysema. *Thorax* 1969;24:176-179

11. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls, AF, Roche WR, Howarth PH, Holgate ST: Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990;142:863-871

12. Corrigan CJ, Hartnell A, Kay AB: T lymphocyte activation in acute severe asthma. *Lancet* 1988;1:1129-1132

13. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T: Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985;131:599-606

14. Holgate ST, Wilson JR, Howarth PH: New insights into airway inflammation by endobronchial biopsy. *Am Rev Respir Dis* 1992;145:S2-6

15. Ollerenshaw SL, Woolcock AJ: Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. *Am Rev Respir Dis* 1992;145:922-927

16. Barnes PJ, Djukanovic R, Holgate ST: Pathogenesis, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine*. London, Saunders, 2000, pp 1108-1153

17. Gell PGH, Coombs RRA (eds): *Clinical aspects of immunology.* Oxford, Blackwell, 1968,

18. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman, OP, Howarth RH, Holgate ST: Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434-457

19. Filley WV, Holley KE, Kephart GM, Gleich GJ: Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 1982;2:11-16

20. de Monchy JG, Kauffman HF, Venge P, Koeter GH, Jansen, HM, Sluiter HJ, De Vries K: Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-376

21. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls, AF, Roche WR, Howarth PH, Holgate ST: Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992;145:669-674

22. Joseph M, Tonnel AB, Torpier G, Capron A, Arnoux B, Benveniste J: Involvement of immunoglobulin E in the secretory processes of alveolar macrophages from asthmatic patients. *Journal of Clinical Investigation* 1983;71:221-230

23. Gosset P, Tsicopoulos A, Wallaert B, Vannimenus C, Joseph M, Tonnel AB, Capron A: Increased secretion of tumor necrosis factor alpha and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J Allergy Clin Immunol* 1991;88:561-571

24. Page CP, Guerreiro D, Sanjar S, Morley J: Platelet activating factor (Paf-acether) may account for late-onset reactions to allergen inhalation. *Agents & Actions* 1985;16:30-32

25. Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, Kay AB: Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991;88:661-674

26. Gajewski TF, Joyce J, Fitch FW: Antiproliferative effect of IFN-gamma in immune regulation. III. Differential selection of TH1 and TH2 murine helper T lymphocyte clones using recombinant IL-2 and recombinant IFN-gamma. *Journal of Immunology* 1989;143:15-22

27. Fiorentino DF, Bond MW, Mosmann TR: Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *Journal of Experimental Medicine* 1989;170:2081-2095

28. Street NE, Mosmann TR: Functional diversity of T lymphocytes due to secretion of different cytokine patterns. *FASEB Journal* 1991;5:171-177

29. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, Hunninghake GW: Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. *Journal of Clinical Investigation* 1987;79:1197-1203

30. Jarjour NN, Calhoun WJ, Schwartz LB, Busse WW: Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with increased airway obstruction. *Am Rev Respir Dis* 1991;144:83-87

31. Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey-Sobotka A, Bleecker ER, Lichtenstein LM: Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and permeability changes. *Am Rev Respir Dis* 1991;144:51-58

32. Wenzel SE, Fowler AA, Schwartz LB: Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* 1988;137:1002-1008

33. Finney MJ, Karlsson JA, Persson CG: Effects of bronchoconstrictors and bronchodilators on a novel human small airway preparation. *British Journal of Pharmacology* 1985;85:29-36

34. Evans TW, Rogers DF, Aursudkij B, Chung KF, Barnes PJ: Inflammatory mediators involved in antigen-induced airway microvascular leakage in guinea pigs. *Am Rev Respir Dis* 1988;138:395-399

35. Shelhamer JH, Marom Z, Kaliner M: Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *Journal of Clinical Investigation* 1980;66:1400-1408

36. Lipworth BJ: Leukotriene-receptor antagonists. *Lancet* 1999;353:57-62

37. O'Byrne PM: Leukotrienes in the pathogenesis of asthma. *Chest* 1997;111:27S-34S

38. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT: Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991;337:690-694

39. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne, PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. *J Allergy Clin Immunol* 1998;102:177-183

40. Christie PE, Smith CM, Lee TH: The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991;144:957-958

41. Dahlen B, Kumlin M, Margolskee DJ, Larsson C, Blomqvist, H, Williams VC, Zetterstrom O, Dahlen SE: The leukotriene-receptor antagonist MK-0679 blocks airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics. *European Respiratory Journal* 1993;6:1018-1026

42. Knorr B, Matz J, Bernstein JA, Nguyen H, Seidenberg BC, Reiss TF, Becker A: Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. *JAMA* 1998;279:1181-1186

43. Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB: Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast Clinical Research Study Group. *Arch Intern Med* 1998;158:1213-1220

44. Fish JE, Kemp JP, Lockey RF, Glass M, Hanby LA, Bonuccelli CM: Zafirlukast for symptomatic mild-to-moderate asthma: a 13-week multicenter study. The Zafirlukast Trialists Group. *Clinical Therapeutics* 1997;19:675-690

45. Suissa S, Dennis R, Ernst P, Sheehy O, Wood-Dauphinee S: Effectiveness of the leukotriene receptor antagonist zafirlukast for mild-to-moderate asthma. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1997;126:177-183

46. Smith HR, Larsen GL, Cherniack RM, Wenzel SE, Voelkel, NF, Westcott JY, Bethel RA: Inflammatory cells and eicosanoid mediators in subjects with late asthmatic responses and increases in airway responsiveness. *J Allergy Clin Immunol* 1992;89:1076-1084

47. Hardy CC, Robinson C, Tattersfield AE, Holgate ST: The bronchoconstrictor effect of inhaled prostaglandin D2 in normal and asthmatic men. *N Engl J Med* 1984;311:209-213

48. Thomson NC, Roberts R, Bandouvakis J, Newball H, Hargreave FE: Comparison of bronchial responses to prostaglandin F2 alpha and methacholine. *J Allergy Clin Immunol* 1981;68:392-398

49. Fuller RW, Dixon CM, Dollery CT, Barnes PJ: Prostaglandin D2 potentiates airway responsiveness to histamine and methacholine. *Am Rev Respir Dis* 1986;133:252-254

50. O'Byrne PM, Fuller RW: The role of thromboxane A2 in the pathogenesis of airway hyperresponsiveness. *European Respiratory Journal* 1989;2:782-786

51. Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, Life P, Talabot D, Flores-Romo L, Thompson J, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993;365:340-343

52. Aubas P, Cosso B, Godard P, Michel FB, Clot J: Decreased suppressor cell activity of alveolar macrophages in bronchial asthma. *Am Rev Respir Dis* 1984;130:875-878

53. Spiteri MA, Knight RA, Jeremy JY, Barnes PJ, Chung KF: Alveolar macrophage-induced suppression of peripheral blood mononuclear cell responsiveness is reversed by in vitro allergen exposure in bronchial asthma. *European Respiratory Journal* 1994;7:1431-1438

54. Richardson JB: Nerve supply to the lungs. *Am Rev Respir Dis* 1979;119:785-802

55. Barnes PJ: Neural mechanisms in asthma. *British Medical Bulletin* 1992;48:149-168

56. Fox AJ, Barnes PJ, Urban L, Dray A: An in vitro study of the properties of single vagal afferents innervating guinea-pig airways. *Journal of Physiology* 1993;469:21-35

57. Barnes PJ: Modulation of neurotransmission in airways. *Physiological Reviews* 1992;72:699-729

58. Barnes PJ: Neural control of human airways in health and disease. *Am Rev Respir Dis* 1986;134:1289-1314

59. Harrison BDW: Acute severe asthma in adults. *Medicine* 1999;27:64-68(Abstract)

60. Bellamy D, Collins JV: "Acute" asthma in adults. *Thorax* 1979;34:36-39

61. Harrison BDW: Acute Severe Asthma, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine*. London, Saunders, 2000, pp 1221-1238

62. Rubinfeld AR, Pain MC: Perception of asthma. *Lancet* 1976;1:882-884

63. Burdon JG, Juniper EF, Killian KJ, Hargreave FE, Campbell EJ: The perception of breathlessness in asthma. *Am Rev Respir Dis* 1982;126:825-828

64. Eason J, Markowe HL: Controlled investigation of deaths from asthma in hospitals in the North East Thames region. *British Medical Journal Clinical Research Ed* 1987; 294:1255-1258

65. Neville RG, Clark RC, Hoskins G, Smith B: National asthma attack audit 1991-2. General Practitioners in Asthma Group. *BMJ* 1993;306:559-562

66. Guidelines on the management of asthma. Statement by the British Thoracic Society, the Brit. Paediatric Association, the Research Unit of the Royal College of Physicians of London, the King's Fund Centre, the National Asthma Campaign, the Royal College of General Practitioners, the General Practitioners in Asthma Group, the Brit. Assoc. of Accident and Emergency Medicine, and the Brit. Paediatric Respiratory Group. *Thorax* 1993;48:S1-24

67. McFadden ER, Jr., Lyons HA: Arterial-blood gas tension in asthma. *N Engl J Med* 1968;278:1027-1032

68. Jenkins PF, Benfield GF, Smith AP: Predicting recovery from acute severe asthma. *Thorax* 1981;36:835-841

69. Edelson JD, Rebuck AS: The clinical assessment of severe asthma. *Arch Intern Med* 1985;145:321-323

70. British Thoracic Society: The British Guidelines on Asthma Management 1995 Review and Posistion Statement. *Thorax* 1997;52:S1-S20

71. Stephens NL, Meyers JL, Cherniack RM: Oxygen, carbon dioxide, H^+ ion and bronchial length-tension relationships. *J Appl Physiol* 1968;25:376-383

72. Stephens NL, Chiu BS: Mechanical properties of tracheal smooth muscle and effects of O2, CO2, and pH. *American Journal of Physiology* 1970;219:1001-1008

73. Stephens NL, Kroeger E: Effect of hypoxia on airway smooth muscle mechanics and electrophysiology. *J Appl Physiol* 1970;28:630-635

74. Wadsworth RM: Vasoconstrictor and vasodilator effects of hypoxia. *TiPS* 1994;15:47-53

75. Clayton RA, Nally JE, Thomson NC, McGrath JC: The effect of oxygen tension on responses evoked by methacholine and bronchodilators in bovine isolated bronchial rings. *Pulmonary Pharmacology* 1996;9:123-128

76. Nally JE, Bunton DC, Martin D, Thomson NC: The role of cyclooxygenase and 5-lipoxygenase metabolites in potentiated endothelin-1-evoked contractions in bovine bronchi. *Pulmonary Pharmacology* 1996;9:211-217

77. Clayton RA, Nally JE, Thomson NC, McGrath JC: Changing the oxygen tension alters the ability of bronchodilators to protect against methacholine-induced challenge in bovine isolated bronchial rings. *Pulmonary Pharmacology & Therapeutics* 1997;10:51-60

78. Clayton RA, Nally JE, Thomson NC, McGrath JC: Reversal of methacholine-induced contraction in isolated bronchial rings fron chronically hypoxic and control rats. *Br J Pharmacol* 1995;116:418

79. Fernandes LB, Stuart-Smith K, Croxton TL, Hirshman CA: Role of Ca2+ entry in the modulation of airway tone by hypoxia. *American Journal of Physiology* 1993;264:L284-9

80. Vannier C, Croxton TL, Farley LS, Hirshman CA: Inhibition of dihydropyridine-sensitive calcium entry in hypoxic relaxation of airway smooth muscle. *American Journal of Physiology* 1995;268:L201-6

81. Paterson NA, Hamilton JT, Yaghi A, Miller DS: Effect of hypoxia on responses of respiratory smooth muscle to histamine and LTD4. *J Appl Physiol* 1988;64:435-440

82. Gao YS, Vanhoutte PM: Lowering PO_2 induces epithelium-dependent relaxation in isolated canine bronchi. *American Journal of Physiology* 1989;257:C1034-7

83. Roy CS, Brown G: On bronchial contraction. *J Physiol* 1885;6:221-225

84. Dixon WE, Brodie TG: Contributions to the physiology of the lung. Part I. The bronchial muscles, their innervation, and the action of drugs upon them. *J Physiol* 1903;29:97-173

85. Nadel JA, Widdicombe JG: Effect of changes in blood gas tensions and carotid sinus pressure on tracheal volume and total lung resistance to airflow. *J Physiol* 1962;163:13-33

86. Green M, Widdicombe JG: The effects of ventilation of dogs with different gas mixtures on airway calibre and lung mechanics. *J Physiol* 1966;186:363-381

87. Loofbourrow GN, Wood WB, Baird IL: Tracheal constriction in the dog. *Am J Physiol* 1957;411-415

88. Vidruk EH, Sorkness RL: Histamine-induced reflex tracheal constriction is attenuated by hyperoxia and exaggerated by hypoxia. *Am Rev Respir Dis* 1985;132:287-291

89. Ahmed T, Marchette B: Hypoxia enhances nonspecific bronchial reactivity. *Am Rev Respir Dis* 1985;132:839-844

90. Wetzel RC, Herold CJ, Zerhouni EA, Robotham JL: hypoxic bronchodilation. *J Appl Physiol* 1992;73(3):1202-1206

91. Denjean A, Canet E, Praud JP, Gaultier C, Bureau M: Hypoxia-induced bronchial responsiveness in awake sheep: role of carotid chemoreceptors. *Respir Physiol* 1991;83:201-210

92. D'Brot J, Ahmed T: Hypoxia-induced enhancement of nonspecific bronchial reactivity: role of leukotrienes. *J Appl Physiol* 1988;65(1):194-199

93. D'Brot J, Ahmed T: Hyperoxia prevents hypoxia-induced bronchial hyperreactivity via a cyclooxygenase independent mechanism. *J Appl Physiol* 1991;70(2):740-747

94. Thomson NC, Dagg KD, Ramsay SG: Humoral control of airway tone. *Thorax* 1996;51:461-464

95. Milic-Emili G, Petit JM: Effets de la teneur en oxygene de l'air inspire sur les proprietes mecaniques des poumons chez l'homme normal. *J Physiol (paris)* 1960;52:175-177

96. Goldstein RS, Zamel N, Rebuck AS: Absence of effects of hypoxia on small airway function in humans. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 1979;47:251-256

97. Denjean A, Roux C, Herve P, Bonniot JP, Comoy E, Duroux, P, Gaultier C: Mild isocapnic hypoxia enhances the bronchial response to methacholine in asthmatic subjects. *Am Rev Respir Dis* 1988;138:789-793

98. Sterling GM: The mechanism of bronchoconstriction due to hypoxia in man. *Clin Sci* 1968;35:105-114

99. Saunders NA, Betts MF, Pengelly LD, Rebuck AS: Changes in lung mechanics induced by acute isocapnic hypoxia. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 1977;42:413-419

100. England SJ, Bartlett D, Jr., Knuth SL: Comparison of human vocal cord movements during isocapnic hypoxia and hypercapnia. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 1982;53:81-86

101. Julia-Serda G, Molfino NA, Furlott HG, McClean PA, Rebuck AS, Hoffstein V, Slutsky AS, Zamel N, Chapman KR: Tracheobronchial dilation during isocapnic hypoxia in conscious humans. *J Appl Physiol* 1993;75:1728-1733

102. Wollner A, Ben-Dov I, Bar-Yishay E: Effect of hyperoxia on bronchial response to inhaled methacholine. *Allergy* 1991;46:35-39

103. Inoue H, Inoue C, Okayama M, Sekizawa K, Hida W, Takishima T: Breathing 30 per cent oxygen attenuates bronchial responsiveness to methacholine in asthmatic patients. *European Respiratory Journal* 1989;2:506-512

104. Libby DM, Briscoe WA, King TK: Relief of hypoxia-related bronchoconstriction by breathing 30 per cent oxygen. *Am Rev Respir Dis* 1981;123:171-175

105. Beckett WS, Wong ND: Effect of normobaric hyperoxia on airways of normal subjects. *J Appl Physiol* 1988;64:1683-1687

106. Astin TW: The relationships between arterial blood oxygen saturation, carbon dioxide tension, and pH and airway resistance during 30 per cent oxygen breathing in patients with chronic bronchitis with airway obstruction. *Am Rev Respir Dis* 1970;102:382-387

107. Schiffman PL, Ryan A, Whipp BJ, Hansen JE, Wasserman K: Hyperoxic attenuation of exercise-induced bronchospasm in asthmatics. *Journal of Clinical Investigation* 1979;63:30-37

108. Resnick AD, Deal EC, Jr., Ingram RH, Jr., McFadden ER, Jr. A critical assessment of the mechanism by which hyperoxia attenuates exercise-induced asthma. *Journal of Clinical Investigation* 1979;64:541-549

109. Tam EK, Geffroy BA, Myers DJ, Seltzer J, Sheppard D, Boushey HA: Effect of eucapnic hypoxia on bronchomotor tone and on the bronchomotor response to dry air in asthmatic subjects. *Am Rev Respir Dis* 1985;132:690-693

110. Knudsen RJ, Lebowitz MD, Holberg CJ, Burrows B: Changes in the normal maximal expiratory flow-volume curve with growth and ageing. *Am Rev Respir Dis* 1983;127:725-734

111. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE: Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clinical Allergy* 1977;7:235-243

112. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J: Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981;68:347-355

113. Goldstein DS, Feuerstein G, Izzo JL, Jr., Kopin IJ, Keiser, HR. Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. *Life Sciences* 1981;28:467-475

114. Brink C, Grimaud C, Guillot C, Orehek J: The interaction between indomethacin and contractile agents on human isolated airway muscle. *British Journal of Pharmacology* 1980;69:383-388

115. Voelkel NF, Hegstrand L, Reeves JP, McMurty IF, Molinoff PB: Effects of hypoxia on density of β-adrenergic receptors. *J Appl Physiol* 1981;50:363-366

116. Richalet JP: The heart and adrenergic systems in hypoxia. in Sutton JR, Coates G, Remmers JE (eds): *The Adaptations.* Toronto, Decker, 1990, pp 231-240

117. Lindeman KS, Fernandes LB, Croxton TL, Hirshman CA: Role of potassium channels in hypoxic relaxation of porcine bronchi in vitro. *American Journal of Physiology* 1994;266:L232-7

118. Miura M, Belvisi MG, Stretton CD, Yacoub MH, Barnes PJ: Role of potassium channels in bronchodilator responses in human airways. *Am Rev Respir Dis* 1992;146:132-136

119. Jennet S: Hypoxia and hyperoxia in man: immediate effects on ventilation and heart rate. PhD Thesis (University of Glasgow);1964

120. Rebuck AS, Campbell EJM: A clinical method for assessing the ventilatory response to hypoxia. *Am Rev Respir Dis* 1974;109:345350

121. Dripps RD, Comroe JJH: The effect of the inhalation of high and low oxygen concentration on respiration, pulse rate, ballistocardiogram and arterial oxygen saturation (oximeter) of normal individuals. *Am J Physiol* 1947;149:277-291

122. Igarashi T, Nishimura M, Kobayshi S, Miyamoto K, Kawakami Y: Dependency on the rate of change in PaO_2 of the ventilatory response to progressive hypoxia. *Am J Respir Crit Care Med* 1995;151:1815-1820

123. Dejours P: Chemoreflexes in breathing. *Physiol Rev* 1962;42:335-358

124. Bernthal T: Respiration. *Ann Rev Physiol* 1944;6:155-194

125. Comroe JH, Schmidt CF: The part played by reflexes from the carotid body in the chemical regulation of respiration in the dog. *Am J Physiol* 1938;121:75-97

126. Newhouse MT, Becklake MR, Macklem PT, McGregor M: Effect of alterations in end-tidal CO_2 tension on flow resistance. *J Appl Physiol* 1964;1994:745-749

127. O'Cain CF, Hensley MJ, McFadden ER, Jr., Ingram RH, Jr. Pattern and mechanism of airway response to hypocapnia in normal subjects. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 1979;47:8-12

128. Sterling GM: The mechanism of bronchoconstriction due to hypocapnia in man. *Clin Sci* 1968;277-285

129. Elshout FJ, Herwaarden C, Folgering H: Effects of hypercapnia and hypocapnia on respiratory resistance in normal and asthmatic subjects. *Thorax* 1991;46:28-32

130. Hughes JMB: Pulmonary Gas Exchange, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine*. London, Saunders, 2000, pp 134-156

131. Watt JG, Dumke PR, Comroe JJH: Effects of inhalation of 100 per cent and 14 per cent oxygen upon respiration of unanaesthetised dogs before and after chemoreceptor denervation. *Am J Physiol* 1942;138:610-617

132. Chakrin LW, Krell RD: Histamine receptors in the respiratory system: a review of current evidence. in Torsoli A, Luccelli PE, Brimblecombe RW (eds): H_2-antagonists in peptic ulcer disease and progress in histamine research. *Excerpta Medica*, 1980, pp 338-346

133. Rodger IW, Pyne NJ: Airwy Smooth Muscle, in Barnes PJ, Roger IW, Thomson NC (eds): *Asthma Basic Mechanisms and Clinical Management*. London, Academic Press, 1992, pp 59-84

134. Bai TR: Abnormalities in airway smooth muscle in fatal asthma. *Am Rev Respir Dis* 1990;141:552-557

135. Pare PD, Hogg JC: The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989;139:242-246

136. Chalmers GW, Thomson NC: The role of endothelin-1 in asthma. *Monaldi Archives for Chest Disease* 1999;54:280-286

137. White FE, Brewis RAL: Anatomy of the Thorax, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine*. London, Saunders, 2002, pp 22-53

138. Wagner PD, Saltzman HA, West JB: Measurement of continuous distributions of ventilation-perfusion ratios: theory. *J Appl Physiol* 1974;36:588-599

139. Wagner PD, Rodriguez-Roisin R: Clinical Advances in Pulmonary Gas Exchange. *Am Rev Respir Dis* 1991;143:883-888

140. Rodriguez-Roisin R, Ferrer A, Navajas D, Agusti AGN, Wagner PD, Roca J: Ventilation-Perfusion Mismatch after Methacholine Challenge in Patients with Mild Bronchial Asthma. *Am Rev Respir Dis* 1991;144:88-94

141. Wagner PD, Hedenstierna G, Bylin G: Ventiltion-Perfusion Inequality in Chronic Asthma. *Am Rev Respir Dis* 1987;136:605-612

142. Ferrer A, Roca J, Wagner PD, Lopez FA, Rodriguez-Roisin R: Airway Obstruction and Ventilation-Perfusion Relationships in Acute Severe Asthma. *Am Rev Respir Dis* 1993;147:579-584

143. Wagner PD, Dantzker DR, Iacovoni VE, Tomlin WC, West JB: Ventilation-Perfusion Inequality in Asymptomatic Asthma. *Am Rev Respir Dis* 1978;118:511-524

144. Roca J, Ramis LI, Rodriguez-Roisin R, Ballester E, Montserrat JM, Wagner PD: Serial Relationships between Ventilation-Perfusion Inequality and Spirometry in Acute Severe Asthma Requiring Hospitilization. *Am Rev Respir Dis* 1988;137:1055-1061

145. Ballester E, Reyes A, Roca J, Guitart R, Wagner PD, Rodriguez-Roisin R: Ventilation-perfusion mismatching in acute severe asthma: effects of salbutamol and 100% oxygen. *Thorax* 1989;44:258-267

146. Rodriguez-Roisin R: Pathophysiology, in Brewis RAL, Corrin B, Geddes DM, Gibson GJ (eds): *Respiratory Medicine*. London, Saunders, 2000, pp 1161-1177

147. Millar EA: The renin angiotensin system in acute severe asthma. MD Thesis (University of Glasgow);1996

148. Millar EA, Angus RM, Hulks G, Morton JJ, Thomson NC: The activity of the renin-angiotensin system in acute severe asthma and the effect of angiotensin II on airway function. *Thorax* 1994;42:492-495

149. Millar EA, Nally JE, Thomson NC: Angiotensin II potentiates methacholine induced bronchoconstriction in mild asthmatic patients. *Eur Resp J* 1995;8:1838-1841

150. Richardson JB: State of the art. Nerve supply to the lungs. *Am Rev Respir Dis* 1979;119:785-802

151. Nadel JA: Autonomic regulation of airway smooth muscle. in Nadel JA (ed): *Physiology and Pharmacology of the Airways.* New York, Dekker, 1980, pp 215-257

PUBLICATIONS ARISING FROM THE WORK IN THIS THESIS

1. Thomson NC, Dagg KD, Ramsay SG. Humoral control of airway tone. Thorax 1996;51(5):461-464.
2. Dagg KD, Thomson LJ, Ramsay SG, Thomson NC. Effect of acute hyperoxia on the bronchodilator response to salbutamol in stable asthmatic patients. Thorax 1996;51(8);853-854.
3. Dagg KD, Thomson LJ, Clayton RA, Ramsay SG, Thomson NC. Effect of acute alterations in inspired oxygen tension on methacholine induced bronchoconstriction in patients with asthma. Thorax 1997;52(5):453-457.
4. Dagg KD, Clayton RA, Thomson LJ, Chalmers GW, McGrath JC, Thomson NC. The effect of acute alterations in oxygen tension on the bronchodilator response to salbutamol in-vitro and in-vivo in man. Pulm Pharm 2001;14:99-105.
5. Dagg KD, Clayton RA, Thomson LJ, Chalmers GW, McGrath JC, Thomson NC. The effect of acute alterations in oxygen tension on airway responsiveness to histamine in-vitro and in-vivo in man. (submitted).

APPENDIX

	Oxygen tension used to drive the nebuliser											
	Oxygen tension 1.0				Oxygen tension 0.21				Oxygen tension 0.15			
Subjects	5 (l/min)	6 (l/min)	7 (l/min)	8 (l/min)	5 (l/min)	6 (l/min)	7 (l/min)	8 (l/min)	5 (l/min)	6 (l/min)	7 (l/min)	8 (l/min)
1	0.08	0.12	0.14	0.15	0.10	0.12	0.13	0.15	0.10	0.12	0.14	0.15
2	0.09	0.13	0.14	0.14	0.11	0.13	0.17	0.16	0.11	0.12	0.13	0.14
3	0.09	0.12	0.13	0.15	0.10	0.11	0.13	0.16	0.10	0.12	0.13	0.15
4	0.09	0.11	0.15	0.14	0.10	0.12	0.13	0.17	0.10	0.11	0.13	0.16
5	0.08	0.10	0.13	0.16	0.11	0.11	0.13	0.16	0.11	0.11	0.14	0.14
Mean (SEM)	**0.086 (0.006)**	**0.116 (0.005)**	**0.138 (0.004)**	**0.148 (0.004)**	**0.104 (0.002)**	**0.117 (0.003)**	**0.132 (0.002)**	**0.160 (0.003)**	**0.104 (0.002)**	**0.115 (0.002)**	**0.131 (0.003)**	**0.148 (0.004)**

Table 1A: Nebuliser output (mls/min) at a flow rate of 5, 6, 7 and 8 litres per minute using oxygen tensions (expressed as a fraction) of 1.0, 0.21 and 0.15 repeated 5 times using a micro cirrus nebuliser.

	Heart rate (beats per minute)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	70	69	72	77	89	71	71	77	89	69	70	74	87	72	69	76	89
2	81	82	81	82	93	82	81	83	89	81	83	79	88	81	79	78	89
3	75	74	78	78	76	74	79	77	86	77	82	78	84	79	80	78	85
4	84	82	80	83	93	87	82	83	93	80	79	85	93	83	75	82	91
5	63	67	72	70	75	67	72	69	78	67	68	70	79	68	67	68	77
6	77	72	84	80	94	69	84	80	94	72	75	74	93	72	78	74	92
7	59	58	62	65	74	53	58	65	77	56	60	67	74	60	59	62	70
Mean (SEM)	**73 (9.2)**	**72 (8.5)**	**76 (7.5)**	**76 (6.6)**	**85 (9.4)**	**72 (6.8)**	**75 (9.1)**	**76 (6.9)**	**87 (6.8)**	**72 (8.8)**	**74 (8.4)**	**75 (6.0)**	**85 (7.0)**	**74 (8.1)**	**72 (7.7)**	**74 (6.8)**	**85 (8.2)**

Table 2A: Mean (SEM) measurements of heart rate (beats per minute) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction (FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Oxygen saturation (SaO_2%)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	96	97	95	90	79	97	95	90	78	96	95	91	77	97	96	91	75
2	95	95	94	88	77	96	93	86	72	96	93	86	74	95	93	90	73
3	95	94	95	88	87	95	93	88	80	95	92	87	80	94	93	88	81
4	97	97	96	93	79	97	95	89	78	97	96	92	76	96	96	91	77
5	97	97	94	91	84	97	96	91	81	97	94	91	79	97	95	90	76
6	97	97	95	92	80	97	95	93	71	97	96	92	76	97	94	92	76
7	96	97	97	93	85	97	97	95	80	98	96	93	76	98	97	92	75
Mean (SEM)	**96 (0.9)**	**96 (1.3)**	**95 (1.1)**	**91 (2.1)**	**82 (3.7)**	**97 (0.8)**	**95 (1.5)**	**90 (3.0)**	**78 (3.0)**	**97 (1.0)**	**95 (2.0)**	**90 (2.7)**	**77 (2.0)**	**96 (1.4)**	**95 (1.6)**	**91 (1.4)**	**76 (2.5)**

Table 3A: Mean (SEM) measurements of oxygen saturation % (SaO_2%) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction (FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	End-tidal CO_2% levels ($PETCO_2$%)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	5.9	6.0	5.9	5.9	5.8	5.9	5.8	5.9	5.8	5.8	5.9	5.9	5.8	5.9	6.0	6.1	5.8
2	6.0	6.2	6.2	6.1	5.9	6.3	6.0	6.1	5.9	6.1	6.3	6.1	5.9	6.3	6.2	6.2	5.2
3	5.4	5.3	5.2	5.2	4.5	5.2	5.3	5.2	3.7	5.1	5.2	5.1	4.8	5.2	5.0	5.1	4.9
4	5.2	5.2	5.3	5.5	5.3	5.3	5.2	5.3	5.2	5.2	5.3	5.2	5.1	5.4	5.4	5.3	5.0
5	5.9	5.8	5.7	5.7	5.5	5.7	5.6	5.8	5.5	5.7	5.9	5.9	5.5	5.7	5.8	5.8	5.5
6	5.8	5.5	5.7	5.7	5.4	5.7	5.7	5.5	5.3	5.7	5.4	5.6	5.1	5.7	5.7	5.6	5.3
7	5.5	5.6	5.4	5.3	5.1	5.5	5.6	5.3	5.0	5.5	5.6	5.3	5.1	5.5	5.4	5.3	5.1
Mean (SEM)	**5.7 (0.3)**	**5.7 (0.4)**	**5.6 (0.4)**	**5.6 (0.3)**	**5.4 (0.5)**	**5.7 (0.4)**	**5.6 (0.3)**	**5.6 (0.3)**	**5.2 (0.7)**	**5.6 (0.3)**	**5.7 (0.4)**	**5.6 (0.4)**	**5.3 (0.4)**	**5.7 (0.4)**	**5.6 (0.4)**	**5.6 (0.4)**	**5.3 (0.3)**

Table 4A: Mean (SEM) measurements of end-tidal CO_2% levels ($PETCO_2$%) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction (FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Respiratory rate (breaths per minute)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	17	18	17	15	11	19	11	15	15	18	14	10	11	18	14	13	16
2	11	10	10	10	7	9	12	9	7	13	11	8	6	10	11	7	7
3	16	14	12	12	12	16	14	13	13	13	15	12	12	14	13	11	12
4	14	13	12	8	10	14	13	10	12	12	12	13	15	11	10	11	16
5	16	14	16	17	19	11	17	17	13	12	18	19	15	16	18	19	15
6	15	15	15	18	17	16	16	15	16	14	18	16	16	16	15	17	17
7	17	15	15	18	18	14	17	16	19	18	17	18	18	17	17	16	18
Mean (SEM)	**15 (2.1)**	**14 (2.4)**	**14 (2.5)**	**14 (4.0)**	**13 (4.6)**	**14 (3.3)**	**14 (2.4)**	**14 (3.0)**	**14 (3.7)**	**14 (2.6)**	**15 (2.8)**	**14 (4.1)**	**13 (4.0)**	**15 (3.0)**	**14 (2.9)**	**13 (4.2)**	**14 (3.8)**

Table 5A: Mean (SEM) measurements of respiratory rate (breaths per minute) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction(FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Mean arterial blood pressure (mm/Hg)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	90	95	83	84	87	85	82	93	91	82	83	83	87	83	82	88	88
2	114	127	108	104	107	109	106	108	108	107	104	104	106	116	104	107	103
3	104	104	99	102	96	102	108	104	92	106	106	106	93	104	108	108	101
4	106	108	105	99	108	113	104	102	101	104	108	108	95	102	112	102	99
5	99	99	99	94	93	96	95	93	96	97	97	89	99	94	92	98	98
6	85	90	93	94	101	92	92	91	93	95	95	97	93	91	86	98	91
7	87	86	88	84	90	86	81	84	83	82	82	83	84	88	85	86	85
Mean (SEM)	**99 (11)**	**101 (8.5)**	**96 (7.9)**	**94 (11)**	**97 (11)**	**98 (11)**	**95 (11)**	**96 (8.5)**	**95 (7.9)**	**96 (11)**	**95 (11)**	**99 (9.5)**	**94 (7.3)**	**97 (11)**	**96 (12)**	**98 (10)**	**95 (7.0)**

Table 6A: Mean (SEM) measurements of mean arterial blood pressure (mm/Hg) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction(FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Diastolic blood pressure (mm/Hg)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	64	71	63	61	62	60	63	72	60	58	63	66	60	64	62	62	62
2	90	91	84	76	83	90	77	80	82	84	81	84	80	87	81	84	76
3	90	90	84	87	79	92	95	88	79	89	93	94	81	90	97	97	88
4	86	84	82	80	78	91	86	79	69	83	86	84	67	85	88	73	71
5	82	84	83	74	75	78	80	76	79	79	74	82	84	82	76	80	82
6	72	75	73	72	74	75	70	74	75	75	72	74	78	69	71	81	78
7	72	68	66	72	72	68	68	72	71	65	65	68	71	74	71	71	70
Mean (SEM)	**79 (10)**	**80 (9.1)**	**76 (9.0)**	**74 (8.0)**	**74 (6.7)**	**79 (12)**	**77 (11)**	**77 (5.7)**	**74 (7.6)**	**76 (11)**	**76 (11)**	**79 (10)**	**74 (8.7)**	**79 (9.8)**	**78 (12)**	**78 (11)**	**75 (8.5)**

Table 7A: Mean (SEM) measurements of diastolic blood pressure (mm/Hg) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction (FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Systolic blood pressure (mm/Hg)																
	Base	3 minutes				6 minutes				9 minutes				12 minutes			
Subject	0.21	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12	0.21	0.18	0.15	0.12
1	133	159	128	127	134	139	129	143	139	127	135	139	138	132	133	135	148
2	163	152	146	149	158	142	156	148	154	152	144	144	161	139	134	155	148
3	131	129	116	132	133	136	132	133	128	134	136	137	131	127	115	112	123
4	146	148	139	151	147	151	145	137	148	143	149	142	152	138	156	149	146
5	134	128	130	134	128	130	124	128	130	134	118	126	129	118	124	134	130
6	117	120	126	131	136	120	128	127	123	127	136	133	129	126	121	131	132
7	114	109	107	108	110	112	109	108	104	114	109	107	108	114	106	109	113
Mean (SEM)	**134 (17)**	**135 (18)**	**127 (13)**	**133 (14)**	**135 (15)**	**133 (13)**	**132 (15)**	**132 (13)**	**132 (17)**	**132 (15)**	**132 (14)**	**133 (13)**	**135 (18)**	**128 (9)**	**127 (16)**	**132 (17)**	**134 (14)**

Table 8A: Mean (SEM) measurements of systolic blood pressure (mm/Hg) at baseline (base), 3, 6, 9 and 12 minutes after breathing inspired oxygen fraction(FiO_2) of 0.21, 0.18, 0.15 and 0.12 in 7 normal subjects (chapter 4).

	Heart rate (beats per minute)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	88	87	77	89	80	88	82	88	77	89	79
2	70	76	69	75	73	75	71	73	72	71	69
3	71	73	69	72	68	74	68	73	69	71	68
4	73	60	58	61	57	60	61	60	58	61	58
5	84	68	58	63	61	66	58	64	55	66	58
6	77	78	72	77	73	77	76	74	75	76	72
7	89	78	72	73	74	77	69	72	71	75	68
8	74	74	71	79	70	79	73	72	70	73	73
Mean (SEM)	**78 (3.6)**	**74 (2.8)**	**68 (2.4)**	**74 (3.1)**	**69 (2.6)**	**74 (3.0)**	**70 (2.7)**	**72 (2.9)**	**68 (2.8)**	**73 (2.9)**	**68 (2.5)**

Table 9A: Mean (SEM) measurements of heart rate (beats per minute) at baseline (base), 3, 6, 9, 12 and 15 minutes in 8 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Oxygen saturation (SaO_2%)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	96	96	97	96	98	96	97	96	98	96	97
2	98	99	100	99	100	99	100	99	100	99	100
3	97	96	97	96	97	96	97	97	97	96	97
4	97	97	98	97	98	97	98	97	98	97	98
5	98	96	97	96	97	96	97	97	98	96	97
6	96	96	98	96	98	96	98	96	98	96	98
7	96	98	100	98	99	99	99	98	99	99	100
8	98	98	99	98	99	98	98	98	100	98	99
Mean (SEM)	**97 (0.36)**	**97 (0.42)**	**98 (0.45)**	**97 (0.42)**	**98 (0.37)**	**97 (0.48)**	**98 (0.38)**	**97 (0.37)**	**99 (0.38)**	**97 (0.48)**	**98 (0.45)**

Table 10A: Mean (SEM) measurements of oxygen saturation (SaO_2%) at baseline (base), 3, 6, 9, 12 and 15 minutes in 8 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	End-tidal CO_2% levels ($PETCO_2$%)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	5.8	5.6	5.9	5.7	5.6	5.8	5.2	5.8	5.5	5.7	5.6
2	5.0	4.8	4.9	4.7	4.6	4.6	4.8	4.8	4.6	4.9	4.8
3	5.5	5.7	5.1	5.5	5.1	5.2	4.9	5.1	4.7	5.2	4.9
4	6.2	6.2	5.9	6.1	5.8	6.2	5.7	6.2	5.9	6.1	5.8
5	5.8	5.9	5.8	5.7	5.3	5.7	5.7	5.6	5.9	5.6	5.6
6	6.3	6.7	6.6	6.6	6.3	6.7	6.4	6.7	6.3	6.6	6.2
7	3.5	3.8	4.0	3.7	3.6	4.0	3.6	3.9	3.6	4.1	3.8
8	5.7	5.9	5.7	5.7	5.4	5.7	5.3	5.8	5.3	5.5	5.2
Mean (SEM)	**5.5 (0.36)**	**5.7 (0.30)**	**5.6 (0.27)**	**5.6 (0.30)**	**5.3 (0.28)**	**5.7 (0.28)**	**5.3 (0.29)**	**5.6 (0.30)**	**5.4 (0.30)**	**5.6 (0.26)**	**5.3 (0.26)**

Table 11A: Mean (SEM) measurements of end-tidal CO_2% levels ($PETCO_2$%) at baseline (base), 3, 6, 9, 12 and 15 minutes in 8 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Respiratory rate (breaths per minute)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	14	11	7	10	9	8	10	9	8	8	6
2	12	13	14	14	17	15	15	14	16	16	14
3	11	12	14	13	15	14	15	14	14	13	14
4	10	10	12	12	10	11	11	14	12	10	9
5	16	14	17	17	18	16	16	13	12	11	16
6	9	7	5	6	6	6	6	6	7	6	6
7	10	12	12	12	10	11	8	12	12	16	11
8	14	16	14	16	13	13	17	14	19	13	18
Mean (SEM)	**12 (1.3)**	**12 (1.0)**	**12 (1.4)**	**12 (1.2)**	**12 (1.5)**	**12 (1.2)**	**12 (1.4)**	**12 (1.1)**	**12 (1.4)**	**12 (1.3)**	**12 (1.6)**

Table 12A: Mean (SEM) measurements of diastolic respiratory rate (breaths per minute) at baseline (base), 3, 6, 9, 12 and 15 minutes in 8 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Diastolic blood pressure (mm/Hg)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	78	77	84	78	83	81	83	78	85	81	83
2	66	66	63	68	63	55	60	62	59	52	63
3	74	82	78	84	79	74	65	81	83	81	73
4	68	65	73	62	66	64	69	66	75	64	67
5	84	86	86	85	87	85	91	85	88	82	91
6	69	68	68	72	67	70	68	68	67	66	74
Mean (SEM)	**73 (3.9)**	**74 (3.6)**	**75 (3.7)**	**75 (3.7)**	**74 (4.1)**	**72 (4.5)**	**73 (4.8)**	**73 (3.8)**	**76 (4.6)**	**71 (5.0)**	**75 (4.2)**

Table 13A: Mean (SEM) measurements of diastolic blood pressure (mm/Hg) at baseline (base), 3, 6, 9, 12 and 15 minutes in 6 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Systolic blood pressure (mm/Hg)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	120	127	123	122	131	120	131	122	121	123	129
2	126	129	126	123	143	126	135	122	122	121	121
3	135	146	136	143	132	145	144	143	143	144	136
4	121	117	127	117	120	122	122	121	131	121	126
5	157	146	145	151	147	153	152	163	148	143	155
6	136	133	127	135	128	133	126	128	131	131	135
Mean (SEM)	**132 (4.8)**	**133 (4.6)**	**131 (3.4)**	**132 (5.5)**	**134 (4.1)**	**133 (5.4)**	**135 (4.6)**	**133 (6.8)**	**133 (4.5)**	**130 (4.4)**	**134 (4.8)**

Table 14A: Mean (SEM) measurements of systolic blood pressure (mm/Hg) at baseline (base), 3, 6, 9, 12 and 15 minutes in 6 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Mean arterial blood pressure (mm/Hg)										
	Base	3 minutes		6 minutes		9 minutes		12 minutes		15 minutes	
Subject	0.21	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0	0.21	1.0
1	92	94	97	93	99	94	99	93	97	95	98
2	86	87	84	86	90	79	85	82	80	75	82
3	94	103	97	104	97	98	91	102	103	102	94
4	83	82	91	80	84	83	87	84	94	83	87
5	108	106	106	103	107	108	111	111	108	102	112
6	91	90	88	93	87	91	87	88	88	88	94
Mean (SEM)	**92 (4.4)**	**94 (4.6)**	**93 (3.8)**	**93 (4.7)**	**95 (3.9)**	**92 (5.2)**	**95 (4.8)**	**94 (5.5)**	**96 (4.8)**	**91 (5.4)**	**95 (5.2)**

Table 15A: Mean (SEM) measurements of mean arterial blood pressure (mm/Hg) at baseline (base), 3, 6, 9, 12 and 15 minutes in 6 normal subjects breathing inspired oxygen fraction (FiO_2) of 0.21 and 1.0 (chapter 4).

	Heart Rate (beats per minute)						Oxygen Saturation (SaO_2 %)						End Tidal CO_2% (PET CO_2%)					
	Normoxia			Hyperoxia			Normoxia			Hyperoxia			Normoxia			Hyperoxia		
Subject	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met
1	81	76	73	67	67	53	97	97	96	98	99	99	3.6	2.8	3.3	3.3	3.2	3.4
2	64	61	68	81	75	67	97	97	96	96	97	97	3.7	3.6	3.7	4.7	3.6	3.8
3	95	94	97	95	91	90	97	97	95	97	97	97	4.7	3.6	3.9	5.0	4.5	4.2
4	76	74	78	76	73	72	98	99	99	98	99	98	5.3	4.8	4.8	5.3	4.8	4.8
5	66	54	66	64	50	56	98	98	98	98	99	99	3.6	3.8	3.5	4.4	4.0	3.8
6	76	74	78	73	63	64	96	97	95	98	98	98	4.8	4.9	4.6	5.1	4.8	4.3
7	85	85	86	92	83	85	96	97	97	98	99	98	4.0	3.5	3.1	2.6	2.6	2.2
8	48	47	48	49	46	46	97	97	97	97	99	99	5.0	4.6	4.5	5.1	4.8	4.7
9	54	54	51	53	53	66	97	98	98	97	99	99	4.7	3.6	2.8	3.7	4.1	2.6
10	94	91	92	84	76	77	95	95	94	95	98	98	4.3	4.3	4.4	4.3	4.2	4.4
11	98	98	103	92	88	90	94	94	92	94	97	97	5.2	5.0	4.4	4.8	5.0	4.5
12	98	99	98	74	76	76	98	98	98	97	98	98	3.9	4.3	3.6	4.8	3.3	3.8
13	67	70	72	68	67	65	96	96	94	96	98	98	5.1	4.9	3.6	4.7	3.8	3.0
14	81	78	79	79	80	83	95	95	95	95	97	98	5.0	4.8	4.9	4.8	4.4	4.7
Mean (SEM)	**77 (4.3)**	**75 (4.5)**	**78 (4.4)**	**75 (3.7)**	**71 (3.7)**	**71 (3.6)**	**96 (0.3)**	**97 (0.4)**	**96 (0.5)**	**97 (0.4)**	**98 (0.2)**	**98 (0.2)**	**4.5 (0.17)**	**4.2 (0.18)**	**3.9 (0.18)**	**4.5 (0.21)**	**4.1 (0.19)**	**3.9 (0.22)**

Table 16A: Mean (SEM) measurements of heart rate, oxygen saturation and end-tidal carbon dioxide levels on the normoxic (FiO_2 inspired oxygen fraction 0.21) and hyperoxic (FiO_2 inspired oxygen fraction 1.0) study days at baseline (base), prior to (pre-met) and following (post-met) a methacholine inhalation challenge test in 14 mild asthmatic patients (Chapter 5)

	Plasma noradrenaline (nmol/l)				Plasma adrenaline (nmol/l)				Methacholine PC20 values (mg/ml)	
	Normoxia		Hyperoxia		Normoxia		Hyperoxia		Normoxia	Hyperoxia
Subject	Baseline	Post met	Baseline	Post met	Baseline	Post met	Baseline	Post met		
1	1.76	1.44	1.82	1.38	0.14	0.10	<0.10	<0.10	3.38	2.57
2	2.28	1.66	0.92	0.68	0.17	<0.10	<0.10	<0.10	0.69	1.02
3	1.21	1.77	1.83	1.10	0.10	0.10	<0.10	<0.10	2.22	1.60
4	3.39	3.90	3.68	3.12	0.14	0.15	0.21	0.10	0.02	0.13
5	2.33	1.95	3.08	3.30	0.15	0.10	0.12	0.14	3.72	5.13
6	8.02	3.19	2.30	2.72	0.21	0.20	0.14	0.15	4.26	1.39
7	1.48	1.39	1.00	0.95	0.10	0.11	<0.10	<0.10	5.90	1.96
8	0.65	0.72	0.59	0.45	0.12	<0.10	<0.10	<0.10	3.83	15.9
9	1.10	0.92	2.06	1.43	<0.10	<0.10	0.16	0.18	0.17	0.54
10	1.07	0.62	1.65	2.04	<0.10	<0.10	0.10	0.17	0.38	1.50
11	0.96	1.28	1.04	0.80	<0.10	<0.10	<0.10	<0.10	10.6	14.3
12	1.37	1.11	1.64	1.57	0.17	0.10	0.12	0.12	0.34	1.11
13	-	-	-	-	-	-	-	-	0.79	0.40
14	-	-	-	-	-	-	-	-	0.08	0.14
Mean (SEM)	**2.14 (0.58)**	**1.66 (0.28)**	**1.80 (0.26)**	**1.63 (0.28)**	**0.11 (0.02)**	**0.07 (0.02)**	**0.07 (0.02)**	**0.07 (0.02)**	**0.96***	**1.38***

Table 17A: Mean (SEM) measurements of plasma adrenaline and noradrenaline levels (nmol/l) in 12 asthmatic patients at baseline and following a methacholine inhalation challenge (post met) breathing air (FiO_2 inspired oxygen fraction 0.21) and oxygen (FiO_2 inspired oxygen fraction 1.0). Absolute and * geometric mean PC_{20} values (mg/ml) in 14 asthmatic patients breathing air (FiO_2 inspired oxygen fraction 0.21) and oxygen (FiO_2 inspired oxygen fraction 1.0.) (chapter 5)

	Heart Rate (beats per minute)						Oxygen Saturation (SaO_2 %)						End Tidal CO_2% (PET CO_2%)					
	Normoxia			Hypoxia			Normoxia			Hypoxia			Normoxia			Hypoxia		
Subject	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met	Base	Pre met	Post met
1	57	58	58	50	51	50	96	97	96	97	94	92	4.8	4.8	3.1	4.8	3.6	2.9
2	73	74	77	74	75	79	96	94	97	96	90	92	4.4	4.4	3.3	4.7	4.8	4.1
3	71	72	76	64	75	71	96	97	95	95	89	90	4.5	4.8	4.7	4.7	4.6	4.6
4	55	52	51	57	60	57	97	97	97	97	91	90	4.7	4.7	4.1	4.0	3.8	3.9
5	94	93	97	92	94	96	97	96	93	96	92	87	4.9	4.7	4.7	4.9	5.2	4.4
6	82	86	83	94	87	82	97	96	96	95	91	95	4.5	4.0	3.6	4.8	4.4	3.2
7	60	57	59	63	63	64	96	97	95	97	93	89	5.2	4.6	2.9	4.8	4.5	4.4
8	72	66	62	63	61	70	97	97	97	97	92	87	4.2	4.0	3.0	4.0	3.9	3.9
9	90	90	90	94	92	90	96	96	95	96	89	87	4.3	4.4	3.9	4.0	3.9	3.8
10	71	72	75	64	69	67	97	96	97	97	88	89	4.4	5.0	3.7	5.4	5.3	4.1
11	57	59	65	62	69	69	97	96	98	96	92	93	5.3	5.4	3.7	4.9	3.8	3.2
Mean (SEM)	**71 (4.0)**	**71 (4.2)**	**72 (4.3)**	**71 (4.7)**	**72 (4.2)**	**72 (4.1)**	**96.5 (0.16)**	**96.2 (0.27)**	**96.0 (0.43)**	**96.3 (0.24)**	**91.0 (0.56)**	**90.5 (1.0)**	**4.7 (0.11)**	**4.6 (0.12)**	**3.7 (0.19)**	**4.6 (0.14)**	**4.4 (0.18)**	**3.9 (0.17)**

Table 18A: Mean (SEM) measurements of heart rate, oxygen saturation and end-tidal carbon dioxide levels on the normoxic (FiO_2 inspired oxygen fraction 0.21) and hypoxic (FiO_2 inspired oxygen fraction 0.15) study days at baseline (base), prior to (pre-met) and following (post-met) a methacholine inhalation challenge test in 11 mild asthmatic patients (Chapter 6)

	Plasma noradrenaline (nmol/l)				Plasma adrenaline (nmol/l)				Methacholine PC20 values (mg/ml)	
	Normoxia		Hypoxia		Normoxia		Hypoxia		Normoxia	Hypoxia
Subject	Baseline	Post met	Baseline	Post met	Baseline	Post met	Baseline	Post met		
1	1.10	1.06	0.66	0.62	0.10	0.10	<0.10	<0.10	1.22	0.53
2	1.60	1.21	1.30	1.26	0.11	<0.10	0.14	<0.10	0.89	0.48
3	1.50	1.17	1.27	1.40	0.17	0.11	0.14	<0.10	1.40	0.30
4	1.36	1.82	1.20	0.94	<0.10	0.15	0.11	<0.10	3.48	1.80
5	1.42	1.61	1.60	1.11	0.11	<0.10	0.13	<0.10	14.7	15.5
6	1.91	2.11	1.20	1.65	0.14	0.16	<0.10	0.16	1.77	1.14
7	2.24	1.97	1.41	1.25	0.16	0.15	0.15	0.11	1.24	0.04
8	1.80	0.93	0.62	0.44	0.10	<0.10	<0.10	<0.10	1.91	1.78
9	1.33	1.50	1.82	1.67	<0.10	<0.10	0.20	0.11	0.26	0.27
10	2.57	2.66	3.88	3.06	0.10	0.15	0.15	0.12	23.0	7.73
11	2.10	1.57	2.75	1.44	0.13	0.13	0.17	0.14	9.84	0.76
Mean (SEM)	**1.70 (0.14)**	**1.60 (0.16)**	**1.61 (0.29)**	**1.40 (0.25)**	**0.10 (0.02)**	**0.08 (0.02)**	**0.11 (0.02)**	**0.06 (0.02)**	*** 2.45**	*** 0.89**

Table 19A: Mean (SEM) measurements of plasma adrenaline and noradrenaline levels (nmol/l) in 11 asthmatic patients at baseline and following a methacholine inhalation challenge (post met) breathing air (FiO_2 inspired oxygen fraction 0.21) and a hypoxic gas mixture (FiO_2 inspired oxygen fraction 0.15). Absolute and * geometric mean PC_{20} values (mg/ml) in 11 asthmatic patients breathing air FiO_2 0.21 and a hypoxic gas mixture FiO_2 0.15.(chapter 6)

	Heart Rate (beats per minute)									Oxygen saturation (SaO_2%)								
	Normoxia			Hyperoxia			Hypoxia			Normoxia			Hyperoxia			Hypoxia		
Subject	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Base	Pre hist	Post hist
1	85	83	90	85	80	79	88	96	91	97	97	98	97	98	98	97	90	93
2	98	98	99	83	85	86	81	78	81	96	95	96	98	99	98	97	87	91
3	49	52	55	52	51	52	52	55	55	96	96	96	97	99	99	96	87	85
4	54	57	62	70	58	69	59	56	64	98	97	97	97	98	97	98	95	93
5	65	67	62	66	57	57	69	73	68	97	96	97	96	98	99	97	91	91
6	64	66	64	61	57	61	74	78	74	97	97	97	97	99	99	98	91	90
7	64	60	73	66	60	66	70	68	71	97	97	94	97	98	98	98	90	81
8	64	61	60	64	54	51	65	69	68	96	97	96	96	97	98	96	88	87
9	66	65	68	63	58	66	63	70	68	97	97	97	96	97	98	97	87	88
10	73	74	74	64	57	62	56	67	57	96	97	96	96	98	96	94	88	91
11	96	95	98	94	88	89	86	97	96	96	96	96	96	98	98	96	90	89
12	73	74	73	82	83	72	85	82	73	95	95	97	96	97	97	96	89	93
13	70	74	82	69	66	63	73	69	80	97	97	95	97	99	99	97	90	87
14	72	71	72	71	64	73	67	72	68	96	96	94	96	97	97	95	87	87
Mean (SEM)	**71 (3.7)**	**71 (3.6)**	**74 (3.7)**	**71 (3.0)**	**66 (3.4)**	**68 (3.1)**	**71 (3.0)**	**74 (3.3)**	**72 (3.1)**	**96 (0.2)**	**96 (0.2)**	**96 (0.3)**	**96 (0.2)**	**98 (0.2)**	**98 (0.2)**	**97 (0.3)**	**89 (0.6)**	**89 (0.9)**

Table 20A: Mean (SEM) measurements of heart rate and oxygen saturation on the normoxic (FiO_2 inspired oxygen fraction 0.21), hyperoxic (FiO_2 inspired oxygen fraction 1.0) and hypoxic (FiO_2 inspired oxygen fraction 0.15) study days at baseline (base), prior to (pre-hist) and following (post-hist) a histamine inhalation challenge test in 14 mild asthmatic patients (Chapter 7)

	End-tidal CO_2% ($PETCO_2$%)									PC_{20} histamine (mg/ml)		
	Normoxia			Hyperoxia			Hypoxia					
Subject	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Base	Pre hist	Post hist	Normoxia	Hyperoxia	Hypoxia
1	5.3	4.9	3.9	4.5	3.7	3.4	5.3	4.7	3.7	5.24	6.60	1.65
2	5.0	4.8	3.3	4.3	4.0	2.8	4.3	4.4	3.2	0.37	0.33	0.76
3	4.7	4.3	4.3	4.3	4.0	4.2	4.1	4.2	4.1	2.96	2.18	0.95
4	4.3	4.2	4.6	4.4	4.5	3.9	4.6	4.4	3.9	5.87	4.91	2.97
5	4.3	4.4	3.2	5.2	4.9	3.9	4.1	4.1	3.1	6.40	5.71	7.03
6	4.7	4.6	4.8	4.7	4.4	4.5	4.9	4.6	4.4	1.64	0.77	0.53
7	4.7	4.5	3.0	4.4	3.9	3.7	4.5	4.4	4.1	0.92	0.78	0.45
8	5.2	5.0	5.3	5.2	5.2	4.8	5.2	5.2	2.3	0.30	0.74	1.22
9	5.3	5.4	3.8	5.4	4.9	3.9	5.5	5.0	4.0	2.61	3.45	2.19
10	5.2	5.2	4.7	5.3	4.5	3.8	4.9	5.3	3.7	13.1	18.5	17.4
11	4.5	4.4	4.3	4.7	4.3	3.9	4.2	4.1	4.0	1.11	2.47	0.27
12	5.1	5.1	4.1	5.2	4.7	4.3	5.2	4.8	4.1	0.54	2.57	4.54
13	5.0	5.1	5.1	5.3	5.3	4.7	5.7	5.4	4.3	15.4	19.0	27.4
14	4.9	4.8	4.7	5.1	4.9	4.7	5.2	5.0	4.8	1.87	0.29	0.93
Mean (SEM)	**4.9 (0.1)**	**4.8 (0.1)**	**4.2 (0.2)**	**4.9 (0.1)**	**4.5 (0.1)**	**4.0 (0.1)**	**4.8 (0.1)**	**4.7 (0.1)**	**3.8 (0.2)**	***2.19**	***2.32**	***1.91**

Table 21A: Mean (SEM) measurements of end-tidal carbon dioxide levels ($PETCO_2$%) on the normoxic (FiO_2 inspired oxygen fraction 0.21), hyperoxic (FiO_2 inspired oxygen fraction 1.0) and hypoxic (FiO_2 inspired oxygen fraction 0.15) study days at baseline (base), prior to (pre-hist) and following (post-hist) a histamine inhalation challenge test in 14 mild asthmatic patients. Absolute and geometric mean PC_{20} values for histamine (mg/ml) on normoxic, hyperoxic and hypoxic study days in 14 mild asthmatic patients (Chapter 7)

	Heart rate (beats per minute)															
	Normoxia/Placebo				Hyperoxia/Placebo				Normoxia/Salbutamol				Hyperoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	79	81	80	77	75	69	69	70	75	70	82	66	79	72	71	71
2	80	85	79	78	94	90	80	82	99	93	92	91	86	77	78	80
3	68	68	65	66	75	71	72	73	66	64	72	65	78	73	74	75
4	84	84	83	82	79	78	76	73	83	82	81	76	86	86	86	85
5	83	74	73	76	81	67	67	70	85	81	86	84	90	80	80	78
6	87	86	85	84	75	71	60	70	83	81	79	79	77	72	72	73
7	81	76	81	75	73	70	70	69	79	77	80	75	78	74	73	75
8	64	62	64	64	69	70	64	81	62	64	62	66	68	64	67	66
9	87	89	87	86	78	70	69	69	108	108	106	111	84	77	74	75
10	81	73	72	73	74	70	70	70	74	73	70	73	67	61	65	69
11	54	55	54	53	54	50	50	56	54	61	51	54	57	48	50	48
12	75	72	71	72	72	61	62	63	70	68	68	73	69	63	60	56
Mean (SEM)	**77 (2.9)**	**75 (3.0)**	**74 (2.9)**	**74 (2.7)**	**75 (2.6)**	**70 (2.7)**	**71 (2.9)**	**71 (2.0)**	**78 (4.4)**	**77 (3.9)**	**77 (4.1)**	**75 (4.5)**	**76 (3.1)**	**71 (2.9)**	**71 (2.7)**	**71 (3.0)**

Table 22A: Mean (SEM) measurements of heart rate (beats per minute) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing air (FiO_2 inspired oxygen fraction 0.21) or oxygen (FiO_2 inspired oxygen fraction 1.0) in 12 patients with asthma (chapter 8).

	Oxygen Saturation (SaO_2%)															
	Normoxia/Placebo				Hyperoxia/Placebo				Normoxia/Salbutamol				Hyperoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	95	94	95	95	95	97	97	97	96	95	95	95	96	97	97	97
2	97	97	97	98	97	98	98	97	97	98	97	98	97	98	98	98
3	98	98	98	98	97	98	99	99	99	99	99	99	97	98	99	99
4	95	95	94	94	95	98	98	98	96	95	94	95	97	97	97	93
5	95	91	95	94	95	98	98	98	94	93	93	94	95	97	97	97
6	96	97	97	96	96	98	97	97	96	97	97	96	96	98	98	98
7	92	92	93	93	94	97	97	97	94	93	94	93	93	98	97	98
8	98	98	98	99	97	99	99	99	98	99	99	99	98	99	98	99
9	94	96	95	95	97	99	99	100	94	94	94	96	94	99	99	99
10	97	97	97	97	97	98	97	98	98	99	98	96	97	98	98	97
11	97	98	97	97	97	98	98	99	95	95	95	96	96	98	99	98
12	96	96	96	96	97	98	97	97	95	95	96	95	97	98	99	99
Mean (SEM)	**96 (0.5)**	**96 (0.7)**	**96 (0.5)**	**96 (0.5)**	**96 (0.3)**	**98 (0.2)**	**98 (0.2)**	**98 (0.3)**	**96 (0.5)**	**96 (0.7)**	**96 (0.6)**	**96 (0.5)**	**96 (0.4)**	**98 (0.2)**	**98 (0.2)**	**98 (0.5)**

Table 23A: Mean (SEM) measurements of oxygen saturation (SaO_2%) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing air (FiO_2 inspired oxygen fraction 0.21) or oxygen (FiO_2 inspired oxygen fraction 1.0) in 12 patients with asthma (chapter 8).

	End-tidal CO_2 level ($PETCO_2$%)															
	Normoxia/Placebo				Hyperoxia/Placebo				Normoxia/Salbutamol				Hyperoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	4.0	4.7	3.8	4.4	4.7	4.2	4.0	4.1	3.7	5.0	5.1	4.5	4.6	4.0	4.1	4.1
2	3.9	3.7	4.1	3.6	3.5	3.8	3.6	3.6	3.3	3.0	3.2	3.0	4.1	4.4	4.3	3.6
3	3.8	3.8	4.2	3.5	3.2	3.7	3.3	3.2	3.7	3.3	3.4	2.8	3.8	3.6	4.1	3.3
4	5.1	5.0	5.0	5.0	5.1	4.5	4.6	4.7	4.6	4.8	4.8	4.8	5.0	4.4	3.4	5.1
5	4.9	4.8	5.1	4.9	5.0	4.7	4.6	4.4	4.7	4.7	4.5	4.6	4.8	4.4	4.4	4.3
6	3.4	3.3	3.2	3.3	3.3	2.7	2.9	2.8	3.1	2.8	2.9	2.7	3.5	3.3	2.9	2.8
7	4.6	4.5	4.6	4.7	5.0	4.0	4.6	4.5	4.7	4.5	4.6	4.5	4.8	4.4	4.3	4.4
8	5.2	5.0	4.9	5.0	5.8	4.3	4.5	4.3	5.1	4.9	5.0	5.0	5.0	4.2	4.3	3.9
9	4.3	4.6	4.4	4.6	4.7	4.2	4.1	4.2	3.9	4.6	4.6	4.8	4.9	4.6	4.3	4.9
10	2.9	2.9	2.9	2.8	3.5	3.4	2.9	2.4	4.0	3.7	3.3	3.4	4.0	3.9	3.7	4.0
11	4.6	3.3	4.8	3.7	5.0	4.6	4.7	3.6	4.6	4.5	4.5	3.8	4.7	4.7	4.3	4.5
12	5.0	5.1	5.0	4.8	5.1	4.7	4.8	4.8	5.0	4.9	4.9	4.8	4.9	4.7	4.6	4.6
Mean (SEM)	**4.3 (0.21)**	**4.3 (0.23)**	**4.3 (0.21)**	**4.2 (0.22)**	**4.5 (0.25)**	**4.1 (0.17)**	**4.1 (0.20)**	**3.9 (0.22)**	**4.2 (0.19)**	**4.3 (0.23)**	**4.2 (0.23)**	**4.1 (0.25)**	**4.5 (0.15)**	**4.2 (0.13)**	**4.1 (0.14)**	**4.12 (0.19)**

Table 24A: Mean (SEM) measurements of end-tidal carbon dioxide levels ($PETCO_2$%) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing air (FiO_2 inspired oxygen fraction 0.21) or oxygen (FiO_2 inspired oxygen fraction 1.0) in 12 patients with asthma (chapter 8).

	FEV_1 (litres)															
	Normoxia/Placebo				Hyperoxia/Placebo				Normoxia/Salbutamol				Hyperoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	1.40	1.39	1.43	1.33	0.82	0.82	0.84	0.85	1.44	1.80	1.96	1.94	1.71	1.44	1.57	1.79
2	3.40	3.28	3.19	2.96	3.20	3.22	3.22	3.18	3.45	3.62	3.63	3.50	2.68	3.24	3.09	3.04
3	2.53	2.60	2.76	2.50	2.98	2.86	2.73	2.57	3.15	2.80	3.10	3.19	2.91	2.79	2.58	2.91
4	2.19	2.18	2.21	2.11	2.22	2.33	2.35	2.34	2.19	2.31	2.45	2.57	2.16	2.24	2.52	2.58
5	1.93	1.96	1.96	1.93	1.77	1.61	1.59	1.49	1.75	2.06	2.14	2.32	1.64	2.02	2.27	2.45
6	1.84	1.78	1.76	1.79	2.11	2.02	2.00	1.98	2.13	2.32	2.62	2.78	1.52	2.07	2.37	2.48
7	0.82	0.82	0.82	0.80	0.96	0.92	0.93	0.91	0.90	0.99	0.98	1.06	0.99	1.12	1.25	1.22
8	2.24	1.86	1.97	1.71	2.24	2.47	2.33	1.99	1.88	2.26	2.29	2.00	2.14	2.04	2.37	2.46
9	1.45	2.05	1.93	1.95	1.85	2.41	2.35	2.45	1.68	1.81	2.10	2.30	1.61	1.43	2.08	1.83
10	1.98	1.53	1.39	1.30	1.85	1.88	1.76	1.51	1.33	1.54	1.67	1.74	1.18	1.26	1.40	1.79
11	1.81	1.80	1.76	1.81	1.78	1.84	1.83	1.73	1.78	1.87	2.02	2.10	1.83	1.78	1.97	1.99
12	3.12	3.43	3.55	3.58	2.88	3.12	3.11	3.15	3.17	3.15	3.50	3.63	3.41	3.54	3.50	3.65
Mean (SEM)	**2.06 (0.21)**	**2.06 (0.22)**	**2.06 (0.22)**	**1.98 (0.22)**	**2.06 (0.21)**	**2.13 (0.22)**	**2.09 (0.22)**	**2.01 (0.22)**	**2.07 (0.23)**	**2.21 (0.21)**	**2.37 (0.22)**	**2.43 (0.22)**	**1.98 (0.21)**	**2.08 (0.22)**	**2.25 (0.19)**	**2.35 (0.19)**

Table 25A: Mean (SEM) measurements of FEV_1 (litres) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing air (FiO_2 inspired oxygen fraction 0.21) or oxygen (FiO_2 inspired oxygen fraction 1.0) in 12 patients with asthma (chapter 8).

	Plasma noradrenaline levels (nmol/l)								Plasma adrenaline levels (nmol/l)							
	Nor/plac		Hyper/plac		Nor/sal		Hyper sal		Nor/plac		Hyper/plac		Nor/sal		Hyper/sal	
Subject	Base	45 min	Base	45 min	Base	45 min	Base	45 min	Base	45 min	Base	45 min	Base	45 min	Base	45 min
1	0.25	0.47	0.43	0.39	0.30	0.19	0.53	0.17	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2	1.63	1.14	6.41	4.46	5.93	10.5	4.86	3.37	0.10	0.12	0.15	0.2	0.30	0.14	0.17	0.16
3	4.66	5.36	2.60	2.65	3.62	3.85	2.88	3.08	0.22	0.20	0.14	0.11	0.17	0.20	0.20	0.14
4	3.09	2.77	1.82	3.82	3.71	4.08	2.57	3.44	0.12	0.10	0.16	0.10	0.14	0.15	0.15	0.14
5	1.48	2.43	1.80	1.29	1.95	1.80	2.34	2.29	0.11	<0.10	<0.10	<0.10	<0.10	<0.10	0.14	0.11
6	4.47	2.50	2.72	1.37	2.76	2.68	3.25	1.40	0.14	0.20	<0.10	0.17	0.15	0.17	<0.10	0.16
7	0.76	0.91	1.53	1.40	1.47	1.30	1.10	1.26	<0.10	<0.10	<0.10	0.12	0.13	0.14	0.10	<0.10
8	0.97	0.81	1.05	0.71	0.88	1.86	1.12	0.95	<0.10	0.12	<0.10	0.12	0.13	<0.10	<0.10	0.10
9	1.08	1.49	2.57	3.38	1.52	2.46	1.12	4.29	0.13	0.13	0.15	0.15	0.14	0.11	0.17	0.14
10	0.48	0.27	0.30	1.76	1.29	0.83	1.44	0.20	<0.10	<0.10	0.12	<0.10	<0.10	0.10	<0.10	0.14
11	2.84	3.11	3.42	3.05	2.58	2.71	3.21	3.15	0.10	0.14	0.15	0.12	0.16	0.17	0.20	0.15
12	1.54	1.64	2.16	2.31	2.09	1.45	1.70	1.71	0.10	0.11	<0.10	0.10	0.10	0.14	0.13	0.15
Mean (SEM)	**1.94 (0.43)**	**1.91 (0.41)**	**2.23 (0.47)**	**2.22 (0.37)**	**2.34 (0.44)**	**2.81 (0.77)**	**2.18 (0.36)**	**2.11 (0.39)**	**0.09 (0.02)**	**0.09 (0.02)**	**0.10 (0.02)**	**0.07 (0.02)**	**0.11 (0.02)**	**0.19 (0.03)**	**0.12 (0.02)**	**0.11 (0.02)**

Table 26A: Mean (SEM) measurements of noradrenaline and adrenaline levels (nmol/l) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing air (FiO_2 inspired oxygen fraction 0.21) or oxygen (FiO_2 inspired oxygen fraction 1.0) in 12 patients with asthma (chapter 8).

	FEV_1 (litres)															
	Hyperoxia/Placebo				Hypoxia/Placebo				Hyperoxia/Salbutamol				Hypoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	1.84	1.97	1.91	2.09	1.76	1.94	1.75	1.85	2.37	2.82	2.83	2.86	1.92	1.84	2.27	2.52
2	2.56	2.39	2.63	2.60	2.59	2.38	2.44	2.53	2.46	2.67	2.92	3.03	2.45	2.68	2.87	2.99
3	2.53	2.55	2.57	2.86	3.08	2.87	2.32	2.32	2.24	2.78	3.21	3.38	2.11	2.19	2.43	2.63
4	2.57	2.45	2.48	2.47	2.51	2.50	2.51	2.55	2.73	2.64	2.72	2.77	2.60	2.71	2.71	2.74
5	2.08	1.73	1.89	1.89	2.88	2.78	2.70	2.60	2.48	2.58	2.81	2.90	2.03	2.25	2.43	2.38
6	3.03	3.12	3.06	2.81	2.60	2.92	2.77	2.70	2.53	3.21	3.48	3.29	2.47	3.05	2.80	3.12
7	1.80	1.87	1.82	1.82	1.94	2.18	1.91	2.08	1.73	1.96	2.07	2.39	1.82	2.15	2.40	2.37
8	2.89	2.99	2.90	2.99	2.55	2.86	2.90	2.82	2.86	2.69	2.87	2.98	2.80	2.87	3.14	3.00
9	3.08	3.12	3.19	2.96	3.00	2.95	3.16	3.08	2.62	3.24	3.33	3.68	2.73	3.01	3.37	3.74
10	2.63	2.48	2.40	2.47	2.74	2.55	2.52	2.59	2.59	2.69	2.67	2.76	1.80	1.71	1.76	1.88
11	2.38	2.16	2.18	2.30	2.51	1.93	2.01	1.73	3.22	3.05	3.00	3.42	2.77	2.75	2.77	2.86
12	2.53	2.62	2.46	2.51	2.53	2.52	2.38	2.4	2.61	2.55	2.82	2.84	2.83	2.76	2.72	2.85
Mean (SEM)	**2.49 (0.12)**	**2.45 (0.14)**	**2.46 (0.13)**	**2.48 (0.11)**	**2.52 (0.12)**	**2.54 (0.10)**	**2.45 (0.12)**	**2.44 (0.11)**	**2.54 (0.10)**	**2.74 (0.10)**	**2.89 (0.10)**	**3.02 (0.10)**	**2.36 (0.12)**	**2.50 (0.13)**	**2.64 (0.12)**	**2.76 (0.13)**

Table 27A: Mean (SEM) measurements of FEV_1 (litres) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing oxygen (FiO_2 inspired oxygen fraction 1.0) or a hypoxic gas mixture (FiO_2 inspired oxygen fraction 0.15) in 12 patients with asthma (chapter 9).

	Heart rate (beats per minute)															
	Hyperoxia/Placebo				Hypoxia/Placebo				Hyperoxia/Salbutamol				Hypoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	74	73	64	59	66	75	71	71	69	67	70	70	73	81	74	85
2	68	63	63	63	77	70	71	68	73	63	62	62	73	74	70	72
3	64	60	58	58	66	72	68	69	73	58	71	62	66	72	64	68
4	78	71	71	67	89	93	92	83	80	72	73	72	77	78	79	81
5	70	63	64	68	83	76	76	80	67	73	71	63	75	79	74	79
6	75	64	67	60	73	77	78	77	86	82	80	81	74	73	70	69
7	85	81	75	79	70	75	72	70	75	73	73	70	62	64	60	62
8	70	66	65	66	66	68	69	59	64	58	59	62	66	74	69	69
9	86	74	67	64	73	70	71	69	80	75	66	71	88	85	80	79
10	81	72	73	67	72	82	78	74	82	78	80	77	82	84	86	84
11	58	58	54	50	57	59	61	60	53	57	54	52	55	54	56	52
12	65	55	54	49	62	69	67	69	59	53	52	52	54	54	55	60
Mean (SEM)	**73 (2.5)**	**67 (2.2)**	**65 (2.0)**	**63 (2.4)**	**71 (2.6)**	**74 (2.4)**	**73 (2.2)**	**71 (2.1)**	**72 (2.8)**	**67 (2.7)**	**68 (2.7)**	**66 (2.6)**	**70 (2.9)**	**73 (3.0)**	**70 (2.8)**	**72 (3.0)**

Table 28A: Mean (SEM) measurements of heart rate (beats per minute) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing oxygen (FiO_2 inspired oxygen fraction 1.0) or a hypoxic gas mixture (FiO_2 inspired oxygen fraction 0.15) in 12 patients with asthma (chapter 9).

	Oxygen saturation (SaO_2%)															
	Hyperoxia/Placebo				Hypoxia/Placebo				Hyperoxia/Salbutamol				Hypoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	96	99	98	99	96	83	87	87	97	99	99	99	97	82	85	79
2	94	96	96	96	95	88	87	90	95	96	95	96	96	86	89	85
3	96	97	97	98	95	85	85	85	95	97	97	98	96	88	89	88
4	96	98	98	98	95	86	87	86	96	98	98	98	95	87	84	86
5	95	98	98	98	95	89	89	89	98	99	98	98	95	89	89	89
6	97	98	98	99	97	87	88	87	96	97	97	97	96	86	88	90
7	94	98	98	98	96	89	90	92	95	97	98	98	96	86	87	88
8	97	98	98	97	96	88	89	92	96	98	98	98	97	89	87	84
9	97	99	99	99	97	92	84	82	97	98	99	98	97	89	89	90
10	95	97	98	98	97	87	87	90	94	97	97	97	96	87	88	88
11	96	98	98	98	96	87	87	87	97	98	98	98	97	87	90	89
12	97	98	98	99	97	86	84	87	97	99	98	99	97	90	89	89
Mean (SEM)	**96 (0.32)**	**98 (0.26)**	**98 (0.21)**	**98 (0.26)**	**96 (0.25)**	**87 (0.65)**	**87 (0.55)**	**88 (0.84)**	**96 (0.34)**	**98 90.31)**	**98 (0.31)**	**98 (0.24)**	**96 (0.22)**	**87 (0.61)**	**88 (0.52)**	**87 (0.92)**

Table 29A: Mean (SEM) measurements of oxygen saturation (SaO_2%) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing oxygen (FiO_2 inspired oxygen fraction 1.0) or a hypoxic gas mixture (FiO_2 inspired oxygen fraction 0.15) in 12 patients with asthma (chapter 9).

	End-tidal CO_2 level ($PETCO_2$%)															
	Hyperoxia/Placebo				Hypoxia/Placebo				Hyperoxia/Salbutamol				Hypoxia/Salbutamol			
Subject	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min	Base	15 min	30 min	45 min
1	5.0	4.6	4.5	4.6	5.3	4.8	5.2	4.8	5.2	4.6	4.6	4.7	5.1	5.1	4.9	5.0
2	4.2	4.7	4.7	4.4	3.4	4.5	3.7	4.2	3.5	4.4	4.8	4.6	3.9	4.2	4.4	4.1
3	5.1	5.2	5.2	5.3	4.9	4.8	4.9	5.0	4.9	4.5	4.9	4.7	4.8	4.7	4.7	4.8
4	4.7	4.4	4.4	4.4	4.3	4.5	4.3	4.4	4.8	4.9	4.7	4.6	5.0	4.5	4.6	4.5
5	4.6	3.8	3.8	3.7	4.7	4.5	4.4	4.4	4.7	4.3	4.5	4.4	4.5	4.4	4.4	4.3
6	5.0	4.6	4.5	4.8	5.5	5.2	5.0	5.1	4.9	4.5	4.5	4.4	5.1	4.7	4.7	4.7
7	4.9	5.0	4.7	4.7	5.2	4.7	5.1	5.1	5.4	4.9	4.9	5.0	5.6	5.4	5.4	4.8
8	5.2	4.9	4.9	5.3	4.7	4.8	4.7	4.6	4.4	4.9	4.9	4.8	5.0	4.7	4.6	4.6
9	4.5	4.1	4.2	4.2	3.9	3.4	4.0	4.0	4.3	4.1	3.8	3.9	3.7	3.9	3.8	3.6
10	4.5	4.1	4.0	4.0	4.8	4.7	4.5	4.6	4.6	4.3	4.4	4.5	4.7	4.4	4.4	4.5
11	4.7	4.2	4.2	4.3	4.9	4.9	4.9	4.8	5.0	4.5	4.7	4.7	4.9	4.7	4.6	4.7
12	5.0	4.7	4.3	4.3	4.9	4.6	4.8	4.4	4.4	4.5	4.3	4.5	4.6	4.5	4.6	4.6
Mean (SEM)	**4.8 (0.09)**	**4.5 (0.12)**	**4.4 (0.11)**	**4.5 (0.14)**	**4.7 (0.17)**	**4.6 (0.12)**	**4.6 (0.13)**	**4.6 (0.10)**	**4.7 (0.14)**	**4.5 (0.08)**	**4.6 90.110**	**4.6 (0.08)**	**4.7 (0.15)**	**4.6 (0.11)**	**4.6 (0.11)**	**4.5 (0.11)**

Table 30A: Mean (SEM) measurements of end-tidal CO_2% levels ($PETCO_2$%) at baseline, 15 minutes, 30 minutes and 45 minutes after incremental doses of nebulised salbutamol or placebo breathing oxygen (FiO_2 inspired oxygen fraction1.0) or a hypoxic gas mixture (FiO_2 inspired oxygen fraction 0.15) in 12 patients with asthma (chapter 9).