A Physiological Model of the Human Cough Reflex: Investigations of the Afferent Pathway and Antitussive Studies.

by

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"Cough is the voice of the lung"

Hippocrates

CONTENTS **PAGE**

CHAPTER 3: CHARACTERISATION OF THE COUGH REFLEX

CHAPTER 4:THE EFFECT OF ALTERING AIRWAY TONE ON COUGH

LIST OF TABLES

LIST OF FIGURES

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I dedicate this thesis to my family and friends.

ABBREVIATIONS

ANOVA	Analysis of variance
ASL	Airway surface liquid
CNS	Central nervous system
FEV_1	Forced expired volume in 1 second
FVC	Forced vital capacity
GSD	Geometric standard deviation
LTD_4	Leukotriene D_4
MCF	Back-transformed mean cough frequency
MDI	Metered dose inhaler
MMAD	Mass median aerodynamic diameter
N	Number of subjects
NEP	Neutral endopeptidase
PD_{20}	Dose causing a 20% fall in FEV_1
PEFR	Peak expiratory flow rate
PGE_2	Prostaglandin E_2
RAR	Rapidly adapting receptor
R_{aw}	Airways resistance
SAR	Slowly adapting receptor
SE	Standard error
sG_{aw}	Specific airways conductance
SO_2	Sulphur dioxide
Tc99m	Technetium 99m
UNCA	Ultrasonically nebulized citric acid
UNDW	Ultrasonically nebulized distilled water
URTI	Upper respiratory tract infection
95% CL	95% confidence limits

ABSTRACT

Cough is a common symptom of respiratory disease. Assessment of antitussives has relied mainly on animal studies and clinical trials in which recording of natural cough is difficult. This thesis describes the use of ultrasonically nebulized distilled water (UNDW) to induce cough in man.

Investigation of the chemosensitivity of this response identified that extremes of pH, a chloride concentration below 75mmol/l, but not changes in osmolarity induce cough which reflects afferent rapidly adapting receptor sensitivity in animal studies. Inhaled beta-adrenergic and anticholinergic bronchodilators, which inhibit cough in asthma, markedly reduced UNDW-induced cough in both healthy and asthmatic volunteers. Bronchoconstriction with inhaled leukotriene D_4, which constricts both asthmatic and non-asthmatic airways, also caused coughing. Inhibition of bronchoconstriction either specifically or non-specifically resulted in inhibition of cough. Nedocromil sodium and the diuretic, frusemide, but not the commonly prescribed opiate, codeine, exhibited antitussive activity. Cough was also induced by inhalation of the C-fibre stimulants, capsaicin and prostaglandin E_2 (PGE_2), which was characterised by studies of adaptation, cross-adaptation and antitussives. UNDW and PGE_2, but not capsaicin, exhibited rapid adaptation of cough. Cross-adaptation, however, did not occur suggesting distinct mechanisms of cough mediation. Nedocromil inhibited capsaicin-induced cough but not PGE_2-induced cough, while fenoterol did not affect either challenge. Oxitropium, which inhibited UNDW-induced cough, did not reduce cough associated with upper respiratory tract infection.

Cough can be induced by a variety of inhaled stimuli. These can identify differences in response which may signal a number of pathways leading to cough. Antitussive activity may also be specific to individual challenges. This diversity in response reflects the complex neurological organisation of cough and may be related to pathological causes of cough.

CHAPTER 1: INTRODUCTION

1.1 AIMS

The aims of this work were to develop a model of cough in humans which could be used to evaluate objectively the efficacy of proposed antitussives. Using a nebulizer to deliver aerosols of potential cough stimulants to subjects, an agent would be sought that was sufficiently irritant to evoke cough in the majority of individuals, be non-toxic and to elicit a reproducible cough response.

Having developed a safe and reliable model of cough, identification of the afferent pathway would be determined by characterising the chemosensitivity of cough and comparing results with those in animal studies. Adaptation of the cough reflex would be studied to provide further information about the afferent nerves stimulated, while cross-adaptation of cough between different stimuli would allow identification of a common receptor.

The model would then be used in healthy volunteers to try and identify antitussive activity of both prescribed and novel treatments, including opiates and bronchodilators.

Patient studies would allow comparison of the effect of bronchodilators in healthy volunteers with that in asthmatics where cough is often the only symptom.

Finally, the model would be tested against naturally occurring cough associated with upper respiratory tract infections. This is a common cause of cough for which medical treatment is frequently sought. This study would assess the relationship between induced cough and natural cough.

1.2 THE COUGH REFLEX ARC

Whilst much of the human body is covered with a thick outer protective layer, the lungs, by their physiological nature, offer a potential port of entry for noxious material. They are therefore armed with a number of powerful, complex and interacting mechanisms which defend the lungs and airways from injury. Cough is one such action and constitutes the primary defence of the lower airways and larynx. It interacts with other defensive reflexes such as bronchoconstriction and mucus secretion to prevent entry of foreign matter into the lungs and to expel secretions and other debris. Like many of the other protective mechanisms, cough is a reflex action mediated by the vagus, confirmed by the fact that bilateral cervical vagotomy in animals blocks cough (Widdicombe, 1964). However, unlike the majority of other reflexes, cough can also be initiated voluntarily, bypassing the central respiratory control centre (Davis, 1974). The cough reflex arc can be divided into five components, namely sensory receptors, afferent pathway, central synapses, efferent pathways and effector end organs. Supra-threshold stimulation of sensory receptors located close to the airway lumen is transmitted via afferent fibres of the vagus and its branches to the central cough centre located in the medulla. The efferent pathway within the somatic nervous system transmits impulses to the skeletal respiratory muscles which carry out the act of coughing. The cough reflex arc is represented in Figure 1.1.

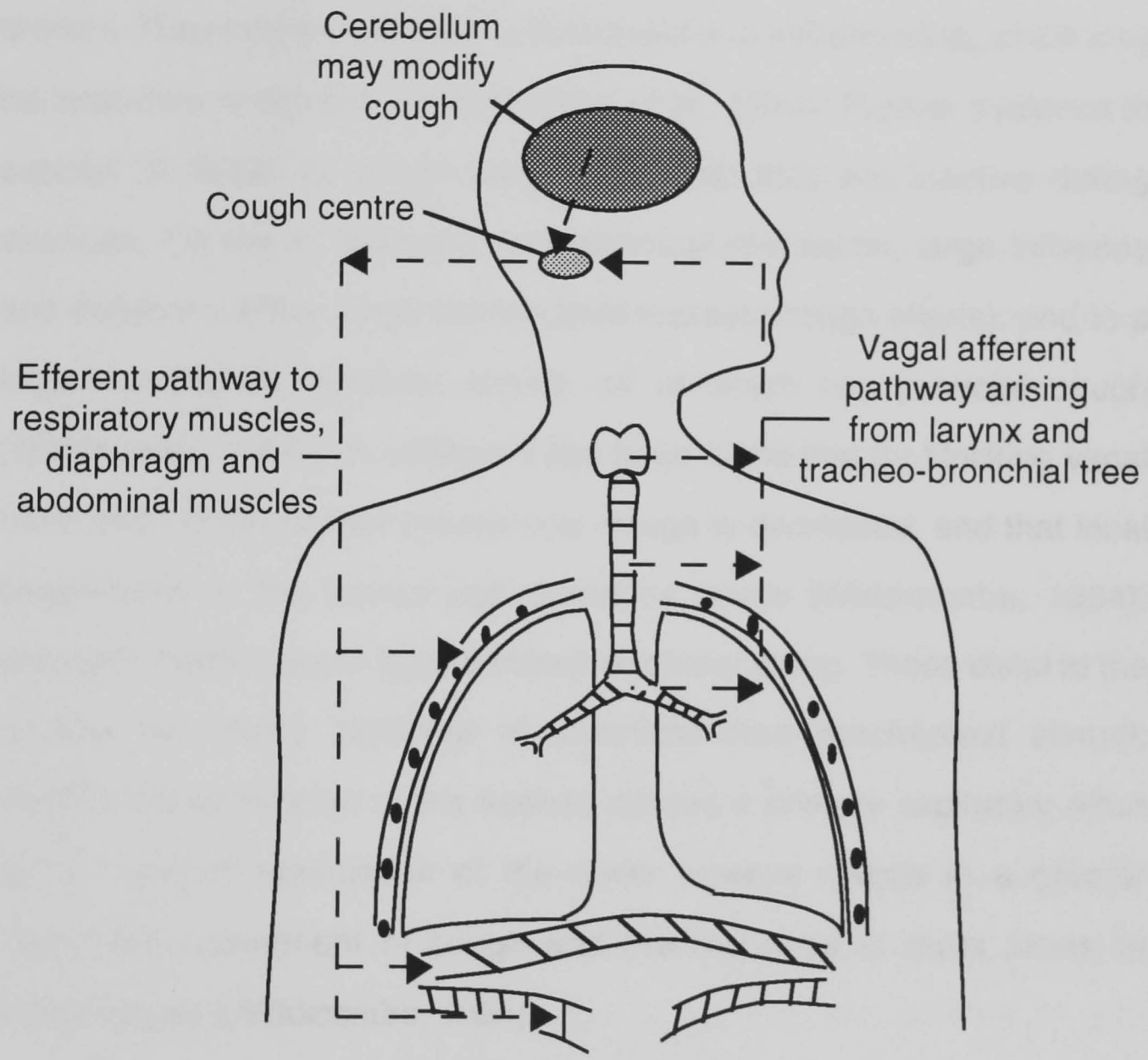
1.2.1 Sensory Receptors and Afferent Pathway

Involuntary cough in humans is entirely mediated by the vagus and can therefore only be initiated from structures it innervates. The main site of cough stimulation is the respiratory tract below the oropharynx, in particular, the larynx, trachea and main bronchi. Receptors responsible for cough are therefore expected to be concentrated in these regions and

to be inactive during tidal breathing (eupnoea). Three main groups of vagal afferent receptors have been identified.

FIGURE 1.1

Cough Reflex Arc



Rapidly adapting 'irritant' stretch receptors (RARs) run to small myelinated fibres with conduction velocities in the range of 4 to 26 ms^{-1} (Widdicombe, 1964). The nomenclature reflects their rapid adaptation of firing frequency in response to maintained stimulation. Both their position and experimental evidence support their role as being responsible for

cough. They are located within the epithelium of the larynx and tracheobronchial tree, concentrated mainly in the carina and points of bronchial branching (Widdicombe, 1964; Mortola *et al.,* 1975). Their endings lose their myelin sheath as they branch between epithelial cells terminating close to the airway lumen beneath the 'tight junctions'. Epithelial axons have been observed histologically within human airways (Laitinen, 1985) in close proximity to the lumen of the trachea and main bronchi. They contain vesicles, neurotubules and mitochondria, which may be indicative of sensory function (King *et al.*, 1974). Further evidence in support of RARs as cough receptors is that they are inactive during eupnoea, but fire in response to mechanical stimulation, large inflations and deflations of the lungs (which could maintain cough efforts), and to a large number of chemical stimuli, all of which can provoke cough (Widdicombe, 1964). In addition it has been found that by blocking vagal conduction in myelinated nerves only, cough is decreased, and that local anaesthesia of the airway wall abolishes cough (Widdicombe, 1964). However, RARs appear to be a mixed functional group. Those distal to the trachea are more sensitive to chemical than mechanical stimuli. Mechanical stimulation of the trachea causes a primary expiratory effort while chemical stimulation of the lower airways results in a greater inspiratory component of cough and the response is more prone to tachyphylaxis (Widdicombe, 1964).

Slowly adapting stretch receptors (SARs) run to large myelinated fibres with conduction velocities of 14 to 59 ms^{-1} (Widdicombe, 1964). They are found in association with airway smooth muscle particularly in large and small bronchi (Bartlett *et al.*, 1976; Miserocchi & Sant'Ambrogio, 1974) and are responsible for the Hering-Breuer inflation reflex, which primarily limits inspiration (Widdicombe, 1964). They are unlikely candidates for cough receptors because of their position within smooth

muscle and because they are insensitive to many cough stimuli (Widdicombe, 1964). However, they may modify the central integration of cough since inhibition of SARs by sulphur dioxide (SO_2) in rabbits inhibits RAR-mediated cough (Hanacek *et al.*, 1984; Sant'Ambrogio *et al.,* 1984).

C-fibre receptors run to unmyelinated fibres with conduction velocities between 0.8 and 2.6 ms^{-1}. In animal studies, they are the most numerous fibre in the vagus accounting for over 80% of fibres in the cat (Jammes *et al.*, 1982). Two categories of C-fibres have so far been distinguished; pulmonary C-fibres responsive to injections of stimulants into the right atrium and bronchial C-fibres responsive to injections of stimulants into the bronchial circulation (Coleridge & Coleridge, 1984). Chemical stimulants of C-fibres include capsaicin, the pungent extract of red pepper, (Coleridge *et al.*, 1965), prostaglandins (Coleridge *et al.*, 1976) and bradykinin (Kaufman *et al.,* 1980). Observations that inhalation of these agents in humans results in cough (Collier & Fuller, 1984; Costello *et al.*, 1985 and Simonsson *et al.*, 1973, respectively) suggest that C-fibres may also mediate cough.

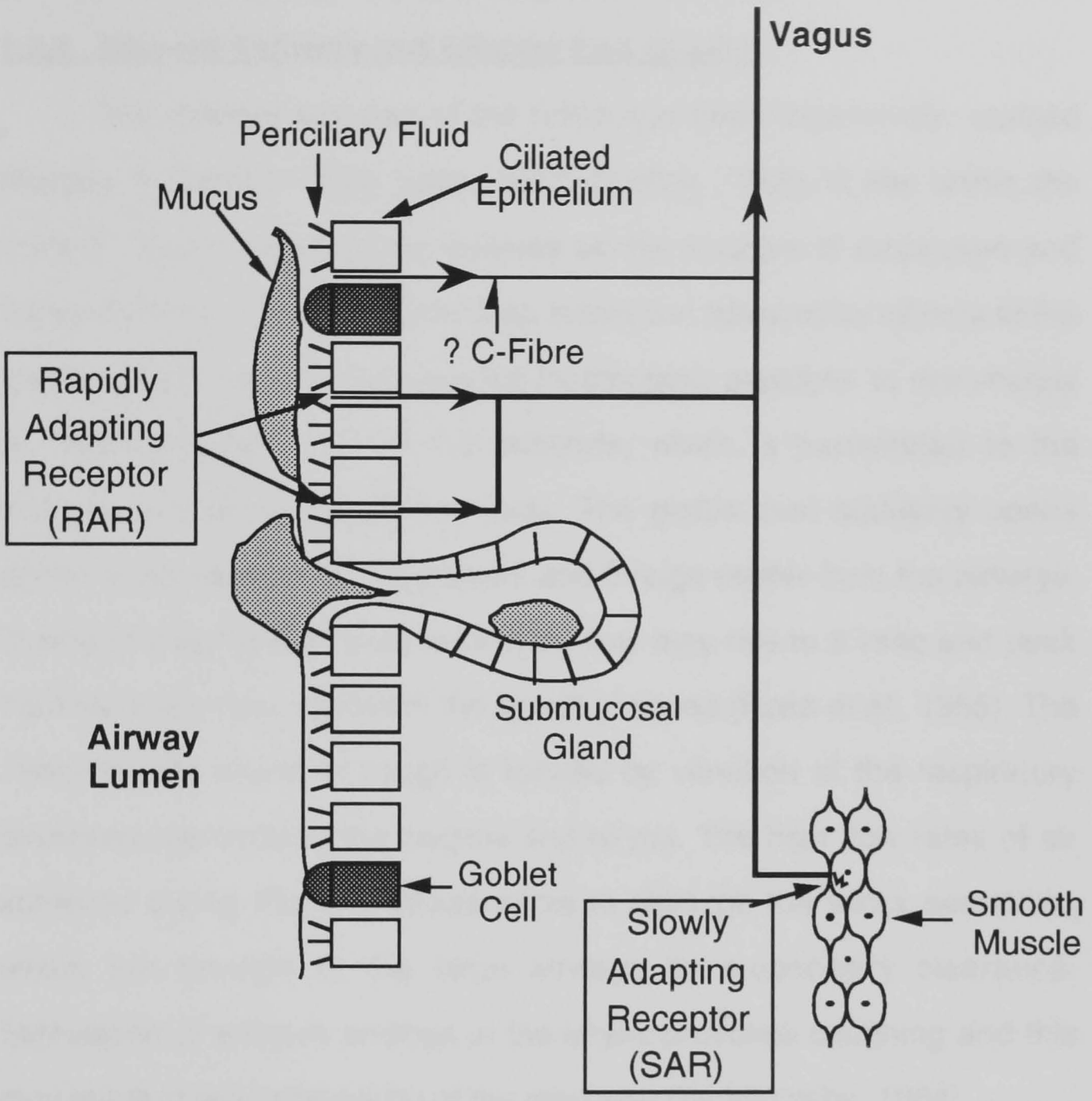
The afferent pathways of the cough reflex are represented schematically in Figure 1.2.

Thus all three groups of receptors may play a role in the cough reflex. In addition to the respiratory tract, cough can be elicited from the external auditory meatus and tympanic membrane, the pericardium and diaphragm (Korpas & Tomori, 1979).

Impulses from supra-threshold stimulation of afferent cough receptors pass up the vagus and its branches including the auricular, pharyngeal and superior laryngeal nerves to the central nervous system (CNS) via the vagal nuclei.

FIGURE 1.2

Vagal Afferent Pathways of the Cough Reflex



1.2.2 Central Integration

A 'cough centre' separate from the respiratory centre which drives the basic mechanisms of respiration has been postulated. The most likely site for this is an area close to the solitary tract nucleus in the medulla oblongata (Chou & Wang, 1975). The central mechanisms are complicated because they are not predetermined but can be modified by

higher centres in the cerebellum to achieve different patterns of cough, for example, suppressing cough during sleep (Jamal *et al.*, 1983; Power *et al.*, 1984) and initiating voluntary cough (Davis, 1974).

1.2.3 Efferent Pathway and Effector End Organs

The efferent pathway of the reflex has been extensively studied (Korpas & Tomori, 1979; Leith, 1977; Bucher, 1958). It lies within the somatic nervous system and involves all the muscles of respiration and the glottis. The result is a rapid deep inspiration followed by closure of the glottis. Forced expiration causes the intrathoracic pressure to rise sharply to 13kPa (100mm Hg) for 0.2 seconds, which is transmitted to the circulatory and cerebrospinal fluids. The glottis then suddenly opens allowing expulsion of air, secretions and foreign matter from the airways. During this last phase, peak expiratory flow may rise to 6 l/sec and peak tracheal flows may approach the speed of sound (Ross *et al.*, 1955). The characteristic sound of cough is formed by vibration of the respiratory structures, particularly the trachea and larynx. The high flow rates of air achieved during this final phase serve to dislodge the sticky secretions which are brought to the large airways by mucociliary clearance. Stimulation of efferent endings in the larynx provokes coughing and this may result in self-potentiation of the response (Widdicombe, 1964).

1.3 CAUSES AND TREATMENT OF COUGH

Cough is the commonest symptom of respiratory disease, in particular, viral infection which accounts for an estimated 50% of visits to General Practitioners during the winter and almost 50% of short-term absences from work (Korpas & Tomori, 1979). It has been estimated that the prevalence of chronic cough in the population may be as high as 20% (Barbee *et al.*, 1991). Based on the last 4 years, approximately 5 million prescriptions for antitussives have been dispensed in England each year

(personal communication, Department of Health, London, UK) and 10 million doses of cough remedies are sold over-the-counter. Cough can occur as a symptom of over 100 pulmonary and systemic disorders reflecting Hippocrates' observation that "cough is the voice of the lung". When cough performs its physiological function of clearing secretions and foreign matter effectively, it should not be treated. However, when cough is unproductive or becomes excessive or persistent, it can become a debilitating symptom serving no useful function. Cough is then described as pathological and can be associated with adverse events such as cough syncope, impaired cardiac activity and circulation, bone fractures and sleep disturbance (Banner, 1986). Treatment of pathological cough depends on making a diagnosis. Common causes of cough are identified in Table 1.1.

TABLE 1.1

Common Causes Of Cough

1. Infectious disease	Viral	Common cold, influenza
	Bacterial	Pneumonia
		Bordetella pertussis
		Tuberculosis
2. Airway disease	Asthma and allergic rhinitis	
	Chronic Bronchitis	
	Bronchiectasis	
	Lung Cancer	
3. Mechanical	Inhaled foreign bodies	
	Auricular hair	
4. Interstitial lung disease	Sarcoidosis	
	Diffuse Pulmonary fibrosis	
	Connective Tissue Disease	
5. Drug Induced	Angiotensin Converting Enzyme Inhibitors	
6. Gastro-intestinal	Gastro-oesophageal reflux	
7. Psychogenic	'Nervous habit' anxiety states	

The most common cause of cough in all age groups is viral upper respiratory tract infection (URTI). The duration of cough however is short, generally less than 14 days. Persistent coughing suggests an alternative diagnosis and warrants further investigation. Treatment directed at the disease (specific therapy) will in most cases alleviate the associated cough. For example, cough associated with asthma is effectively treated with bronchodilators (Ellul-Micallef, 1983), while treatment of allergic rhinitis with either nasal topical steroids or antihistamines will also limit

cough. Similarly, cough associated with sarcoidosis will be reduced by treatment with oral corticosteroids and cough caused by gastro-oesophageal reflux can be treated with antacids. Certain therapies are known to provoke cough, in particular, the antihypertensive angiotensin converting enzyme inhibitors, such as captopril (Sesoko & Kaneko, 1985; Semple & Herd, 1986). This phenomenon appears idiosyncratic, occurring in 2-10% of patients and usually requires switching to alternative therapy.

Treatment is directed toward the symptom of coughing (symptomatic therapy) only when no cause can be identified, no treatment is available to reverse the disease process, or when treatment is unsuccessful. Examples include lung cancer and self limited disease such as upper respiratory tract viral infections.

The aim of an antitussive would be to decrease the frequency and intensity of cough and the sensation of irritation without altering respiratory function or inhibiting beneficial coughing. Antitussives can be classified according to their site of action; centrally acting which act by increasing the threshold of the medullary 'cough centre' neurones, peripherally acting which act directly on airway sensory receptors and locally acting which alter the characteristics of the airway surface liquid and mucus.

The opioid narcotics and their derivatives are the principal centrally acting antitussives. The most commonly used are codeine and pholcodine and products containing these opiates accounted for almost 3 million of a total 4.6 million prescriptions for antitussives in 1992 (personal communication, Department of Health, London, UK). Whilst generally considered to be safe, constipation and more seriously, respiratory depression and coma may be induced and they should be avoided in patients with respiratory disease (Belville & Seed, 1968). They are reserved for the treatment of persistent, troublesome cough while

morphine and methadone are only for treatment of cough in the terminally ill because of their addictive properties.

Peripherally acting antitussives include local topical anaesthetics, bronchodilators and specific sensory opioid μ-receptor antagonists. Local anaesthetics, such as lidocaine, block nervous transmission on all vagal afferents within their area of contact and depend upon their ability to penetrate the airway wall. They are used successfully during investigations including bronchoscopy and are used in some lozenges and sprays for oral use. Whilst these may soothe pharyngeal irritation, they do not deliver the drug to cough-sensitive airways and therefore have limited efficacy. Inhaled anaesthetics, delivered by nebulizer, may become an alternative treatment for cough, but at present they are associated with unacceptable risks and side-effects.

Bronchodilators are thought to have antitussive activity which may be direct or indirect and have been shown to be effective in treating cough associated with bronchoconstriction (Ellul-Micallef, 1983). Alpha-adrenoceptor agonists are reported to be upper respiratory tract decongestants due to their vasoconstricting actions. New agents acting on specific receptors on vagal afferent nerve endings may also acquire a place in the treatment of cough. They are opioid-like but probably act by blocking peripheral μ-receptors, rather than acting centrally.

Antihistamines are effective in treating cough associated with allergic rhinitis and post nasal drip but have no known direct antitussive activity. Their sedative side-effects preclude their use for other forms of cough.

Locally acting antitussives include demulcents, mucolytics and expectorants. Over 1.6 million prescriptions for simple linctus were dispensed in England in 1992 (personal communication, Department of Health, London, UK). This is a demulcent with no pharmacological action

containing mainly syrup and may reflect the importance of the placebo effect in treating undiagnosed cough and perhaps also the lack of experimentally and clinically proven antitussives without side-effects. It acts by lining the mucous surface so lubricating dry airway walls and is purported to soothe a dry irritating cough although, again, treatment will not reach the most cough-sensitive airways. Demulcents nevertheless offer a safe inexpensive treatment for acute self-limiting respiratory disorders and in particular, paediatric simple linctus offers a safe treatment for cough in children.

Mucolytics and expectorants act similarly by changing the physico-chemical characteristics of the airway surface liquid. Expectorants, such as potassium iodide, are reported to increase mucus secretion, thus changing a dry unproductive cough to a productive cough aiding its physiological function. However, their actions have not been proven experimentally and their efficacy is questionable. Mucolytics which reduce sputum viscosity may be beneficial in patients with bronchiectasis and chronic bronchitis.

Many proprietary cough treatments contain mixtures of the above antitussives. Often the individual drugs are present in sub-therapeutic doses and as yet there is no logical place for them in the treatment of cough.

1.4 EVALUATION OF ANTITUSSIVES

The purpose of research into cough has been to develop new treatments which do not put the patient at risk but provide an effective means of reducing pathological cough. Work has centred upon studies of the afferent limb of the cough reflex arc together with the central regulatory control. Early studies of cough and antitussives relied on experimental studies of animals where cough was evoked using mechanical, electrical or chemical stimuli. Mechanical methods are usually performed under anaesthesia and irritants such as nylon fibres (Korpas & Tomori, 1979) and polyethylene tubing (May & Widdicombe, 1954) are introduced via a tracheostomy or pharyngostomy. Electrical methods require surgical procedures in anaesthetised animals where stimuli are applied to the afferent nerves or central cough centre (Chou & Wang, 1975; Chakravarty *et al.*, 1956). Chemical stimuli are applied either into closed cages or directly into the airways via masks or tracheal cannulas. Stimulants include sulphuric acid (Chen *et al.*, 1960), SO_2 (May & Widdicombe, 1954), and citric acid (Forsberg & Karlsson, 1984).

Centrally acting antitussives such as codeine have been effective in treating cough induced by mechanical (May & Widdicombe, 1954) and chemical (Jackson, 1988) stimulation. Anaesthetics have been effective in treating chemically induced cough in guinea-pigs (Karlsson, 1987) and mechanically induced cough in dogs and rabbits (Dain *et al.*, 1975; Cross *et al.*, 1976). The anticholinergic bronchodilator, atropine, reduces acetylcholine-induced cough (Tiffeneau, 1957) but not SO_2-induced cough (Nadel *et al.*, 1965).

These early studies provided the basis of efficacy data for the opiates, in particular, codeine. Codeine is now generally employed as a positive control with which to compare other antitussives. However, there are many disadvantages of animal studies. These include marked inter-

species differences precluding inference to humans, the surgical procedures which may injure the airways and the anaesthetics employed which may themselves modify the cough response. This may help to explain the conflicting evidence of efficacy of antitussives in humans.

More recently, cough has been studied in humans. However, the early clinical studies of pathological cough were often uncontrolled and unreliable owing to the subjectiveness of the patient's own assessment of cough and the variation arising from the changing status of the patient's disease. Effective studies require large numbers of patients, placebo controlled, double-blind and randomised design and an elaborate method of monitoring cough. For example, continual monitoring of hospitalised patients with chronic bronchitis using a tape recorder demonstrated an antitussive action of the antihistamine, phenyltoloxamine, but not the opiate, pholcodine (Edwards *et al.*, 1977). Another comprehensive study demonstrated an antitussive action of the bronchodilator, terbutaline sulphate, in chronic allergic cough (Ellul-Micallef, 1983).

In an attempt to overcome these difficulties, Bickerman and Barach in 1954, evoked cough experimentally in normal and asthmatic volunteers using nebulized aerosols of various substances including organic acids, ethyl alcohol and ether, hydrochloric and sulphuric acids and ammonium hydroxide. All aerosols evoked cough; the organic acids yielding the most consistent response and provoking cough in about 80% of subjects. They then used aerosols of citric acid to evaluate several centrally acting antitussives and found significant activity for codeine, dextromethorphan and noscapine (Bickerman *et al.*, 1957). The introduction of this method of evoking cough in healthy humans with nebulized stimuli appears to offer a means of performing objective placebo controlled studies of antitussives which generate reliable data.

CHAPTER 2 : METHODS

This chapter presents the methods that are common to most studies described in this thesis. Detailed methods relating to individual studies are given in their particular sections.

2.1 AEROSOL GENERATION

Aerosols offer an easy means of administering stimuli direct to the airways of humans and have in recent years been used increasingly in the diagnosis and treatment of respiratory disease. An aerosol consists of liquid particles in a gas and is usually generated by passing air at high velocity with respect to liquid. This is known as 'jet' atomisation and was first used in perfume sprayers over 100 years ago. However, to produce aerosols with smaller particles, it was found necessary to force liquid through a small diameter orifice at high velocity. To achieve this, a high velocity air jet is passed over a liquid feed tube creating an area of negative pressure. This draws liquid up the tube from where it is fragmented into a fine mist. A baffle is placed in front of the jet to remove large particles. Such a device is known as a jet nebulizer.

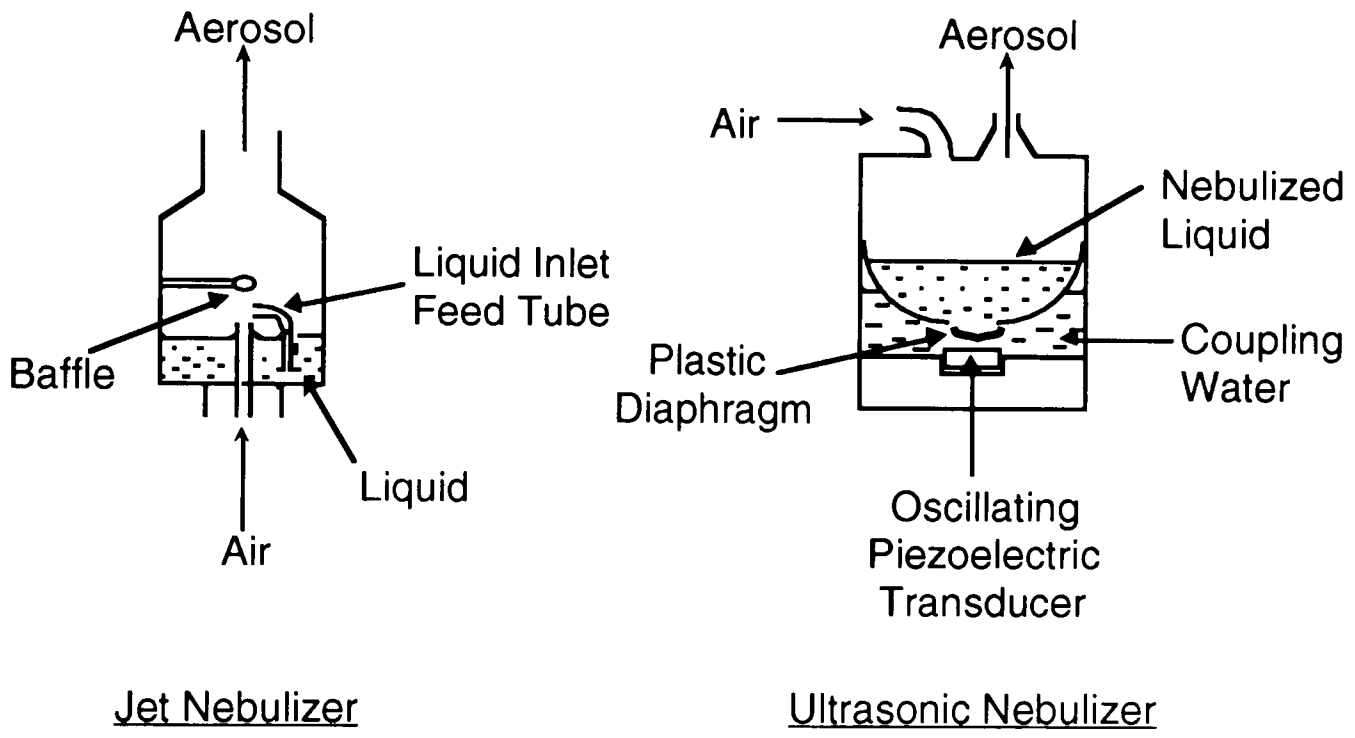
Another form of nebulizer is the ultrasonic nebulizer which generates ultrasonic waves from an oscillating piezoelectric transducer. These are transmitted to the liquid to be atomised through water which is separated from the liquid by a thin plastic sheet or diaphragm. This causes the surface of the liquid to break up to produce large airborne droplets which are picked up by the air flow through the chamber. This produces a well mixed aerosol of high mass concentration of up to 150 mg/l air, called a 'fog'. Ultrasonic nebulizers produce a higher output and larger particles than jet nebulizers (Sterk *et al.*, 1984); the particle size decreasing with increasing frequency of the transducer oscillations. These

high frequency conditions heat the liquid by sound absorption in contrast to jet nebulizers which lose heat through evaporation.

The jet and ultrasonic nebulizers are represented schematically in Figure 2.1.

FIGURE 2.1

Jet and Ultrasonic Nebulizers



Jet Nebulizer

Ultrasonic Nebulizer

2.2 ULTRASONIC CHALLENGE

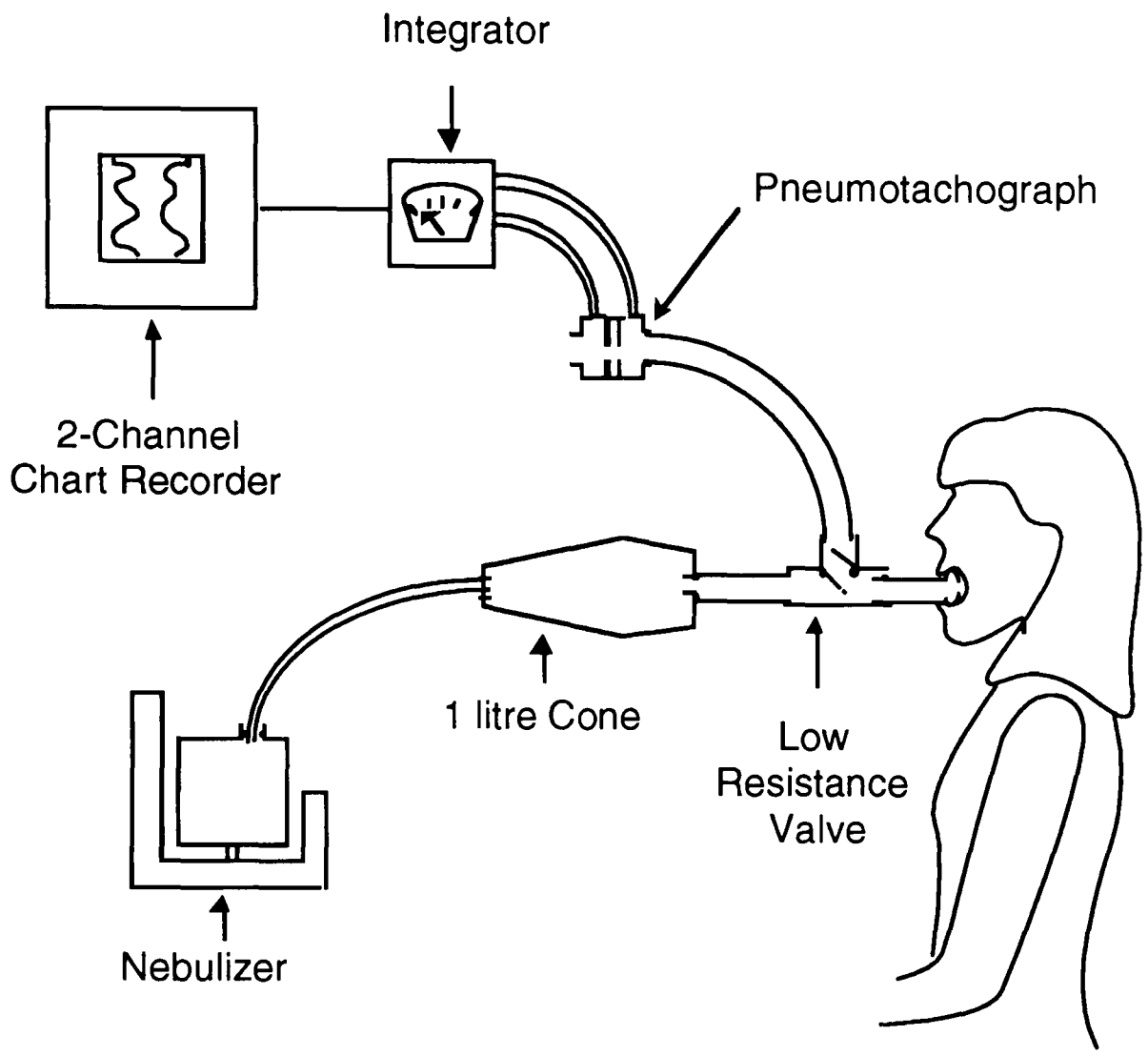
For the majority of studies a DeVilbiss 65 ultrasonic nebulizer (DeVilbiss Healthcare UK Ltd., Feltham, Middlesex, England) operating at maximal setting and with the air vent fully open, was used. This produces a solution output of approximately 3 ml/minute and particles with a mass median aerodynamic diameter (MMAD) of 6-7μm (Sterk *et al.*, 1984). With its high output and large particles, deposition when mouth breathing is likely to occur mainly in the large central airways where most of the cough receptors are thought to be found (Widdicombe, 1954). The aerosol was

passed through a 50 cm length of corrugated tubing into a plastic cone-shaped 1 litre container to ensure a continuous supply of aerosol. Subjects then tidally breathed nebulized solutions through a two-way low resistance valve and rubber mouthpiece for 1 minute. The valve body was made from nickel plated brass and the flaps from flexible soft rubber which produced minimal resistance to breathing, did not invert with the high flow rates of cough and did not stick when wet with the aerosol. Cough was recorded by means of a heated Fleisch pneumotachograph (PK Morgan, Chatham, England) calibrated directly with a Godart flow calibration set (Gould Electronics Ltd., Hainault, Essex, England) and mounted in the expiratory port of the valve. The flow signal obtained was integrated using a respiratory integrator mark II (PK Morgan, Chatham, England) and flow and volume signals were displayed on a two-channel hot pen chart recorder (Brush 220, Gould Electronics Ltd., Hainault, Essex, England). The equipment was arranged as shown in Figure 2.2.

Subjects were instructed prior to all challenges that the aerosol may or may not make them cough and to cough only if it was unavoidable.

FIGURE 2.2

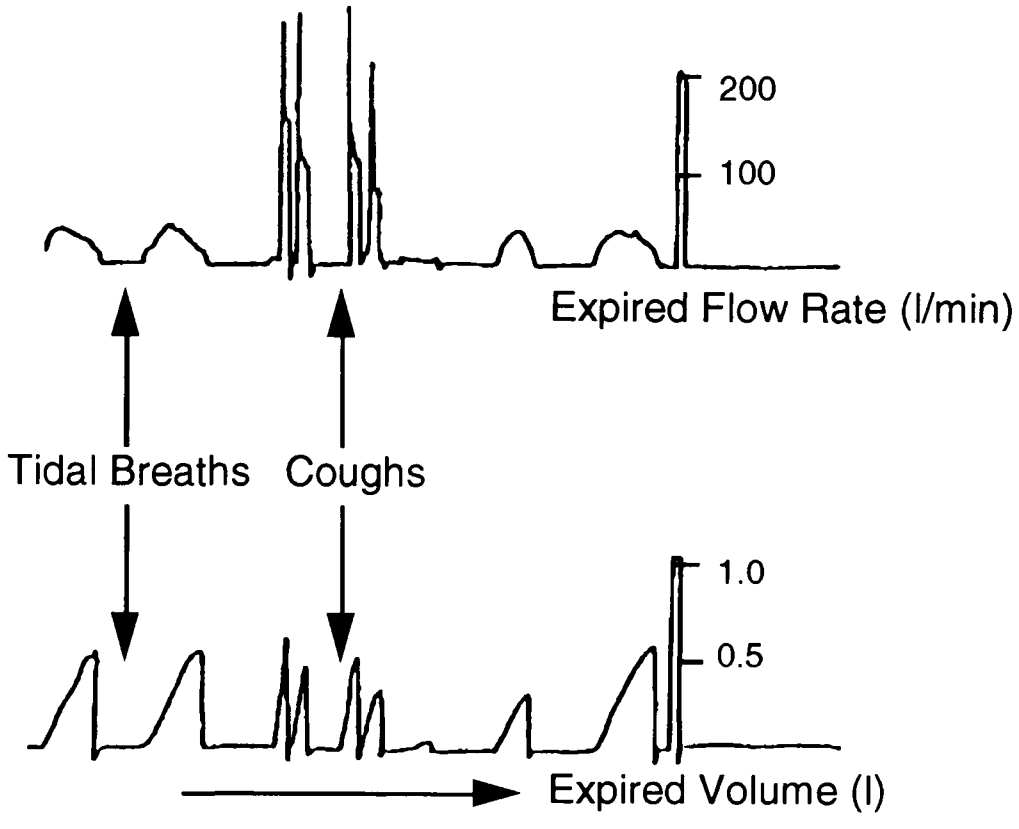
Ultrasonic Cough Challenge Equipment



Coughs were identified by their characteristic flow pattern, with a very rapid initial rise in expiratory flow rate. A typical trace is shown in Figure 2.3. The number of coughs occurring during the one minute inhalations were counted for analysis.

FIGURE 2.3

A Typical Cough Challenge Trace



2.3 JET CHALLENGE

2.3.1 Acorn

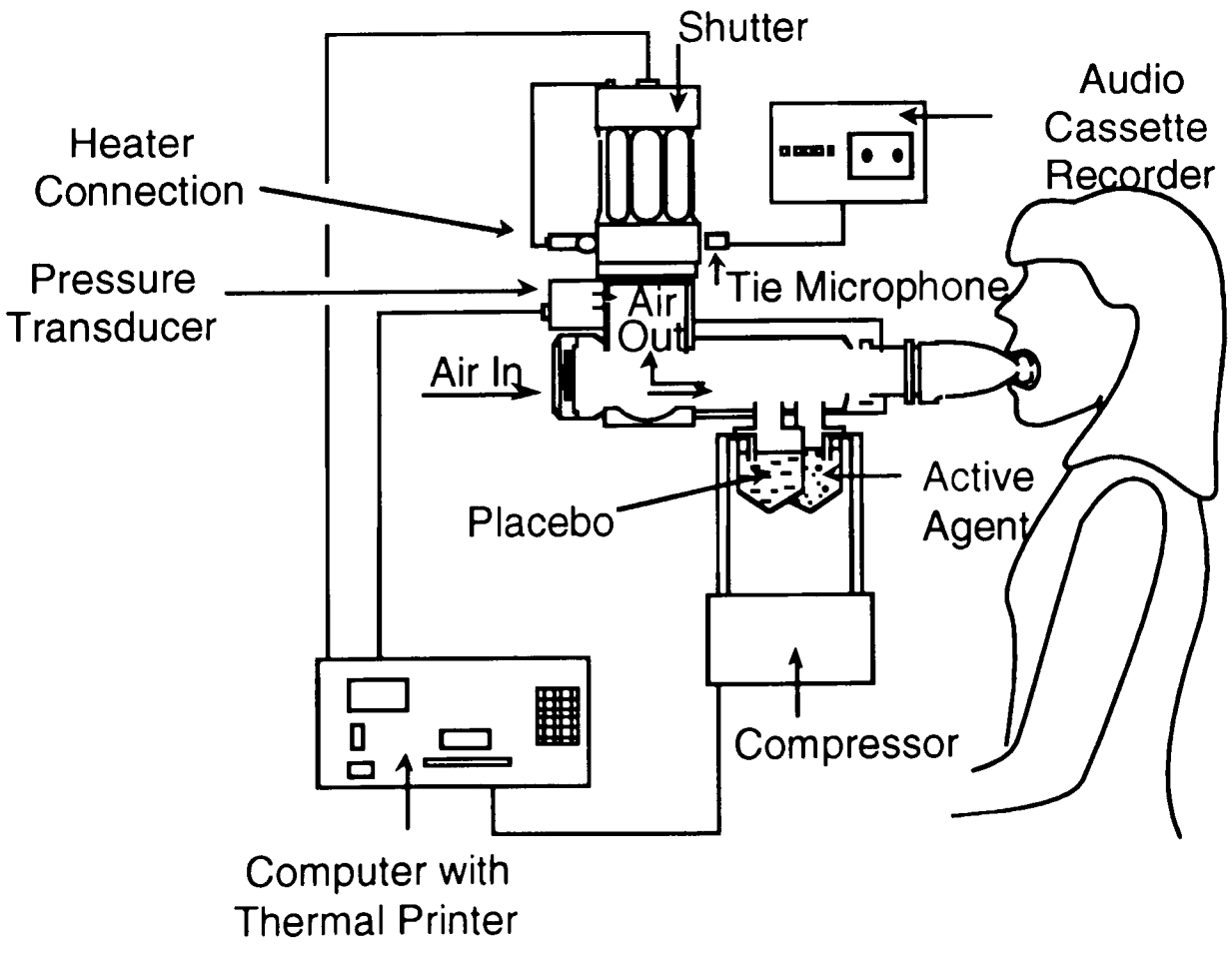
Jet nebulizers were used to administer more noxious substances as they have a lower output than ultrasonic versions. The jet nebulizer used was the Acorn System 22 (Medic Aid Ltd., Pagham, Sussex, England). This has a solution output of 0.2-0.3 ml/min. and a MMAD of 2-4 μm (Sterk *et al.*, 1984). The nebulizer was run using air from a pressurised gas cylinder at 8 l/min. For the challenge, 5 ml of liquid stimulant was placed in the jet nebulizer and the aerosol inhaled using the same equipment as the ultrasonic nebulizer for 1 minute periods during which cough frequency was counted.

2.3.2 Bronchoscreen Dosimeter

Finally a dosimeter was also used (Bronchoscreen, Erich Jaeger UK Ltd., Market Harborough, UK). This is a breath actuated dosimeter illustrated in Figure 2.4, which delivers aerosol from 2 jet nebulizers (Sandoz 1500), each of which is triggered in a pre-set sequence. One nebulizer contains active agent and the other placebo (isotonic saline). In this way, the subject is unaware of when the active agent will be delivered. During a one minute challenge, the machine was set to deliver 3 doses of active agent. Cough frequency was recorded via a tie microphone (RS Components, England) which was connected to an audio tape recorder. The dosimeter activates the nebulizers for 0.5 seconds, which produces an output of approximately 4μl. Data from Sandoz indicates that 52% of these particles are between 1.9 and 5.6 μm.

FIGURE 2.4

Bronchoscreen Dosimeter



2.4 PULMONARY FUNCTION

Basic tests of pulmonary function were performed to assess airway calibre. Spirometric assessment of forced vital capacity (FVC) and forced expired volume in 1 second (FEV_1) were recorded as the best of 3 measurements using a dry wedge bellows spirometer (Vitalograph Ltd., Maids Moreton House, Buckingham, UK).

Peak expiratory flow rate (PEFR) was assessed as the best of 3 measurements using a mini-Wright peak flow meter (Clement Clarke International Ltd., London, UK).

Specific airways conductance (sG_{aw}) was assessed as the average of 2 measurements using a whole body plethysmograph (Gould 2800 Autobox, Coventry, UK).

Airways resistance (R_{aw}) was assessed using the Bronchoscreen dosimeter described in Section 2.3.2 which calculates R_{aw} breath by breath by the occlusion method (Mead & Whiteenberger, 1954). R_{aw} was measured during both air and aerosol breathing over 1 minute periods and the average of the final 5 measurements recorded for analysis. Measurements of R_{aw} during coughing can give artificially high values owing to the high expiratory air flow rates. These were rejected automatically by the Bronchoscreen.

2.5 VOLUNTEERS

Written informed consent was obtained from all volunteers prior to their enrolment. Volunteers were healthy male and female members of hospital staff. Basic demographic data including age, height and smoking history were recorded and the following inclusion and exclusion criteria were applied:

Inclusion Criteria

Aged between 18 and 70 years.

FEV_1 greater than 75% of predicted (Cotes, 1979).

Exclusion Criteria

Females who were pregnant or lactating.

Respiratory disease including an upper respiratory tract infection within 6 weeks of study.

Cardiovascular disease, hyperthyroidism, hypertension or diabetes.

2.6 CLINICAL TRIAL DESIGN

Where appropriate, studies described in this thesis were performed using a double-blind, placebo controlled, crossover and randomised design. Approval from the hospital Ethical Committee was obtained for all studies.

2.7 STATISTICAL ANALYSIS

Analysis of the data was performed in conjunction with the Medical Research Council Biostatistics Unit, Institute of Public Health, Cambridge.

The number of coughs during each 1 minute challenge was counted and recorded for analysis. Initial review of the data revealed that cough frequency follows a skewed distribution rather than a Normal distribution. A square-root transformation (after addition of one), the usual transformation for data with a Poisson distribution, was therefore performed on the data prior to analysis. This was found to stabilise the within-group variance allowing parametric analysis of variance (ANOVA) to be performed. Factors tested in a two-way analysis of variance were subject and treatment/challenge. Pairwise comparisons between means were made using the 'least significant difference' (Snedecor & Cochran.

1967). The residual mean square from the ANOVA was used to compute 95% confidence limits, which together with the resultant means were then back-transformed (i.e., squared and 1 subtracted). ANOVA was also performed on FEV_1, FVC, PEFR, sG_{aw} and R_{aw} data.

CHAPTER 3: CHARACTERISATION OF THE COUGH REFLEX

3.1 INTRODUCTION

Cough can be induced in animals by chemical stimulation of the large central airways (May & Widdicombe, 1954) and peripheral airways (Widdicombe, 1954). Administration of chemical stimuli to human airways *in vivo* can be achieved using aerosols and, by using aerosols of different particle size, it is possible to deposit solutions in the large central airways or in more peripheral regions of the lungs (Lippman *et al.*, 1980). The large particles produced by ultrasonic nebulizers are likely to deposit mainly in the larynx and trachea, while the smaller particles produced by jet nebulizers will deposit more peripherally.

An aerosol of ultrasonically nebulized distilled water (UNDW) has been introduced as a method of documenting bronchial hyperreactivity since it causes a dose-dependent bronchoconstriction in asthmatics but not normals (Allegra & Bianco, 1980; Schoeffel *et al.*, 1981; Anderson & Schoeffel, 1984). However, it has also been reported to evoke cough in both asthmatics and non-asthmatics (Sheppard *et al.*, 1983; Anderson & Schoeffel, 1984; Chadha *et al.*, 1984). Water instilled into the larynx of animals may also evoke cough but in neonates of many species, the result is apnoea (Storey & Johnson, 1975; Harding *et al.*, 1978; Lucier *et al.*, 1979; Boggs & Bartlett, 1982). Since the two responses are mediated by the same receptor group within the superior laryngeal nerve, it has been postulated that the CNS determines the reflex evoked, i.e. cough in adults and apnoea in neonates (Boggs & Bartlett, 1982). The apnoeic reflex has also been documented in human infants and may be implicated in sudden infant death syndrome (Perkett & Vaughan, 1982).

The stimulus for the bronchoconstrictor response to UNDW in asthmatics is thought to be the low osmolarity of water (Schoeffel *et al.*, 1981), while the lack of a permeant anion is responsible for the apnoeic reflex in neonatal puppies (Boggs & Bartlett, 1982).

Based on these observations, the following studies were designed to compare the cough response to inhalation of aerosols of varying ionic content, osmolarity, pH and particle size in order to determine the chemosensitivity and region of stimulation of aqueous aerosol-induced cough in healthy human volunteers.

3.2 METHODS

3.2.1 Chemical Sensitivity

Ten healthy, non-smoking volunteers (5 males and 5 females, age range 18 - 42 yrs) were studied. Each subject was challenged with 6 solutions chosen to provide a range of ionic content. Of these solutions, 5 were isotonic and 1 was hypotonic (distilled water). The isotonic solutions were urea, D-glucose, sodium chloride, sodium acetate and sodium bicarbonate. Solutions were administered as aerosols using the method described in Section 2.2 for the ultrasonic challenge and given in random order, double blind, in duplicate and on separate days at the same time of day. Cough frequency, flow rate and volume for each 1 minute challenge were recorded for analysis.

3.2.2 Anion Sensitivity

To determine whether the lack of a permeant anion is responsible for the cough response to aqueous aerosols, 5 healthy, non-smoking volunteers (3 males and 2 females; age range 25 - 35 yrs) were studied.

Challenges were performed as above with saline solutions containing concentrations of chloride of 145 (isotonic), 112, 75, 52, and 31 mmol/l.

3.2.3 pH Sensitivity

The effect of altered pH of inhaled aerosol on cough was studied in 7 healthy subjects (3 males and 4 females, age range 25 - 57 yrs) using isotonic saline. The pH was manipulated by adding small amounts of phosphate buffers to achieve solutions with pH values of 4.8 and 8.0. To achieve a solution with a pH of 2.6, 2.1ml of concentrated hydrochloric acid and 5.9g of glycerine were made up to 1.5 litres with saline. A pH of 10.0 was achieved by adding 2.2g of sodium hydroxide and 7.1g of glycerine to 1.5 litres of saline. Aerosols were administered on separate days at the same time of day, in random order, single blind and for a period of 1 minute during which cough frequency was recorded.

3.2.4 Osmolarity Sensitivity

(a) To clarify the effect of changes in osmolarity of inhaled aerosol on cough, 7 healthy subjects (1 male and 6 females, age range 23 - 57 yrs, mean FEV_1 = 109%, range 97 - 122% of predicted) inhaled 5 aerosols of D-glucose in a range from 77 - 1232 mosmol/l (calculated values). For comparison, saline solutions of matched osmolarities were also inhaled. To exclude any effect the low pH of D-glucose may have on cough, small amounts of 0.1 mol/l sodium hydroxide were added to each solution to raise the pH to that of saline (pH 5-7). All aerosols were inhaled in random order on separate days and as changes in osmolarity of inhaled aerosols can cause bronchoconstriction in asthmatics (Schoeffel *et al.*, 1981), FEV_1 was recorded immediately after each challenge.

(b) To test further the role of hyperosmolarity in inducing cough, while keeping a chloride concentration close to that of isotonic saline, 2 more solutions were studied as described above by 7 subjects, three of whom had taken part in experiment (a). The solutions were an isotonic and a hypertonic mixture of D-glucose and saline created to keep the concentration of chloride close to that in isotonic saline and thereby excluding an effect of high ionic concentration. The resultant solutions were 1.25% D-glucose in 0.68% saline (308 mosmol/l; 112 mmol/l Cl^-) and 15% D-glucose in 0.9% saline (1232 mosmol/l; 150 mmol/l Cl^-).

3.2.5 Citric Acid-Induced Cough

The mechanism of the irritant property of citric acid, which has been introduced as a stimulus for cough (Bickerman & Barach, 1954), was examined in 7 healthy subjects (3 males and 4 females, age range 25 - 31 yrs). To assess the effect of pH and chloride concentration, the cough response to isotonic citric acid in saline, isotonic sodium citrate and sodium citrate in saline were compared. UNDW and isotonic D-glucose were included for comparison.

3.2.6 Particle Size Dependence

Aerosols of different particle size were used to determine the distribution in humans of receptors causing cough induced by distilled water. This was achieved by comparing the cough response to aerosols produced by ultrasonic and jet nebulizers. Ultrasonic nebulizers are known to produce larger particles than jet nebulizers which will be deposited in larger airways. However, as jet nebulizers also have a lower output than ultrasonics, it was estimated that 4 jet nebulizers would be needed to obtain an equivalent output. The aerosols were matched for output and air

flow rate and their particle size distribution measured by Dr Woolman at the Department of Nuclear Medicine, Addenbrooke's Hospital, Cambridge.

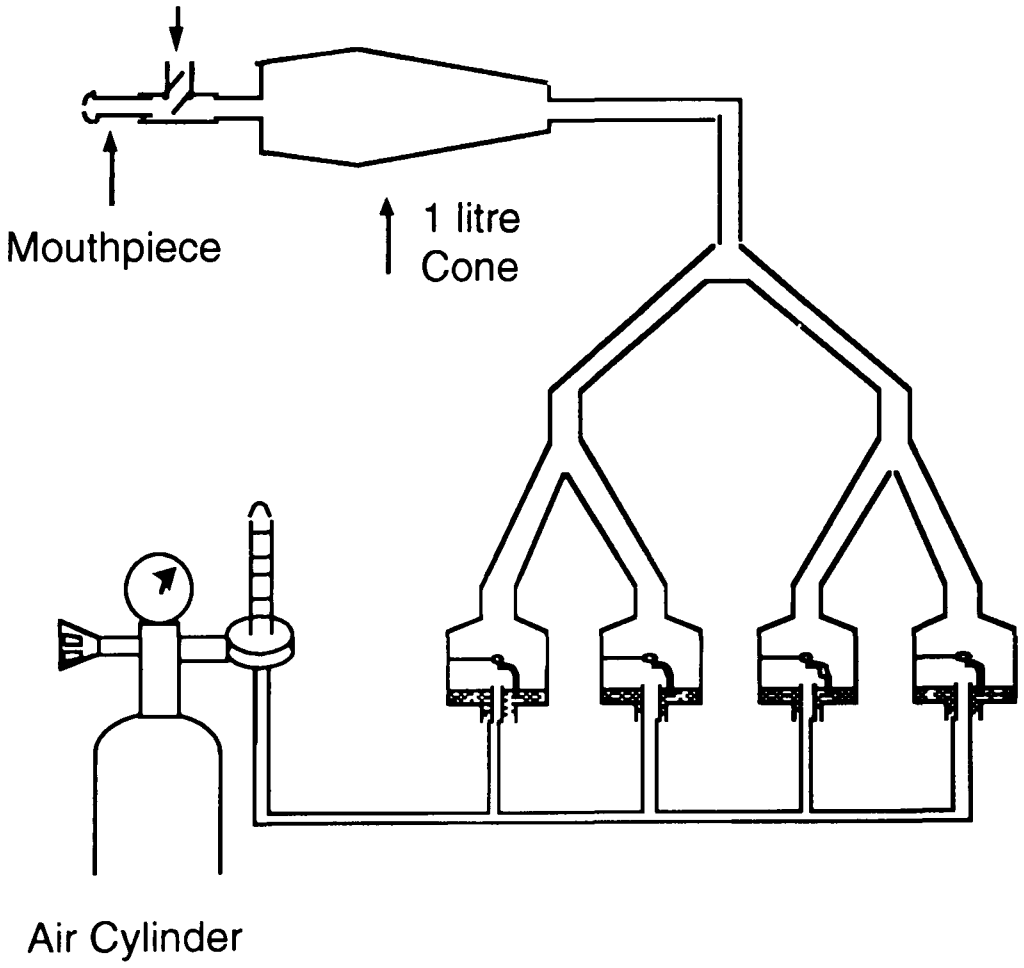
The aerosol output was estimated by placing a corrugated filter, which collected more than 99.9% of the aerosol particles, on the end of the tubing from the nebulizers which were loaded with an aqueous solution containing approximately 50MBq of the radioisotope technetium 99m (Tc99m) in 0.9% saline. 1 ml of fluid was extracted from the nebulizer reservoir and the activity measured. The radioactivity deposited on the filter was measured after running the 2 nebulizer systems for 1 minute. The ultrasonic nebulizer was run at half power (setting 5) with the air vent fully open producing an air flow rate of 27 l/minute as measured with a rotating vane mechanical flow meter (Wrights respirometer, Ferraris, London, England). Four jet nebulizers (Acorn System 22) were set up in parallel with two Y-pieces and short lengths of plastic tubing. The output of all 4 nebulizers passed through the same 50 cm tubing and cone used with the ultrasonic nebulizer as shown in Figure 3.1. The nebulizers were powered from compressed air at a flow rate of 30 l/minute. The output rate was calculated in millilitres of fluid produced as aerosol particles per minute of operation of the nebulizer systems. This technique excludes the output by evaporation as this would have produced an artificially high output for the jet nebulizer system.

Particle sizing of the aerosols was performed using a May cascade impacter (Biral, Bristol, England) which involves the separation and collection of different size fractions of particles. In each stage, the aerosol accelerates through constricting jets and impinges on collecting plates. The first stage collects the largest particles and so on until a final filter collects all the particles below the size of the last stage. The number of particles on each stage are counted to give a cumulative distribution frequency. The ultrasonic nebulizer was run at half power for 30 seconds

and then a sample of aerosol was taken from the cone for the following 30 seconds with the nebulizer still running. The nebulizer fluid contained approximately 50MBq of Tc99m in 0.9% saline. The radioactivity deposited on the plates of the cascade impactor was measured with a calibrated scintillation detector. The counts on each plate were used to determine the particle size distribution from which the MMAD of the aerosol particles was calculated (May, 1975). The particle size distribution was also determined for the jet nebulizer system and these, together with the output rates, were repeated at the end of the study.

FIGURE 3.1

Parallel Arrangement of Jet Nebulizers



Twelve healthy volunteers (8 males and 4 females, age range 22 - 49 yrs) inhaled distilled water from each of the nebulizer systems on separate days at the same time of day, in random order and for 1 minute during which cough frequency was recorded.

3.2.7 Adaptation of Cough

Five healthy subjects (1 male and 4 females, age range 19 - 59 yrs, mean FEV_1 = 145% of predicted) undertook a 1 minute cough challenge to UNDW which was repeated 5 minutes and 3 hours later (This is part of a larger study described in detail in Section 6.2.2). In addition to comparing the cough frequencies at each of the 3 time points, each 1 minute challenge was subdivided into the number of coughs over each of 3 consecutive 20 second periods. This allowed identification of adaptation of cough over a continuous challenge and subsequent challenges. All subjects performed a cough challenge prior to the study to accustom them to the procedure and therefore to exclude any adaptation of cough due to a learning effect.

3.3 RESULTS AND STATISTICAL ANALYSIS

The raw data and ANOVA tables for the following studies are presented in Appendix 1.

Cough, when provoked, was restricted to the duration of aerosol inhalation and no adverse reactions were recorded.

3.3.1 Chemical Sensitivity

Distilled water, urea, D-glucose, sodium acetate and sodium bicarbonate, but not sodium chloride, evoked cough. One subject did not cough in response to any of the aerosols and 1 subject coughed on only one occasion during inhalation of D-glucose. For the 5 aerosols resulting

in cough, ANOVA revealed no significant difference in cough frequency ($p>0.05$). However, there was a difference between subjects ($p<0.001$) indicating intersubject variation in cough responses. The back-transformed mean cough frequencies (MCF) and 95% confidence limits (95% CL) are presented in Table 3.1.

TABLE 3.1

The Chemical Sensitivity of Induced Cough

Solution	Tonicity	pH	MCF (95% CL)
Urea	isotonic	8.2	6.6 (3.7 - 10.2)
Water	hypotonic	8.5	9.4 (6.0 - 13.6)
Sodium Acetate	isotonic	7.5	7.4 (4.3 - 11.2)
D-Glucose	isotonic	3.6	7.3 (4.2 - 11.0)
Sodium Bicarbonate	isotonic	8.9	6.3 (3.5 - 9.8)
Sodium Chloride	isotonic	6.0	0.0

Peak cough flow rates and volume were measured and the mean values and standard deviations (SD) are shown in Table 3.2. The peak expiratory flow rate during cough was probably slightly underestimated owing to the opening pressure of the expiratory valve and the reaction time of the pneumotachograph. Since for practical purposes this was constant, comparison between challenges remained valid. No significant differences in peak cough flow rates or cough volume were detected on occasions where cough occurred ($p > 0.05$).

TABLE 3.2

Cough Flow Rate and Volume During Aerosol Inhalation

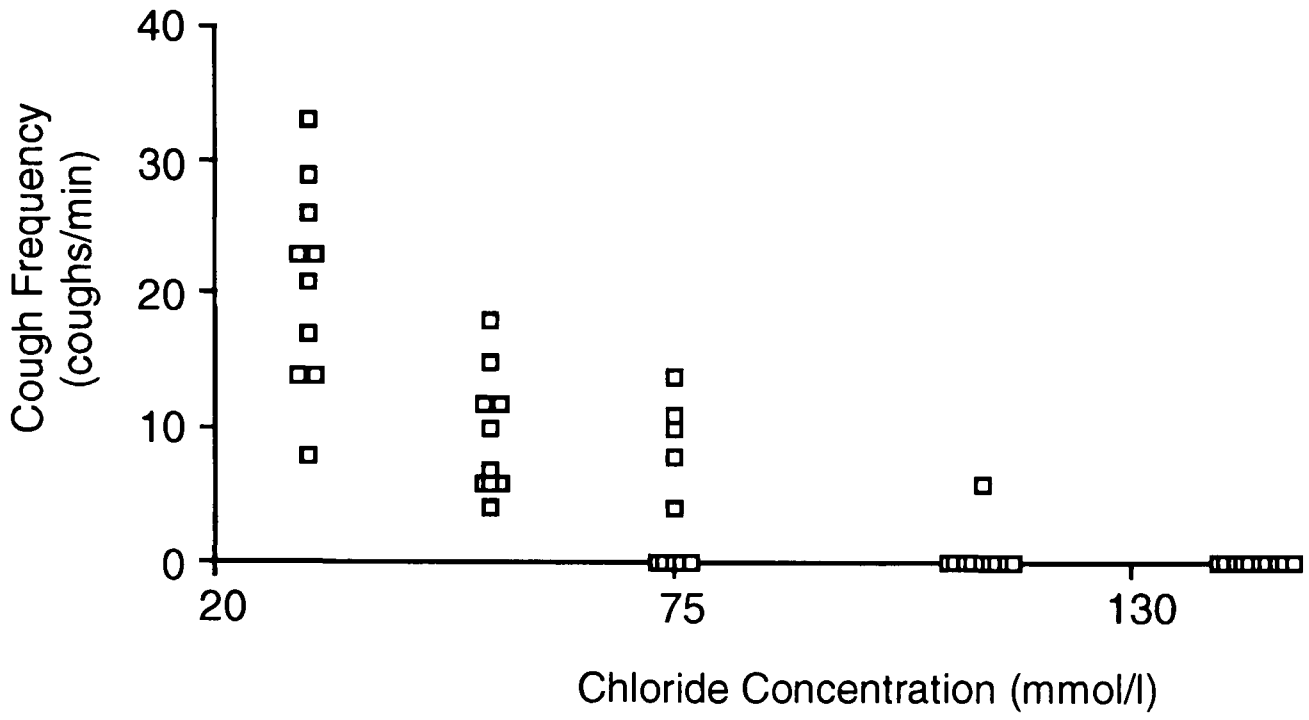
	Cough Flow Rate (l/min)	(SD)	Cough Volume (l)	(SD)
Urea	228	(37)	0.31	(0.10)
Water	246	(61)	0.40	(0.20)
Sodium Acetate	238	(60)	0.42	(0.19)
D-Glucose	242	(56)	0.41	(0.15)
Sodium Bicarbonate	245	(60)	0.43	(0.15)
Sodium Chloride	0	0	0.00	(0.00)

3.3.2 Anion Sensitivity

Cough did not occur with isotonic sodium chloride aerosol confirming the previous observation in Section 3.3.1. However, cough increased in frequency as the chloride concentration of the inhaled aerosol was reduced, signifying a dose response relationship. The individual cough frequencies are presented in Figure 3.2 ($p<0.001$).

FIGURE 3.2

The Effect of Chloride Concentration on Cough



3.3.3 pH Sensitivity

Cough occurred on inhalation of the 2 aerosols of extreme pH, 2.6 and 10.0 only as shown in Table 3.3. ANOVA revealed no significant difference in cough frequency at these 2 levels of pH ($p>0.05$), but the cough response to both aerosols was significantly greater than zero ($p<0.01$).

TABLE 3.3

The Effect of pH on the Cough Response to Saline

pH	2.6	4.8	8.0	10.0
MCF (95%CL)	2.1(0.6 - 4.2)	0.0	0.0	3.6 (1.7 - 6.0)

3.3.4 Osmolarity Sensitivity

(a) ANOVA revealed no significant difference in the cough response between any of the D-glucose aerosols ($p>0.05$). The dose response relationship between decreasing chloride concentration and increasing cough frequency was confirmed ($p<0.01$). At the highest sodium chloride concentration occasional coughing was observed. FEV_1 values measured after each inhalation did not vary by more than 5% in any subject indicating that no significant bronchoconstriction occurred with either challenge across the range of osmolarities studied. Back-transformed mean cough frequencies and 95% confidence limits are presented in Table 3.4 and graphically in Figure 3.3.

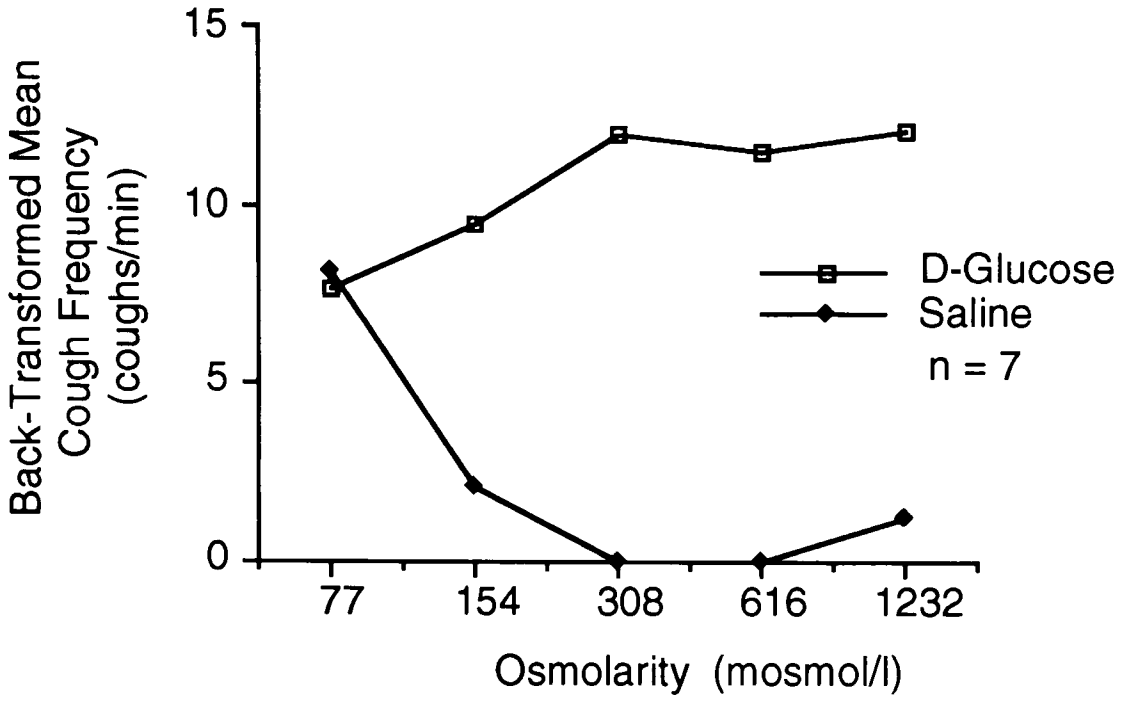
TABLE 3.4

The Effect of Osmolarity on Cough

Osmolarity (mosmol/l)	MCF (95% CL) (coughs/minute)			
	D-Glucose		Saline	
77	7.6	(4.4 - 11.5)	8.1	(4.8 - 12.1)
154	9.5	(6.0 - 13.8)	2.1	(0.4 - 4.6)
308	12.0	(8.0 - 16.6)	0.0	
616	11.5	(7.6 - 16.1)	0.0	
1232	12.1	(8.1 - 16.8)	1.3	(0.0 - 3.6)

FIGURE 3.3

The Effect of Osmolarity on Cough



(b) Cough occurred in response to inhalation of the hypertonic mixture of D-glucose and saline as shown in Table 3.5 but not to the isotonic mixture suggesting that marked hyperosmolarity of aerosol, rather than high ionic concentration, may provoke cough.

TABLE 3.5

The Effect of Hyperosmolarity on Cough

	Cough Frequency (coughs / minute)						
Subject	1	2	3	4	5	6	7
1.25% D-Glucose in 0.68% Saline	0	0	0	0	0	0	0
15% D-Glucose in 0.9% Saline	3	7	0	4	2	6	4

3.3.5 Citric Acid-Induced Cough

Cough occurred in response to inhalation of citric acid in saline and sodium citrate, but not sodium citrate in saline. The cough response to D-glucose and UNDW was confirmed. ANOVA revealed no difference in cough frequency between the aerosols causing cough ($p>0.05$). Back-transformed mean cough frequencies and 95% confidence limits are presented in Table 3.6.

TABLE 3.6

The Cough Response to Citric Acid

Aerosol	Osmolarity (mosmol/l)	pH	Chloride (mmol/l)	MCF(95%CL) (coughs/minute)	
0.68% Citric Acid in 0.79% Saline	308	2.0	130	11.4	(7.2 - 16.5)
Sodium Citrate	308	8.6	0	12.5	(8.1 - 17.7)
Sodium Citrate in 0.9% Saline	616	8.2	150	0.0	
UNDW	0	8.5	0	15.7	(10.8 - 21.6)
D-Glucose	308	3.6	0	18.1	(12.8 - 24.3)

3.3.6 Particle Size Dependence

The fluid volume output rates for the 2 nebulizer systems were matched. That of the ultrasonic system was 0.9 ml/min and that of the jet system was 0.85 ml/min. The aerosols, however, differed in their particle size distribution. The MMAD of the ultrasonic nebulizer was 6 μm (geometric standard deviation (GSD) = 2.0) whereas the jet system MMAD was 3 μm (GSD = 2.4). Deposition studies demonstrated a peripheral to central deposition ratio for the ultrasonic nebulizer to be 0.5 (SD ± 0.1) whereas that for the jet system was 0.9 (SD ± 0.1) suggesting greater central deposition for the ultrasonic nebulizer. The total lung deposition fraction for the ultrasonic nebulizer was 0.14 (SD ± 0.06) compared with 0.08 (SD ± 0.03) for the jet nebulizer system.

ANOVA revealed that coughing with distilled water occurred more frequently when inhaled from the ultrasonic nebulizer than from the jet nebulizer system ($p<0.001$) as shown in Table 3.7.

TABLE 3.7

Ultrasonic versus Jet Nebulized Water-Induced Cough

Subject	Cough Frequency (coughs/minute)	
	Ultrasonic	Jet
1	14	0
2	21	5
3	18	8
4	3	0
5	0	0
6	9	1
7	22	0
8	32	0
9	5	1
10	10	0
11	21	0
12	17	0
MCF (95% CL)	12.6 (8.4 - 17.6)	0.85 (0.0 - 2.9)

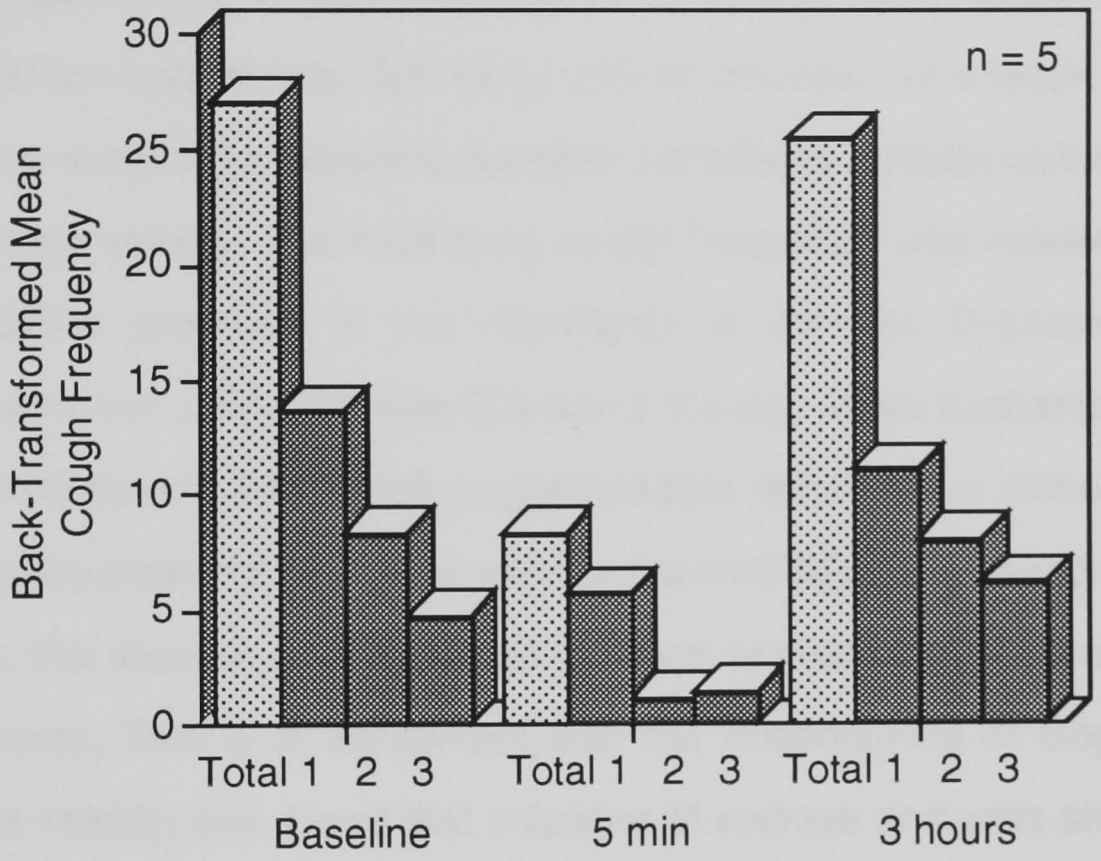
3.3.7 Adaptation of Cough

ANOVA revealed that adaptation of cough frequency occurred when the first UNDW challenge was repeated after 5 minutes ($p<0.001$). but had recovered 3 hours later ($p>0.05$). (A study, not presented in this thesis, suggests that the response recovers after approximately 30 minutes). When the number of coughs during the first 1 minute challenge

were divided into 3 consecutive 20 second periods, adaptation of cough again occurred ($p<0.001$) as shown in Figure 3.4.

FIGURE 3.4

Adapation of UNDW-Induced Cough



Total 1 minute cough frequencies at each time point have been broken down into 3 x 20 second periods.

3.4 DISCUSSION

In the study investigating the chemical sensitivity of induced cough (Section 3.3.1), all aerosols except sodium chloride provoked cough. The importance of the chloride ion was further examined (Section 3.3.2) and cough was found to increase in frequency as the chloride concentration of the inhaled aerosol was decreased below a threshold of approximately 75 mmol/l. In this respect it is analogous to the apnoeic reflex in neonatal puppies which is provoked by instillation of fluid deficient in chloride (below

80 mmol/l) into the larynx (Boggs & Bartlett, 1982). They found that the length of apnoea extended as the chloride concentration was further reduced, in a dose response relationship. They also found that the chloride ion may be replaced by other small permeant anions such as iodide or bromide, but not by larger anions such as bicarbonate or acetate. Similarly, in the study investigating the chemical sensitivity of cough (Section 3.3.1), cough was provoked by aerosols of sodium acetate and sodium bicarbonate, but not by sodium chloride. To assess whether the dose response relationship between decreasing chloride concentration of inhaled aerosol and increasing cough frequency was related to the associated decrease in the osmolarity of aerosol, D-glucose was compared with saline aerosols (Section 3.3.4 (a)). While Eschenbacher *et al.* (1984) have subsequently suggested that alterations in osmolarity as well as absence of a permeant anion in the inhaled aerosol could provoke cough, this study found no change in cough frequency with alterations in osmolarity. This is in agreement with the observations of Boggs and Bartlett (1982), who found that solutions of sucrose and urea stimulated apnoea regardless of their osmolarity. However the 3.6% saline caused some coughing. This could have been due to its high ion content or its hyperosmolarity. In a further study (Section 3.2.4 (b)), a solution of equal osmolarity but only a quarter of the ion content also resulted in occasional cough, suggesting that marked hyperosmolarity of the inhaled aerosol is a further stimulus for cough. The cough response was not associated with bronchoconstriction indicating that the two reflex responses can occur independently.

It is possible that the performance of the nebulizer would be affected by hyperosmolar solutions and this was partly assessed by measuring the osmolarity of isotonic and hypertonic solutions of saline

and D-glucose before and after 1 minute nebulizations. The values did not alter by more than 5%.

Investigating the effect of pH of inhaled aerosol on cough (Section 3.3.3) demonstrated the ability of extremes of pH, even in the presence of chloride, to evoke cough. This pH sensitivity is also similar to that observed by Boggs & Bartlett (1982), who found that saline with a pH lower than 4.5 or higher than 8.1 induced apnoea when instilled into the larynx.

These results suggest that an additive effect of low pH and lack of chloride may be responsible for the tussive properties of citric acid aerosol and this was confirmed (Section 3.3.5). Citric acid, even in the presence of chloride, and sodium citrate stimulated cough while sodium citrate in saline failed to elicit a response. The irritant properties of acetic acid (Mitsuhashi *et al.*, 1984) may also be mediated by a similar mechanism. Calcium chelation, thought to be responsible for the bronchoconstriction observed in Basenji-Greyhound dogs in response to citric acid (Downes & Hirshman, 1983), is unlikely to contribute to cough, as sodium citrate in saline failed to evoke cough.

Isolated nerve studies by Boggs & Bartlett (1982), revealed that all solutions capable of inducing apnoea in neonatal puppies stimulated laryngeal RARs. Other workers have also noted the stimulating effect of water on RARs (Boushey *et al.*, 1974; Anderson *et al.*, 1990). Recently, laryngeal water-sensitive RARs in dogs have been sub-divided into two groups; one responding with a short latency and duration to the low ionic content of water, the other responding with a long latency and duration to the low osmolarity of water (Sant'Ambrogio *et al.*, 1991) However, those in lobar bronchi appear to be only sensitive to low osmolarity (Pisarri *et al.*, 1992) These results suggest that RARs that are sensitive to low ion concentrations mediate cough and apnoea, while those responsive to

changes in osmolarity may mediate the bronchoconstrictor response to UNDW. However, both bronchial and pulmonary C-fibres also respond to the low osmolarity of water and may also mediate bronchoconstriction (Pisarri *et al.*, 1992).

The mechanism of 'low chloride' stimulation of RARs is unclear. RARs lie within the paracellular spaces of epithelial cells (Jeffery & Reid, 1973). These paracellular spaces offer a path across the epithelium which bypasses the cells. Those in airway epithelium, like the small intestine and gall bladder, are classified as 'leaky' (Frizzell *et al.*, 1979) allowing passive transport of small molecular weight solutes and ions between the serosal and luminal surfaces. The luminal surface is covered by a liquid consisting of 2 phases; the inner serous fluid called airway surface liquid (ASL) and the outer viscous mucus phase. The ASL covers the cilia providing a medium in which they can beat freely, so propelling the mucus upwards for expectoration. The interchange between the ASL and paracellular spaces suggests that the RARs are able to respond to changes in the composition of ASL. The ASL in human trachea and bronchi is believed to be 15 to 30 μm thick and consists of approximately 90% water with a sodium and chloride concentration of approximately 80 mmol/l and a pH of 6.8 (Mentz *et al.*, 1984; Joris *et al.*, 1993). The ionic concentration of this hypotonic fluid is maintained by active electrolyte transport involving sodium absorption. Alterations in the humidity of inspired air can affect the composition of ASL; increased humidity increases the volume of ASL and decreases the concentration of sodium chloride (Man *et al.*, 1979). The ionic composition of ASL appears to be important for a number of epithelial cell functions. Ciliary beat frequency is decreased when the luminal concentration of sodium chloride is reduced below 80 mmol/l (Luk & Dulfano, 1983). Such reduced levels of sodium chloride have been observed in severe asthmatics possibly reflecting sodium hyperabsorption

while elevated levels are found in cystic fibrosis patients reflecting decreased sodium absorption (Joris *et al.*, 1993). Thus, inhalation of a large volume of nebulized saline below 80 mmol/l chloride concentration, or water, may reduce the chloride concentration of the ASL which could be detected by RARs leading to cough. The mechanism of 'low chloride' stimulation of RARs could be direct by altering the ion flux across the terminals (Shingai, 1979) or indirect by the fluid shifts causing distortion of the epithelium (Hogg & Eggleston, 1984).

The results of the study investigating the effect of particle size on cough (Section 3.3.6) suggest regional differences exist in the distribution of airway receptors responsible for coughing. In agreement with other workers (Sterk *et al.*, 1984), the ultrasonic nebulizer produced larger particles than the jet nebulizers. On inhalation these are distributed more towards the central airways, including the larynx, than peripherally (Emmett *et al.*, 1982). In contrast, the smaller particles from the jet nebulizer are distributed more peripherally. The small particle aerosol of distilled water induced less coughing than the large particle aerosol suggesting that UNDW stimulates receptors that are predominantly located in the central airways and larynx. However, a reduction in the liquid dose to the airway epithelium caused by an increase in the surface area of the peripheral airways and by the reduced total deposition found with the jet system, may also contribute to the reduced cough frequency. The decreased cough response to UNDW seen in heart-lung transplant patients who have an intact larynx, but are permanently denervated below the tracheal anastomosis, may be due to a loss of RARs in the lower trachea and large bronchi (Higenbottam *et al.*, 1989).

The cough response to UNDW appears to be subject to rapid adaptation (or tachyphylaxis) (Section 3.3.7). The number of coughs decreased over a one minute challenge and remained low when re-

stimulated after five minutes. Cough frequency returned to baseline values within three hours although this response again exhibited a rapid adaptation during the challenge.

The studies described in this Chapter also revealed that individuals exhibit varied susceptibility to the irritancy of inhaled stimuli ranging from 0 to 40 coughs / minute in response to UNDW (Appendix 1; 3.3.1). The reason for this variation and why some individuals appear to be insensitive to UNDW probably arises from differences in the central respiratory control mechanisms (Banner, 1988). Central processes may also modify cough at different levels of consciousness, for example, during sleep, initiate cough voluntarily and suppress cough, which is possible at low levels of irritation.

In summary, these results suggest that cough can be stimulated in approximately 80% of humans by inhalation of aerosols low in chloride concentration, extremes of pH and markedly high osmolarity. These characteristics are similar to those described for the apnoeic reflex in neonatal puppies (Boggs & Bartlett, 1982) and for a group of laryngeal RARs in animal studies (Boushey *et al.*, 1974; Boggs & Bartlett, 1982; Sant'Ambrogio *et al.*, 1991). In humans, intra-epithelial RARs lie close to the airway lumen and are located primarily in the large central airways (Laitinen, 1985), the major site for cough provocation (Widdicombe, 1980). Similarly, the receptors responsible for UNDW-induced cough appear to be situated predominantly in these large airways and larynx and exhibit a rapid adaptation of their reflex response. It may be concluded that cough induced from inhalation of aqueous aerosols results from stimulation of airway RARs which are located in the large conducting airways. Inhalation of hypotonic saline and UNDW appears to offer a safe, physiological stimulus with which to study cough.

CHAPTER 4: THE EFFECT OF ALTERING AIRWAY TONE ON COUGH

4.1 INTRODUCTION

Cough is often associated with bronchoconstriction and it has been suggested that both responses are mediated by RARs since many stimuli which discharge RARs also evoke cough and bronchoconstriction (Widdicombe, 1954). It has also been suggested that bronchoconstrictor agents stimulate RARs indirectly through their action on airway smooth muscle (Coleridge *et al.*, 1978) and that a causal relationship, bronchoconstriction leading to cough, also exists (Salem & Aviado, 1964; Chausow & Banner, 1983). If this is correct, then bronchodilators may be a useful strategy for suppressing cough. In support of this hypothesis, cough is a common symptom of asthma (Corrao *et al*, 1979; Irwin *et al.*, 1981) and this 'allergic' cough is inhibited by treatment with bronchodilators (Ellul-Micallef, 1983; Corrao *et al.*, 1979). Furthermore non-asthmatic volunteers with cough associated with upper respiratory tract infection (URTI) have been shown to have hyper-reactive airways and an enhanced cough response to citric acid aerosol which is inhibited by bronchodilator therapy (Empey *et al.*, 1976). However, in recent years, the relationship between these two reflexes has been questioned. The cough response to UNDW is not associated with bronchoconstriction in non-asthmatics (Schoeffel *et al.*, 1981) and the two responses are mediated by different mechanisms of action confirmed by a differential effects of drugs. Atropine and sodium cromoglycate inhibit bronchoconstriction but not the cough response to UNDW (Sheppard *et al.*, 1983; Fuller & Collier, 1984), while lidocaine inhibits cough but not bronchoconstriction (Sheppard *et al.*, 1983). Furthermore, the cough response to UNDW is immediate and adapts over a 1 minute challenge, while the bronchoconstriction follows

inhalations of UNDW of over 1 minute duration in a dose-dependent manner (Chadha *et al.*, 1984). Cough appears to result from the lack of permeant anions in water (Section 3.3.2) while bronchoconstriction results from its reduced osmolarity (Eschenbacher *et al.*, 1984). Also, studies using citric acid-induced cough have demonstrated conflicting evidence as to the efficacy of bronchodilators as antitussives (Karttunen *et al.*, 1987; Pounsford *et al.*, 1985).

In view of these uncertainties, the aims of the following experiments were to assess the ability of bronchodilation to inhibit cough and bronchoconstriction to promote cough. Bronchodilation can be achieved with two classes of drugs; catecholamines cause bronchodilation by activating beta$_2$-adrenoceptors, while anticholinergic drugs inhibit acetylcholine release from parasympathetic post-ganglionic neuromuscular junctions which causes bronchoconstriction. The studies of bronchodilation were designed to compare the antitussive properties of inhaled and orally administered beta$_2$-adrenergic and anticholinergic bronchodilators, administered in therapeutic doses, on cough induced by inhalation of aqueous aerosols in normal and asthmatic volunteers. Cough challenges were performed at times following treatment to coincide with the peak time of action of the drugs. The relationship between bronchodilation and inhibition of cough was also determined.

The ability of bronchoconstriction to promote cough was investigated using leukotriene D_4 (LTD_4), a 5-lipoxygenase metabolite of arachidonic acid and an inflammatory mediator. LTD_4 is a potent constrictor of non-asthmatic as well as asthmatic airways causing a prolonged bronchoconstriction which is maximal within 2 to 3 minutes (Smith *et al.*, 1985). Coughing has been reported in some (Holroyde *et al.*, 1981; Kern *et al.*, 1986; Ayala *et al.*, 1988) but not all (Bel *et al.*, 1987; Smith *et al.*, 1987) studies and the cough response has not been formally

studied. Therefore, the dose response characteristics of both cough and bronchoconstriction in response to LTD_4 inhalation in healthy subjects were determined. In addition, the effect of pre-treatment with a specific LTD_4 antagonist SK&F 104353, which inhibits LTD_4-induced bronchoconstriction in asthmatics (Evans *et al.*, 1988) and non-specifically with a $beta_2$-adrenergic agonist, salbutamol, was investigated.

4.2 BRONCHODILATION

4.2.1 Beta-Adrenergic Bronchodilators

(a) Inhaled Fenoterol Hydrobromide

This study aimed to investigate the antitussive property of a standard $beta_2$-agonist, fenoterol hydrobromide administered by inhalation. Twenty healthy volunteers (7 males and 13 females, age range 19 to 50 yrs, 10 smokers and 10 non-smokers) completed the study. Volunteers received fenoterol hydrobromide by metered dose inhaler (mdi) (360 µg; 2 x 180 µg / puff) or an identical looking placebo inhaler (2 puffs) in random order, double blind and on separate days 30 minutes before a cough challenge. Saline solutions containing 150, 112, 75, 31 and 0 mmol/l chloride were inhaled from the DeVilbiss 65 ultrasonic nebulizer as described in Section 2.2 for 1 minute periods and at 10 minute intervals during which cough frequency was recorded.

(b) Oral Salbutamol Sulphate

The antitussive property of a standard oral presentation of a $beta_2$-agonist, salbutamol sulphate was investigated using 11 healthy volunteers (4 males and 7 females, age range 19 to 50 yrs). Subjects received salbutamol sulphate (4 mg: 2 x 2 mg tablets) or matched placebo (2 x

tablets) 2 hours before a cough challenge on separate days, in random order and double blind. The challenge consisted of inhaling ultrasonically nebulized saline solutions containing 150, 75, 31 and 0 mmol/l chloride respectively for 1 minute periods and at 10 minute intervals. FEV_1 was recorded pre-tablet and pre- and post-challenge.

(c) Inhaled Salbutamol and Procaterol Hydrochloride

The antitussive properties of inhaled salbutamol were investigated to compare its efficacy with the oral preparation studied above in Experiment 4.2.1(b). Procaterol hydrochloride was also investigated as a new highly selective $beta_2$-adrenergic agonist (Siegel *et al.*, 1985) comparing 2 doses. Twenty healthy volunteers (5 males and 15 females, age range 21 - 60 yrs (mean 40 yrs.), mean FEV_1 = 107%, range 81 - 135% of predicted) completed the study. For this study, it was decided to assess the effect of treatment on just 1 challenge to exclude any adaptation of cough during dose response challenges. Five visits to the laboratory were required within a 14 day period. On visit 1, a cough dose response challenge was performed using ultrasonically nebulized saline containing 150, 75, 31 and 0 mmol/l chloride respectively, which were inhaled for 1 minute periods and at 5 minute intervals until more than 10 coughs occurred during a single inhalation. Subjects who did not cough on more than 10 occasions to any aerosol were excluded from the study. The concentration causing greater than 10 coughs was then used by the volunteer for the remainder of the study. On visits 2 to 5, volunteers received salbutamol (200 µg: 2 x 100 µg/puff), procaterol (10 µg: 1 x 10 µg/puff procaterol + 1 puff placebo), procaterol (20 µg: 2 x 10 µg puffs) or placebo (2 puffs) by mdi in random order derived from 5 Latin squares of order 4 balanced for 1st order carryover (residual) effects (Cochran & Cox, 1966), double blind and on separate days 15 minutes prior to cough

challenge. R_{aw} and FEV_1 were recorded pre-treatment and pre- and post-cough challenge.

4.2.2 Anticholinergic Bronchodilators

(a) Inhaled Ipratropium Bromide

The antitussive efficacy of a standard inhaled anticholinergic agent, ipratropium bromide, was investigated in 14 healthy volunteers (8 males and 6 females, age range 19 - 50 yrs). Subjects inhaled ipratropium bromide mdi (72 µg: 4 x 18 µg/puff) or identical placebo (4 puffs) 45 minutes prior to cough challenge. This consisted of inhaling ultrasonically nebulized saline solutions containing 150, 75, 31 and 0 mmol/l chloride respectively for 1 minute periods and at 10 minute intervals.

(b) Oral Pirenzepine Hydrochloride

This study aimed to investigate the antitussive property of an orally administered anticholinergic agent. Pirenzepine hydrochloride is chemically related to ipratropium bromide and is a selective inhibitor of gastric acid secretion (Stockbrugger *et al.*, 1979). Fourteen healthy volunteers (7 males and 7 females, age range 19 - 50 yrs) were given pirenzepine (50 mg) or matched placebo tablet 2 hours before cough challenge. This consisted of inhaling ultrasonically nebulized saline solutions containing 150, 75 31 and 0 mmol/l chloride respectively for 1 minute periods and at 10 minute intervals.

(c) Inhaled Oxitropium Bromide, Ipratropium Bromide and a Combination of Ipratropium and Fenoterol Hydrobromide.

This study was designed to determine the antitussive efficacy of a new anticholinergic, oxitropium bromide (Peel *et al.*, 1984) compared with

placebo. A compound preparation of a beta-agonist with an anticholinergic was also investigated to determine whether this produced a greater antitussive effect than the anticholinergic component alone. Sixteen healthy non-smoking volunteers (8 males and 8 females, age range 21 - 39 yrs) who coughed in response to UNDW completed this study. FEV_1 was measured in 12 of the volunteers (mean FEV_1 = 119% of predicted, range 103 to 153%). After studying 6 subjects, a learning effect on cough frequency was thought to occur and the data were analysed (author remaining blind). This confirmed a learning effect which was most apparent between days 1 and 2 of the study. Although this was statistically insignificant and was less than the overall treatment effect, an extra open placebo control day on day 1 was incorporated for each subject and the study restarted. Data from this open placebo day were not included in the analysis. Subjects attended the laboratory on 5 consecutive days. On day 1 placebo (2 puffs) was administered. On days 2 - 5, treatment with oxitropium bromide mdi (200µg: 2 x 100 µg/puff), ipratropium bromide (80µg: 2 x 40 µg/puff), ipratropium bromide (80µg) + fenoterol hydrobromide (200µg) (2 x puffs ipratropium bromide 40 µg/puff + fenoterol hydrobromide 100 µg/puff) or placebo (2 puffs) were administered in random order and double-blind 60 minutes before a cough challenge consisting of inhaling ultrasonically nebulized saline solutions containing 53, 31, and 0 mmol/l chloride respectively for 1 minute periods and at 5 minute intervals.

4.2.3 The Association Between Alterations in Airway Tone and the Inhibition of Cough

The aims of this study were to investigate the relationship between the bronchodilation produced by inhaled and orally administered beta-agonists and anticholinergics and the inhibition of cough. Six healthy

volunteers (1 male and 5 females, age range 21 - 39 yrs, mean FEV_1 = 122% of predicted, range 110 - 142%), completed this study. Each underwent whole body plethysmographic measurement of sG_{aw} followed by flow/volume curves from which FEV_1 was derived. These measurements were performed in duplicate before and immediately after a cough challenge and this procedure was repeated after each treatment. Challenge for cough consisted of UNDW delivered for 1 minute duration during which cough frequency was recorded. Treatments were fenoterol hydrobromide mdi (360 μg) 30 minutes prior to challenge; salbutamol sulphate tablets (4 mg) 2 hours prior to challenge, ipratropium bromide mdi (72 μg) 45 minutes prior to challenge, pirenzepine tablets (50 mg) 2 hours prior to challenge, placebo mdi (2 puffs) 30 minutes prior to challenge and placebo tablets 2 hours prior to challenge. Treatments were administered on separate days with the order randomised for each subject using a Latin square design.

4.2.4 The Antitussive Effect of Bronchodilators in Asthmatics

(a) Inhaled Salbutamol and Procaterol Hydrochloride

This study of asthmatics was performed in conjunction with experiment 4.2.1(c) in healthy subjects to determine whether they responded to a greater extent to the antitussive properties of inhaled beta-agonists.

Twenty volunteers with stable bronchial asthma (9 males and 11 females, age range 18 - 61 yrs (mean 33 yrs), mean FEV_1 = 103% of predicted, range 74 - 129%) completed this study. Asthma was confirmed by a 15% or more increase in FEV_1 following salbutamol (200 μg) inhalation. None were taking oral steroids and the previous 3 months had been free of acute exacerbations of their asthma requiring hospital

admission. Inhaled steroids continued unchanged during the study period but all bronchodilators were withheld for 8 hours prior to each visit.

The experimental procedure followed that described in 4.2.1 (c).

(b) Inhaled Oxitropium Bromide, Ipratropium Bromide and a Combination of Ipratropium and Fenoterol Hydrobromide.

This study of asthmatics was performed in conjunction with experiment 4.2.2.(c) which was performed in healthy subjects to determine whether they responded to a greater extent to the antitussive properties of inhaled anticholinergic bronchodilators.

Ten non-smoking subjects with stable bronchial asthma (3 males and 7 females, age range 20 - 32 yrs) who coughed in response to UNDW completed this study. None were taking oral steroids and the previous 3 months had been free of acute exacerbations. Beta agonists were withheld for 8 hours and all other medication for 12 hours prior to each visit. FEV_1 was recorded in 9 of the subjects prior to the study (mean FEV_1 = 107% of predicted, range 95 to 124%).

The experimental procedure followed that described in 4.2.2 (c).

4.3 STATISTICAL ANALYSIS

4.2.1(a),(b) 4.2.2 (a),(b): (Fenoterol, Salbutamol, Ipratropium, Pirenzepine)

Each active drug was compared with placebo for its effect on cough frequency at each concentration of chloride. As the cough response to solutions with chloride concentrations greater than 75 mmol/l was low, these results were not included in the analysis. The justification for this was that to include them would have artificially reduced the residual variance.

The cough frequency values were first transformed as described in Section 2.7. Three factor ANOVA was then performed where the factors

were subjects, drugs (active/placebo) and concentration of chloride. The interaction term was used to compute the 95% confidence limits for the drug means at each chloride concentration. A probability level of p=0.05 was set to test the main factors of the analysis and the interactions. Comparisons between means were however made using a least significant difference with a p value of 0.01 to reduce the risk of a Type 1 statistical error.

4.2.3: (The Association Between Alterations in Airway Tone and the Inhibition of Cough)

Cough frequencies were again transformed prior to analysis. A logarithmic transformation was performed on values of FEV_1 and sG_{aw} as the data was not Normally distributed. Three way ANOVA was used to test whether these values changed with the cough challenge or with treatment. The least significant difference was calculated to enable comparisons to be made between oral and inhaled preparations for each type of drug. Finally the 2 placebo preparations were combined and compared with the oral and inhaled beta agonists using William's test for ordered means (Shirley, 1979) (one tailed) with respect to the mean values of FEV_1, sG_{aw} and cough frequency. This was repeated for the anticholinergics.

The association between the changes in FEV_1 and cough frequency and sG_{aw} and cough frequency were assessed with correlation analysis.

4.2.2 (c), 4.2.4 (b): (Oxitropium Bromide)

After transformation, ANOVA was performed on cough frequency data combining the 2 experiments in healthy and asthmatic subjects in a balanced repeated measures design. Differences between the means were assessed by the method of least significant difference.

4.2.1 (c), 4.2.4 (a): (Salbutamol and Procaterol)

Analysis was performed on the combined data from the 2 experiments in healthy and asthmatic subjects. Cough frequency, FEV_1 and R_{aw} values from the 4 treatment days were recorded for analysis. Initial review of the data confirmed a skewed distribution for cough frequency and also for R_{aw}. A square-root and a logarithmic transformation, respectively, was therefore performed on the data prior to ANOVA to normalise their distributions. Comparisons between means were made using the least significant difference. Correlation analysis was performed on the % change in cough frequencies between active treatments and placebo, and the % change in FEV_1 measured 15 minutes post treatment between active treatments and placebo.

4.4 RESULTS

The raw data and ANOVA tables for the following studies are presented in Appendix 2.

4.4.1 Beta-Adrenergic Bronchodilators

(a) Inhaled Fenoterol Hydrobromide

No cough occurred on any occasion to saline aerosols containing 150 and 112 mmol/l chloride but increased as the chloride concentration was reduced below 75 mmol/l ($p<0.01$) confirming the dose/response relationship described in Section 3.3.2. No difference in cough responses could be detected between smokers and non-smokers (mean UNDW-induced cough frequency = 13.7 coughs / minute for smokers and 13.6 for non-smokers on placebo), but there was a difference in cough responses between subjects in general, with 3 subjects failing to cough on any occasion ($p<0.01$). A statistically significant reduction in cough frequency

occurred with fenoterol hydrobromide compared with placebo for distilled water ($p<0.001$) and 31 mmol/l chloride ($p<0.02$). The back-transformed mean cough frequencies are presented in Table 4.1 and graphically in Figure 4.1.

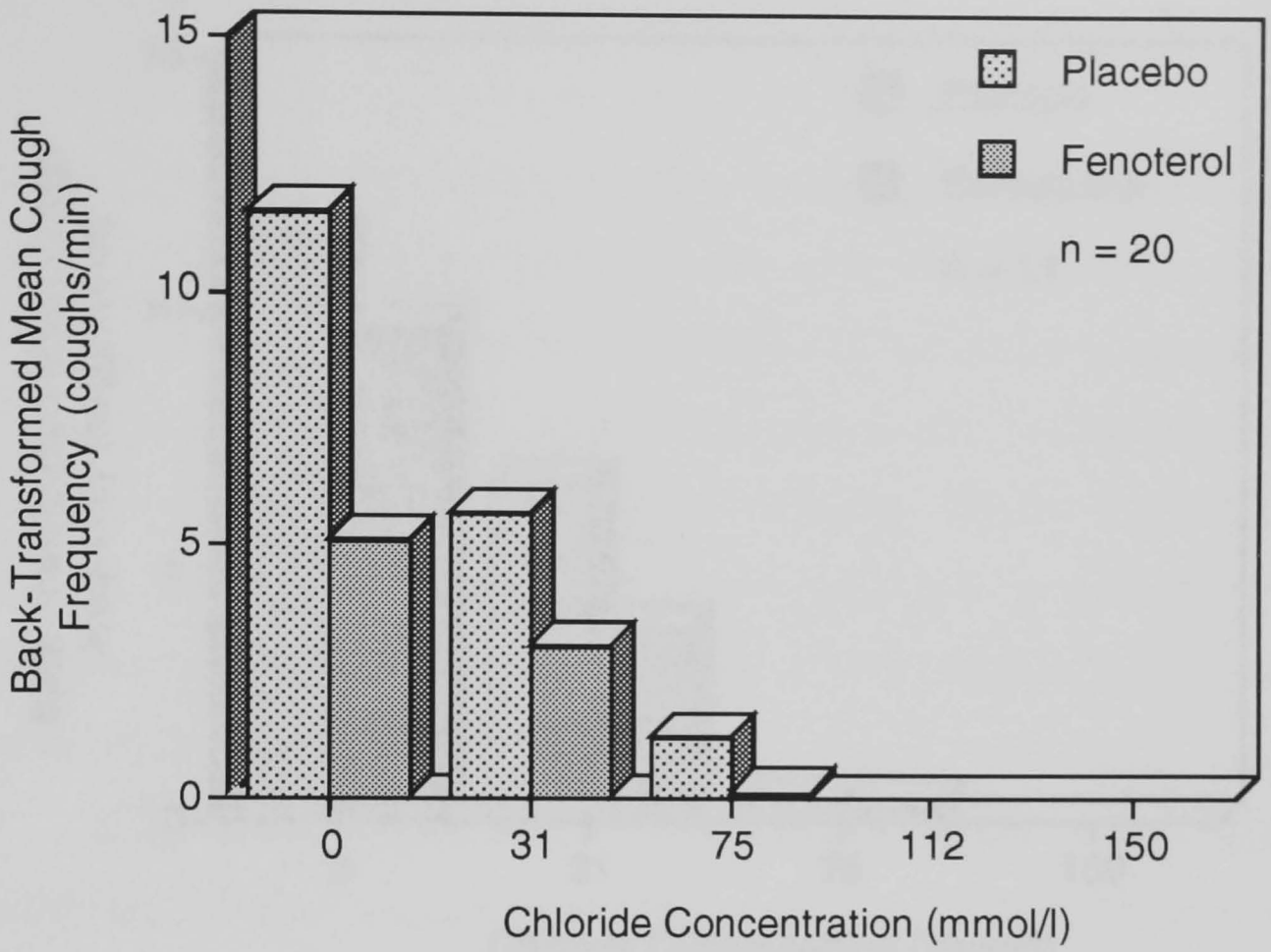
TABLE 4.1

The Effect of Bronchodilators on Cough Frequency

	MCF (95% CL) (coughs/minute)					
	Chloride Concentration (mmol/l)					
	0		31		75	
Placebo	11.6	(9.3-14.2)	5.6	(4.0-7.5)	1.2	(0.3-2.4)
Fenoterol mdi	5.1	(3.5-6.9)	2.9	(1.7-4.4)	0.1	(0.0-0.9)
Placebo	11.5	(7.4-16.3)	6.7	(3.6-10.6)	0.4	(0.0-2.3)
Oral Salbutamol	9.7	(6.0-14.2)	3.8	(1.4-7.0)	0.3	(0.0-2.2)
Placebo	13.3	(10.5-16.3)	3.4	(1.9-5.1)	0.4	(0.0-1.5)
Ipratropium mdi	3.4	(1.9-5.1)	1.3	(0.3-2.6)	0.0	(0.0-0.9)
Placebo	12.8	(9.5-16.5)	4.8	(2.8-7.3)	0.2	(0.0-1.4)
Oral Pirenzepine	7.3	(4.8-10.3)	4.1	(2.2-6.5)	0.1	(0.0-1.3)

FIGURE 4.1

The Antitussive Effect of Inhaled Fenoterol

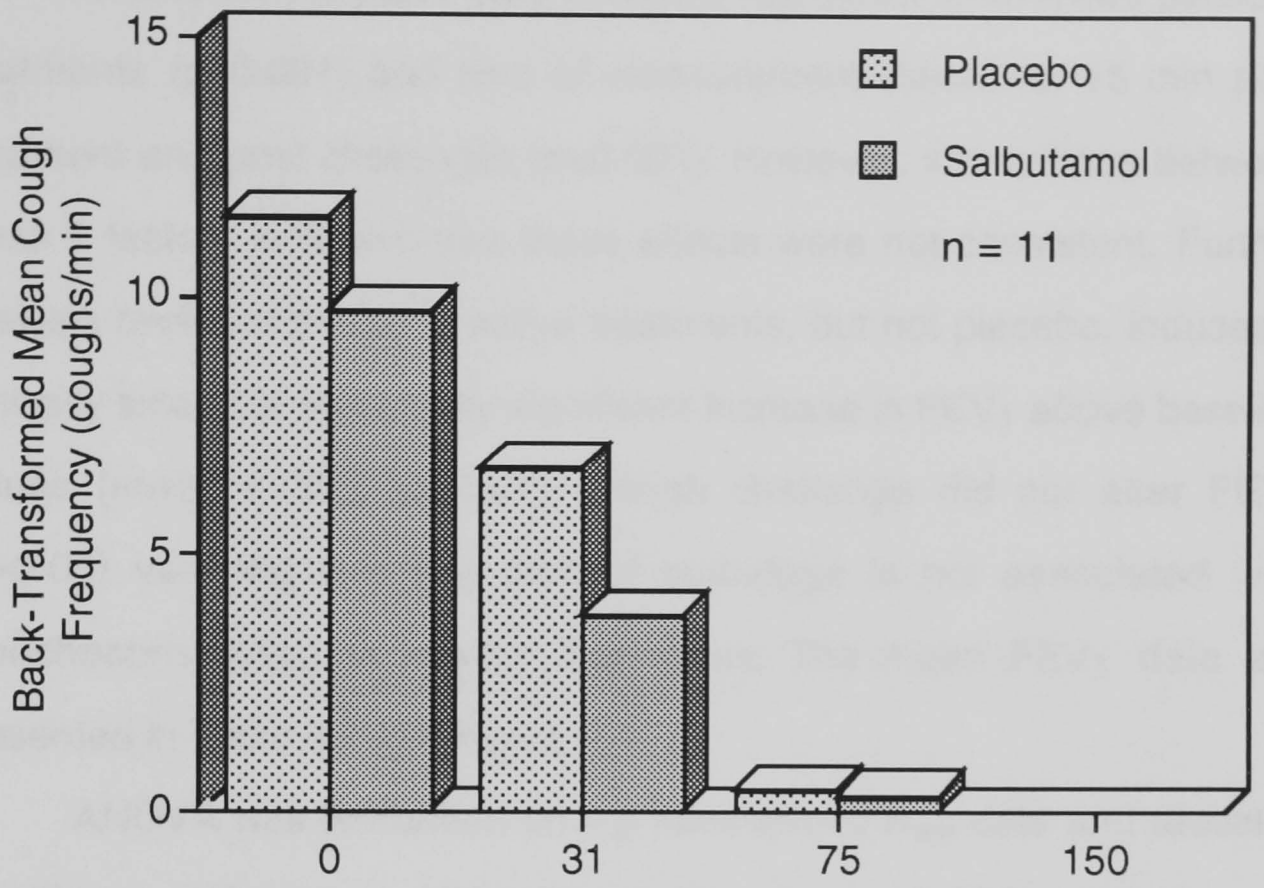


(b) Oral Salbutamol Sulphate

The reduction in cough frequency with oral salbutamol compared with placebo did not reach statistical significance ($p>0.05$). However, the dose / response relationship between decreasing chloride concentration and increasing cough frequency was again confirmed ($p<0.01$). The mean cough frequencies are presented in Table 4.1 and graphically in Figure 4.2. Placebo did not affect FEV_1 but salbutamol increased FEV_1 slightly by an average of 3%.

FIGURE 4.2

The Antitussive Effect of Oral Salbutamol



(c) Inhaled Salbutamol and Procaterol Hydrochloride

Thirty-two healthy volunteers were screened to achieve the target of 20 completing the trial. Of the 12 volunteers who failed to meet the entry criteria, 10 failed to produce more than 10 coughs to UNDW confirming the intersubject variation in cough response, 1 had a recent respiratory tract infection and 1 was on active medication. 17 of the volunteers required UNDW to elicit 10 or more coughs during a 1 minute inhalation, the other 3 required saline containing 31 mmol/l chloride.

ANOVA performed on square-root transformed cough frequency data revealed a highly significant difference between treatments ($p<0.001$). Cough was inhibited by procaterol hydrochloride (10 and 20 μg) and salbutamol (200 μg) compared with placebo ($p<0.001$) with no

statistically significant difference detected between the 3 treatments ($p>0.05$). The back-transformed mean cough frequencies and 95% confidence limits are presented in Figure 4.3.

ANOVA of the FEV_1 data revealed significant differences between treatments ($p<0.001$) and time of measurement (baseline, 15 min post treatment and post challenge) ($p<0.001$). However, interactions between these 2 factors indicated that these effects were not consistent. Further analysis revealed that all 3 active treatments, but not placebo, induced a clinically small but statistically significant increase in FEV_1 above baseline values (mean = 3%) ($p<0.01$). Cough challenge did not alter FEV_1 ($p>0.05$) verifying that this type of challenge is not associated with bronchoconstriction in healthy individuals. The mean FEV_1 data are presented in Table 4.2 and Figure 4.4.

ANOVA was performed on log transformed R_{aw} data and revealed significant differences between treatments ($p<0.001$) and time of measurement ($p<0.001$). Again, interactions between the 2 factors indicated that the responses were not consistent. Only procaterol (20 µg) and salbutamol resulted in a clinically small but statistically significant fall in R_{aw} compared with baseline ($p<0.05$ and $p<0.001$ respectively). Cough challenge produced no change in R_{aw} ($p>0.05$). The mean R_{aw} values are presented in Table 4.2 and Figure 4.5.

The degree of bronchodilation as measured by the % increase in FEV_1 post treatment compared with placebo did not correlate with the % reduction in cough with treatment compared with placebo ($r = -0.19, -0.05$ and 0.29 for procaterol 10 and 20 µg and salbutamol respectively) ($p > 0.05$) as shown in Figure 4.6.

1 volunteer reported mild, transient hand tremor after the 3 active treatments, which is a known and common side-effect of beta-adrenergic therapy.

FIGURE 4.3

The Antitussive Effect of Inhaled Procaterol and Salbutamol

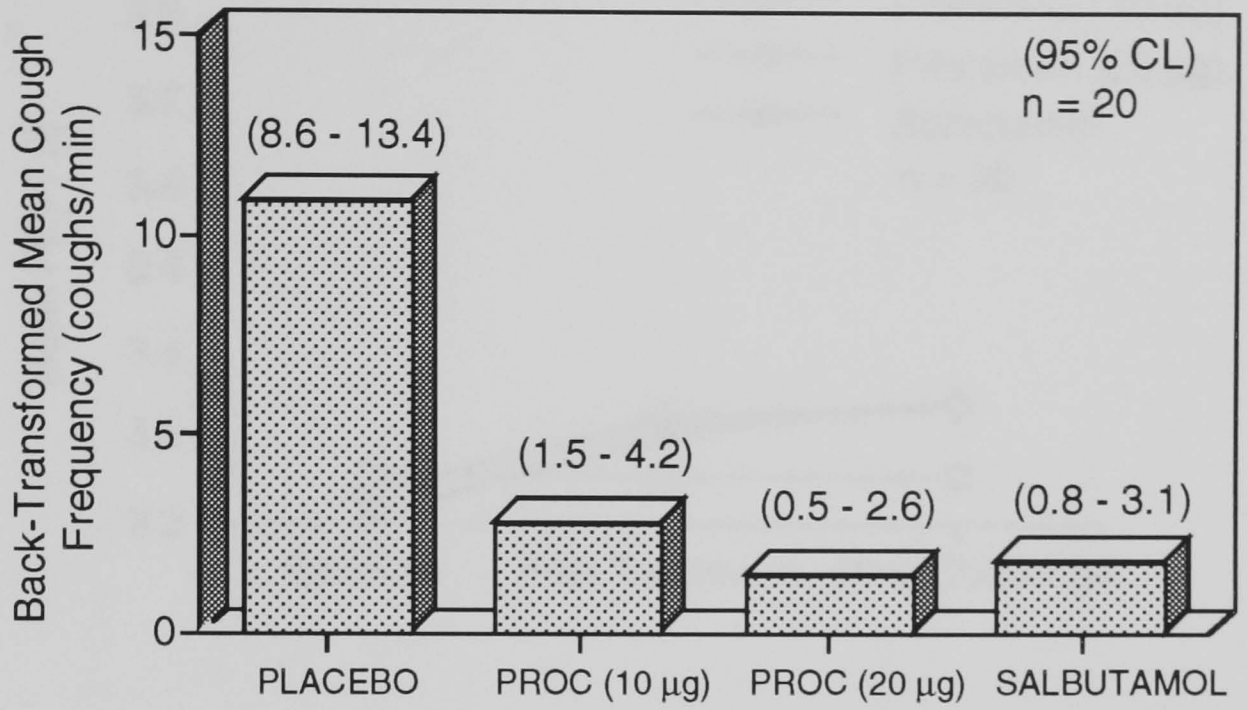


TABLE 4.2

The Effect of Treatment and Challenge on FEV_1 and R_{aw}

	MEAN FEV_1 (l)			BACK-TRANSFORMED MEAN R_{aw} (kPa/l/s)		
	Base	Post Treat	Post Chall	Base	Post Treat	Post Chall
Placebo	3.26	3.26	3.26	0.32	0.30	0.31
Procaterol 10 µg	3.24	3.33	3.34	0.29	0.27	0.26
Procaterol 20 µg	3.24	3.33	3.34	0.29	0.26	0.26
Salbutamol	3.21	3.31	3.35	0.30	0.26	0.26

FEV_1 SE = 0.021

R_{aw} SE (transformed data) = 0.012

FIGURE 4.4

The Effect of Treatment and Challenge on FEV1

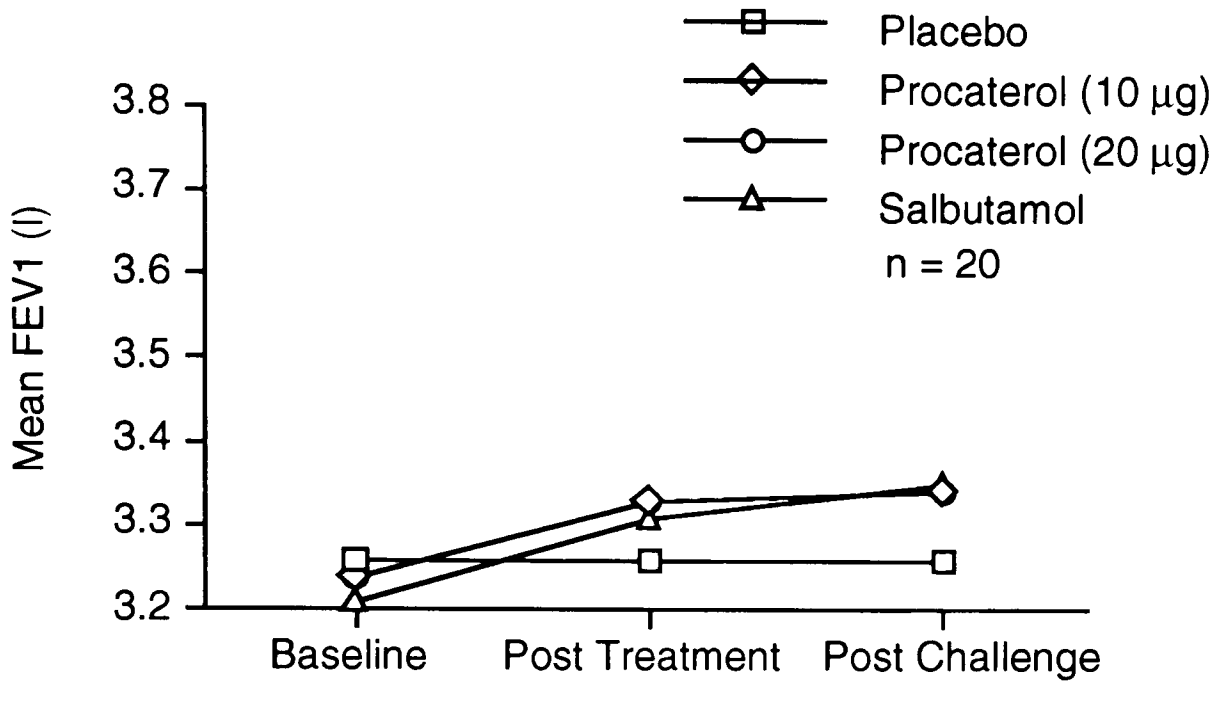


FIGURE 4.5

The Effect of Treatment and Challenge on Raw

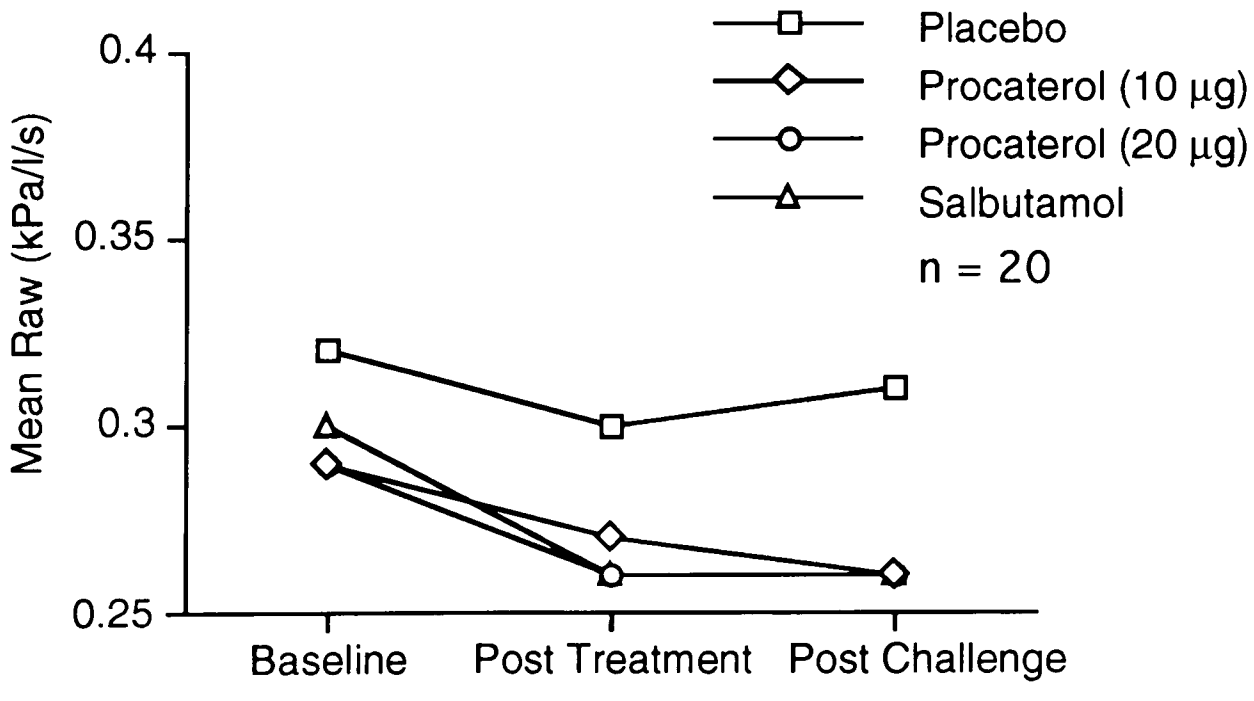
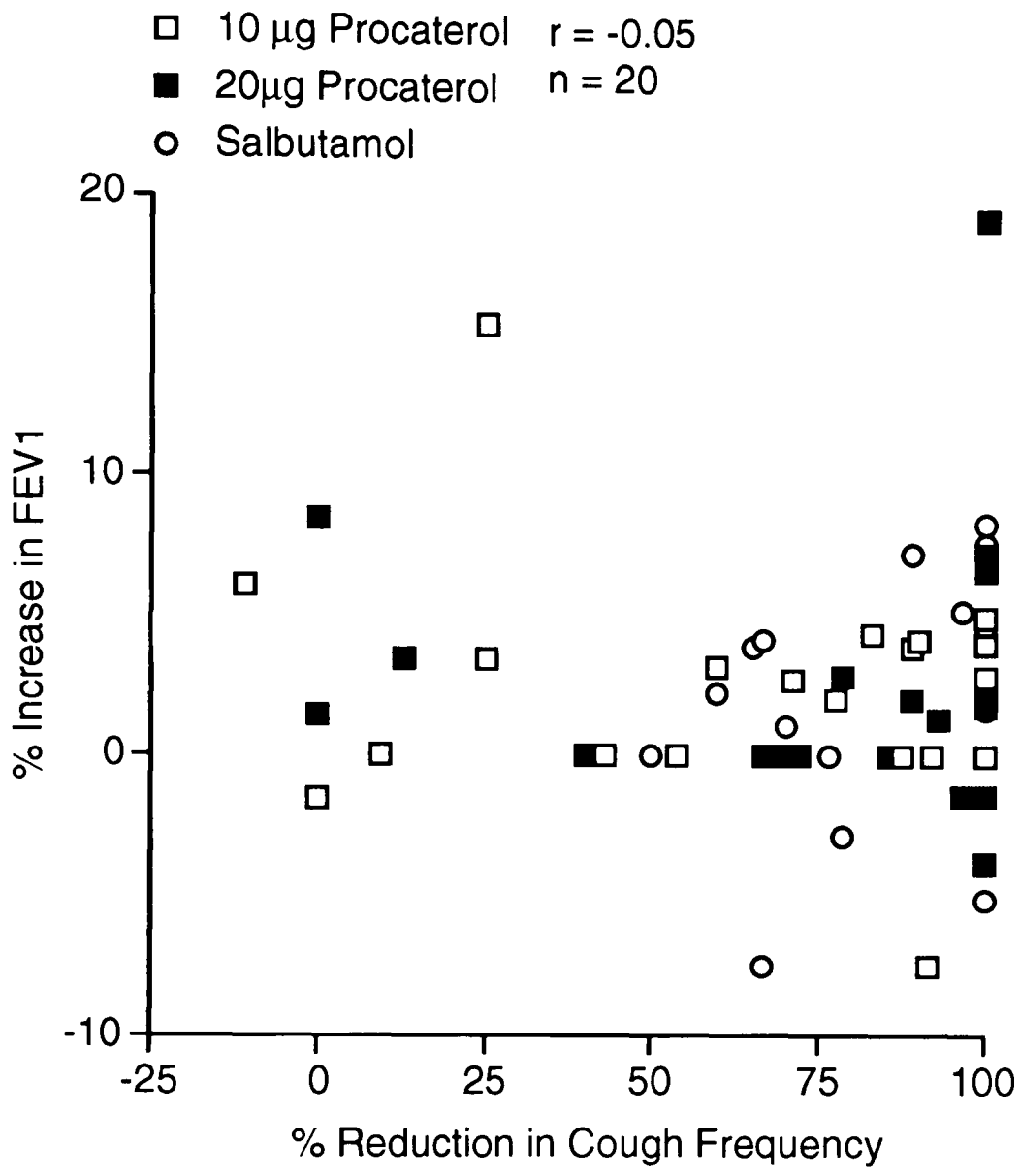


FIGURE 4.6

The Association Between Bronchodilation and Inhibition of Cough



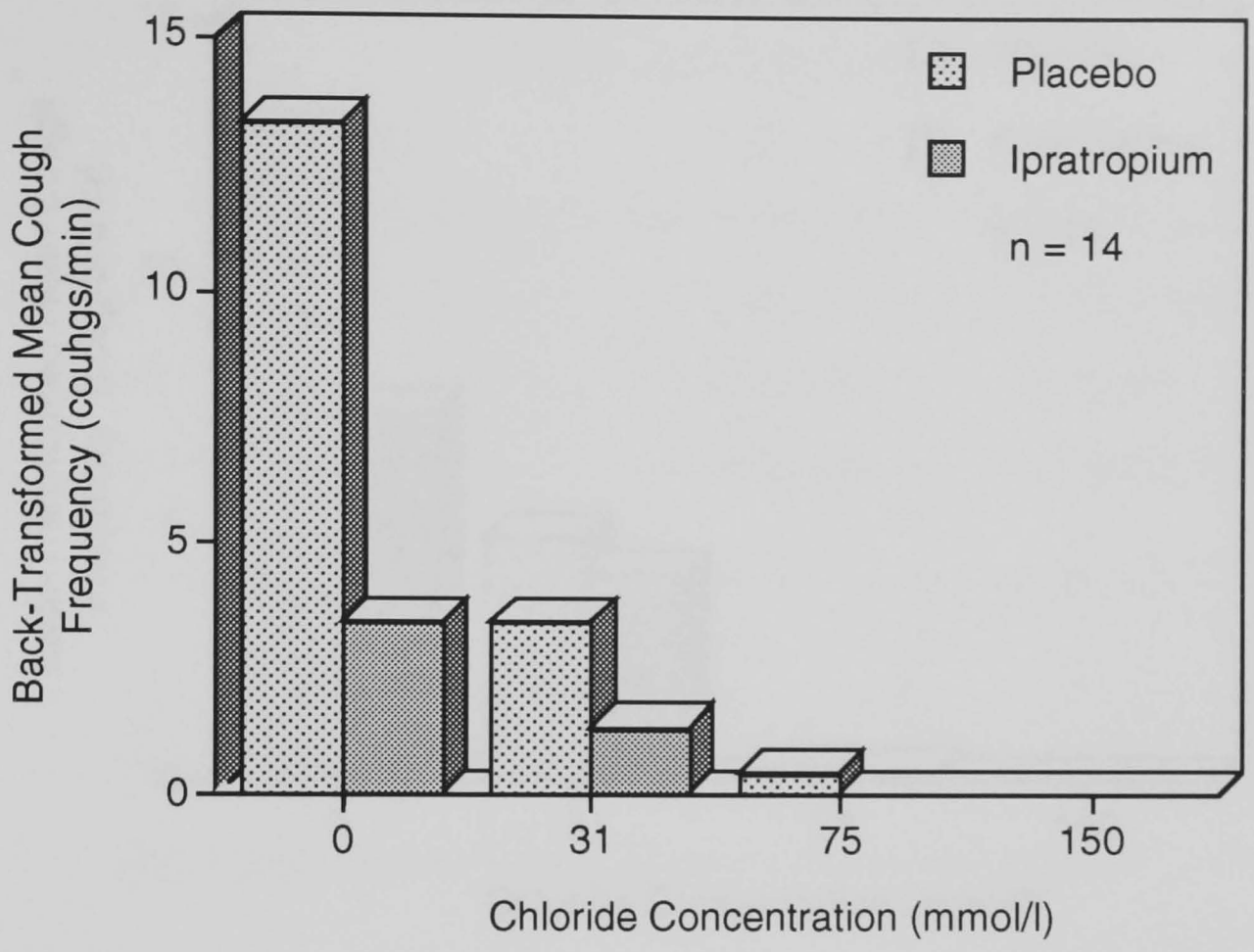
4.4.2 Anticholinergic Bronchodilators

(a) Inhaled Ipratropium Bromide

No cough occurred with saline containing 150 mmol/l chloride but cough increased with decreasing chloride concentration ($p<0.01$). A statistically significant reduction in cough frequency occurred with ipratropium bromide compared with placebo when UNDW was inhaled ($p<0.001$). The mean cough frequencies are presented in Table 4.1 and graphically in Figure 4.7.

FIGURE 4.7

The Antitussive Effect of Inhaled Ipratropium

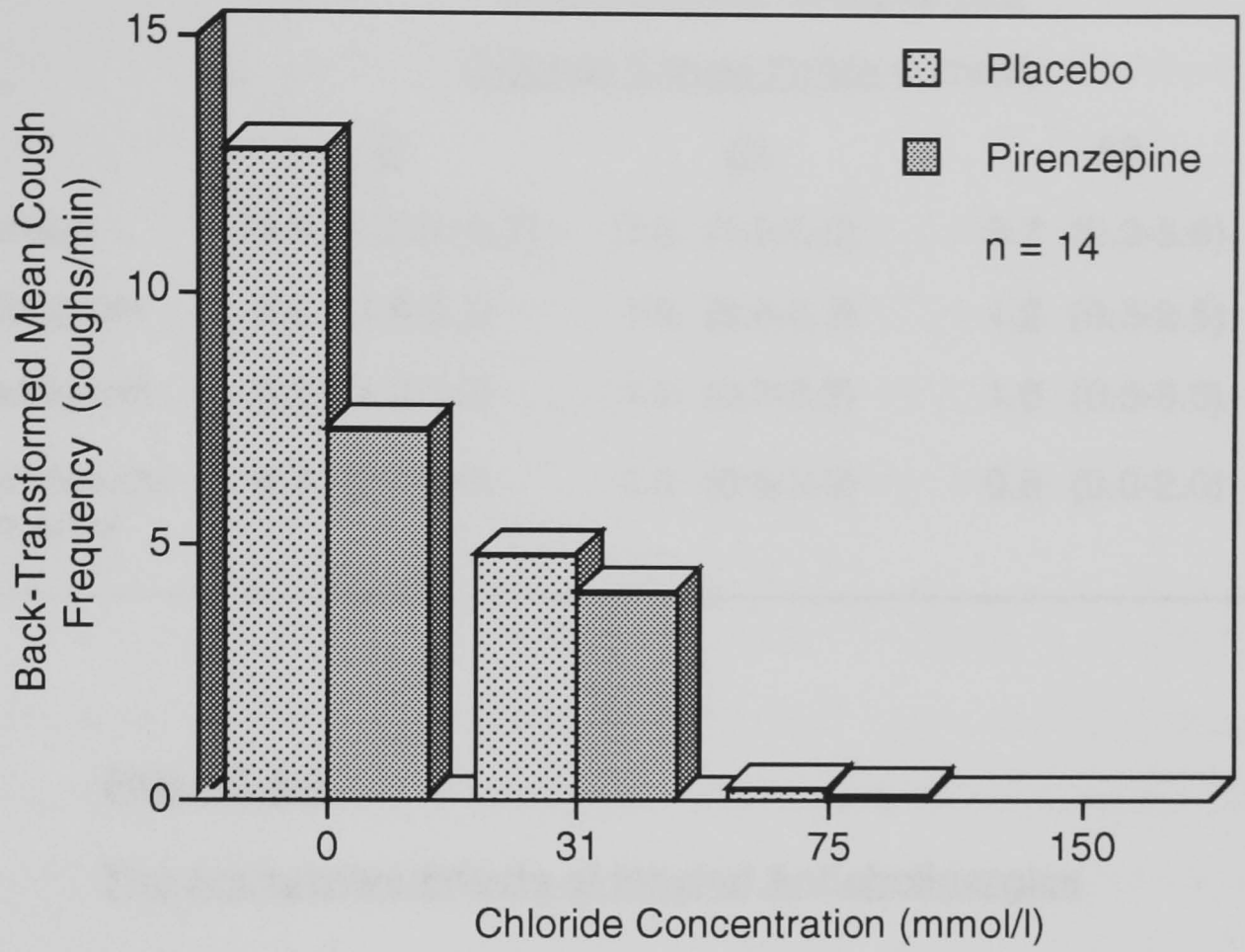


(b) Oral Pirenzepine Hydrochloride

Pirenzepine did not produce a statistically significant reduction in cough frequency compared with placebo ($p>0.05$). However, cough frequency increased with decreasing chloride concentration with both treatments ($p<0.01$). The mean cough frequencies are presented in Table 4.1 and graphically in Figure 4.8.

FIGURE 4.8

The Antitussive Effect of Oral Pirenzepine



(c) Inhaled Oxitropium Bromide, Ipratropium Bromide and a Combination of Ipratropium and Fenoterol Hydrobromide.

Oxitropium bromide, ipratropium bromide and the combination preparation all resulted in fewer coughs than placebo in response to UNDW ($p<0.01$). Cough frequencies in response to the saline solutions were too low to show a statistically significant difference with the active treatments. No important residual effects of treatment between laboratory visits were found. The inhibitory effect of ipratropium bromide on UNDW-induced cough confirms the previous findings in Section 4.4.2.(a). The mean cough frequencies are presented in Table 4.3 and graphically in Figure 4.9

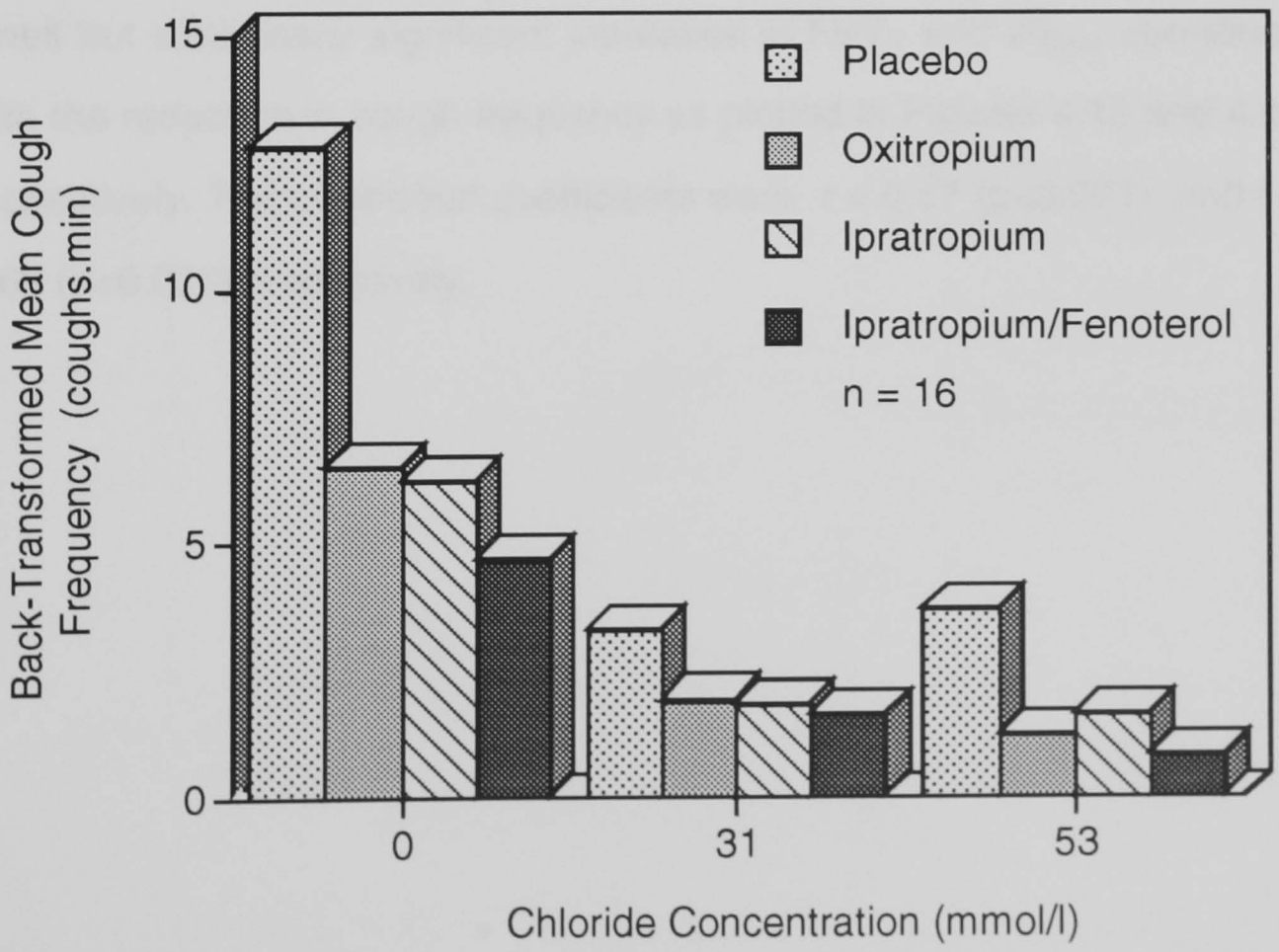
TABLE 4.3

The Antitussive Effects of Inhaled Anticholinergics

	MCF (95% CL) (coughs/min) Chloride Concentration (mmol/l)		
	0	31	53
Placebo	12.8 (10.1-15.7)	3.3 (1.9-5.0)	3.7 (2.2-5.5)
Oxitropium	6.5 (4.6-8.7)	1.9 (0.8-3.3)	1.2 (0.3-2.5)
Ipratropium	6.2 (4.3-8.3)	1.8 (0.7-3.2)	1.6 (0.5-3.0)
Ipratropium/ Fenoterol	4.7 (3.0-6.6)	1.6 (0.5-3.0)	0.8 (0.0-2.0)

FIGURE 4.9

The Antitussive Effects of Inhaled Anticholinergics



4.4.3 The Association Between Alterations in Airway Tone and the Inhibition of Cough

In this study, oral salbutamol but not oral pirenzepine diminished cough frequency, again with lesser effect than the inhaled drugs fenoterol or ipratropium ($p<0.05$).

For FEV_1, sG_{aw} and cough frequency, the differences between treatments were significant ($p<0.01$). Investigations of the sub components of the treatment effect revealed significant differences between presentation (oral vs. inhaled) and between drugs (placebo vs. beta agonist and anticholinergic) for all three variables.

The means for FEV_1 and sG_{aw} are plotted in Figures 4.10 and 4.11 respectively. UNDW challenge did not affect either FEV_1 or sG_{aw} ($p>0.05$) confirming the fact that UNDW-induced cough is not associated with bronchoconstriction. Both inhaled treatments increased these measurements and decreased cough frequency. These physiologically small but statistically significant increases in FEV_1 and sG_{aw} correlated with the reduction in cough frequency as plotted in Figures 4.12 and 4.13 respectively. The correlation coefficients were $r = 0.67$ ($p<0.001$) and $r = 0.68$ ($p<0.001$) respectively.

FIGURE 4.10

The Effect of Treatment and Cough Challenge on FEV1

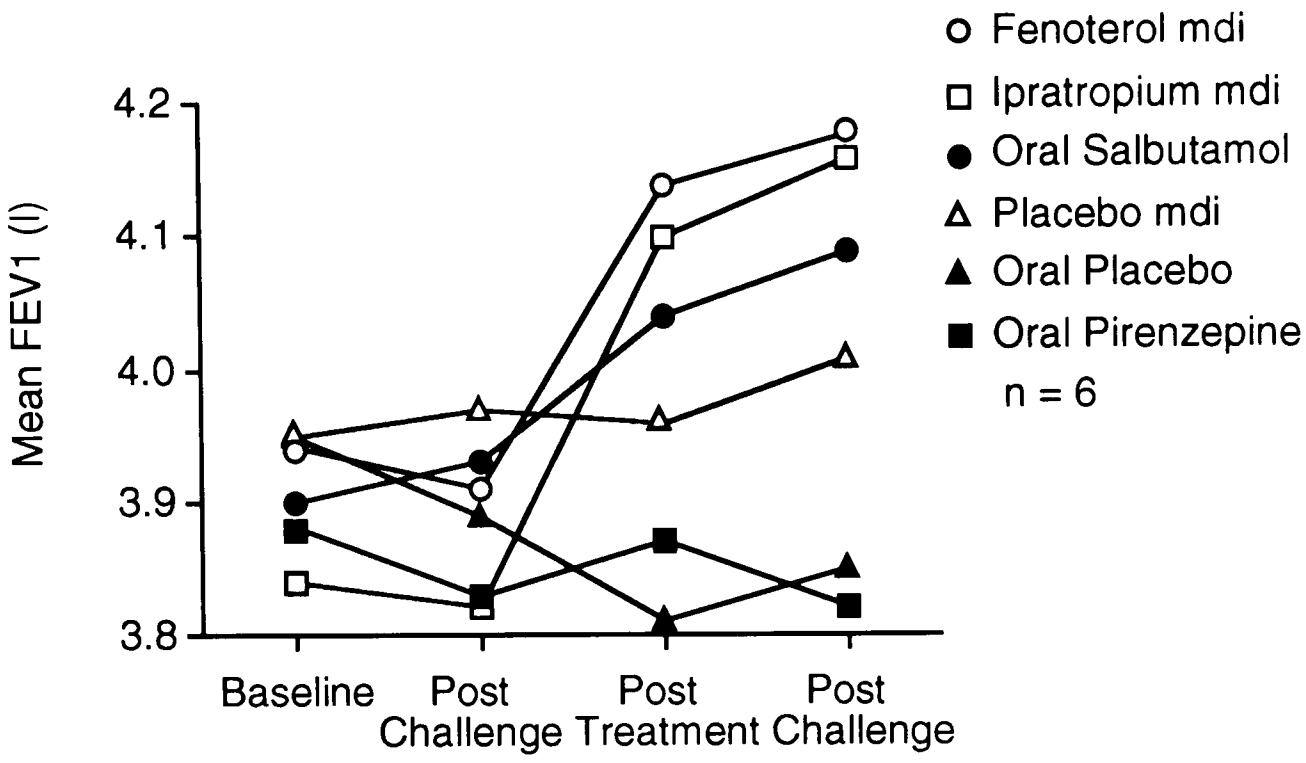


FIGURE 4.11

The Effect of Treatment and Cough Challenge on sGaw

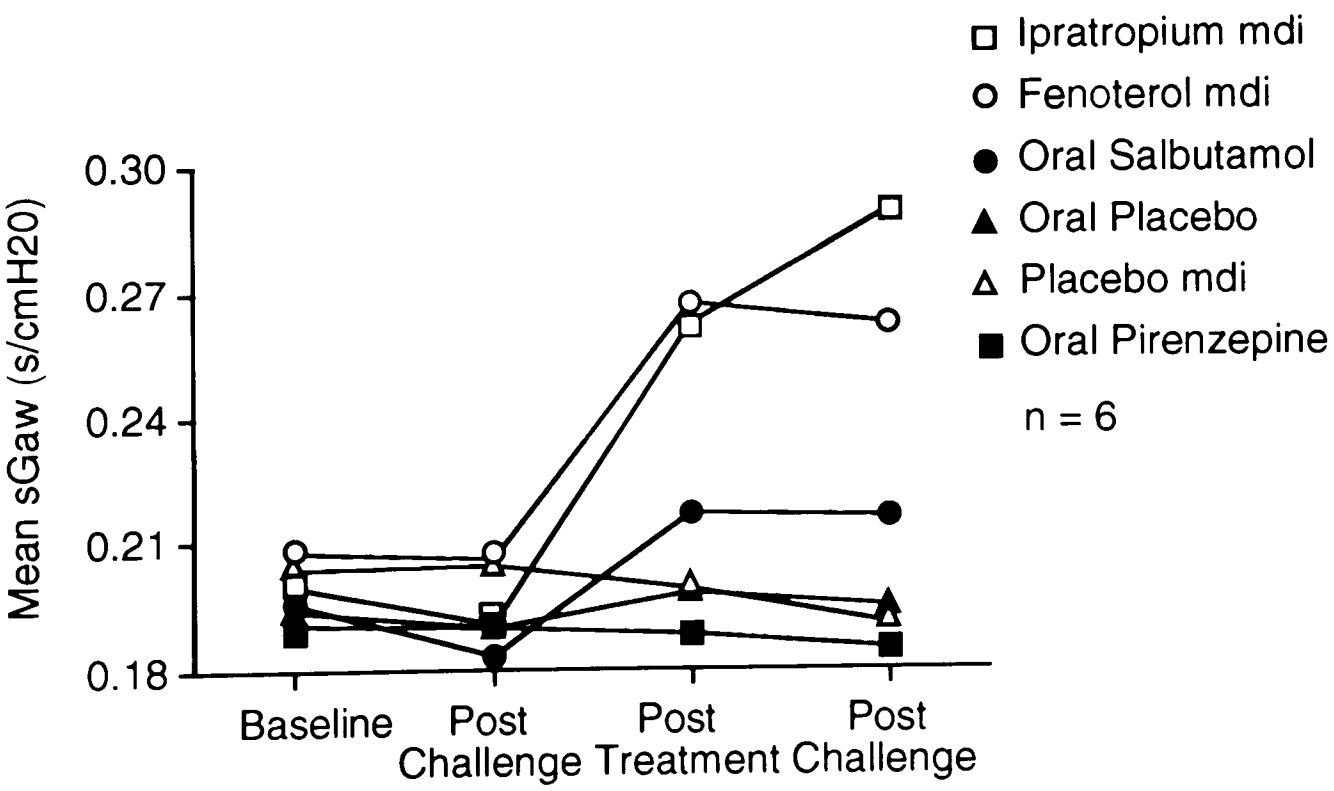
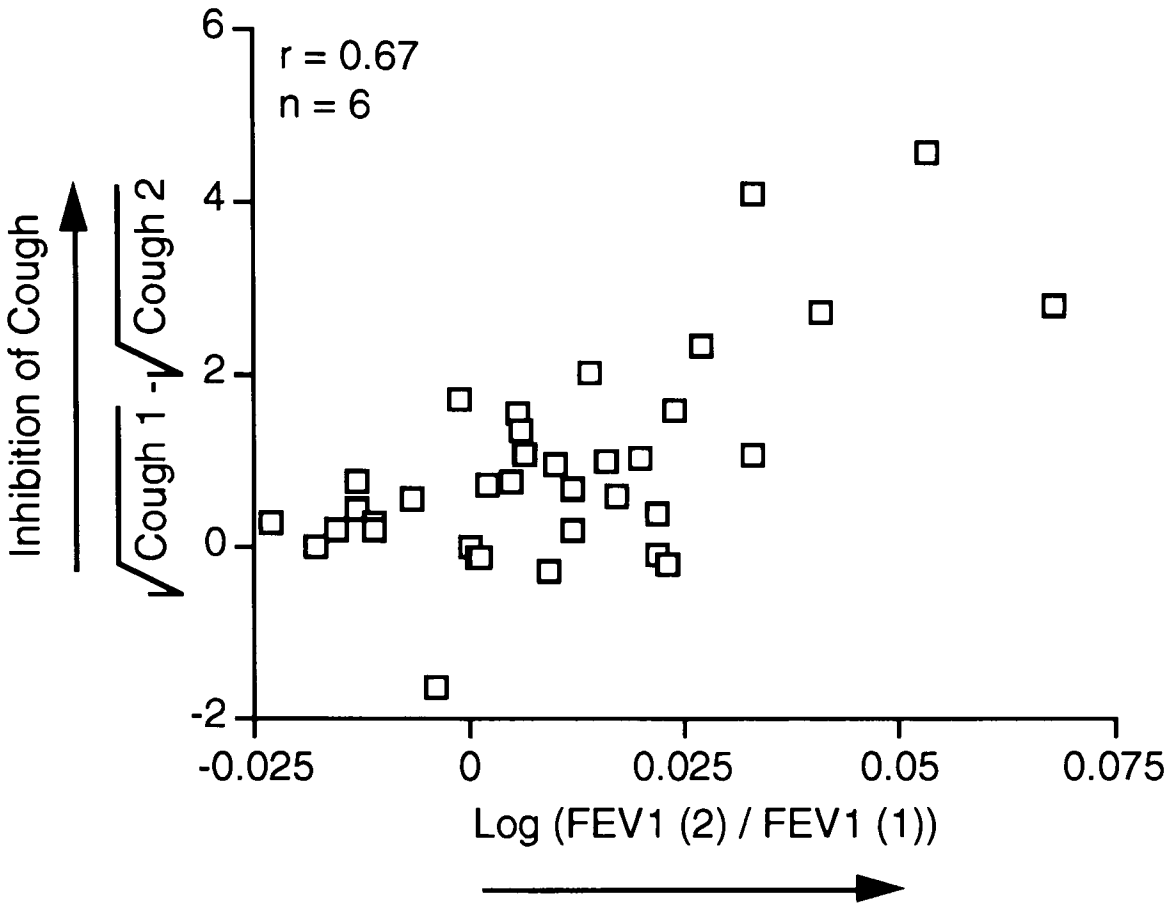


FIGURE 4.12

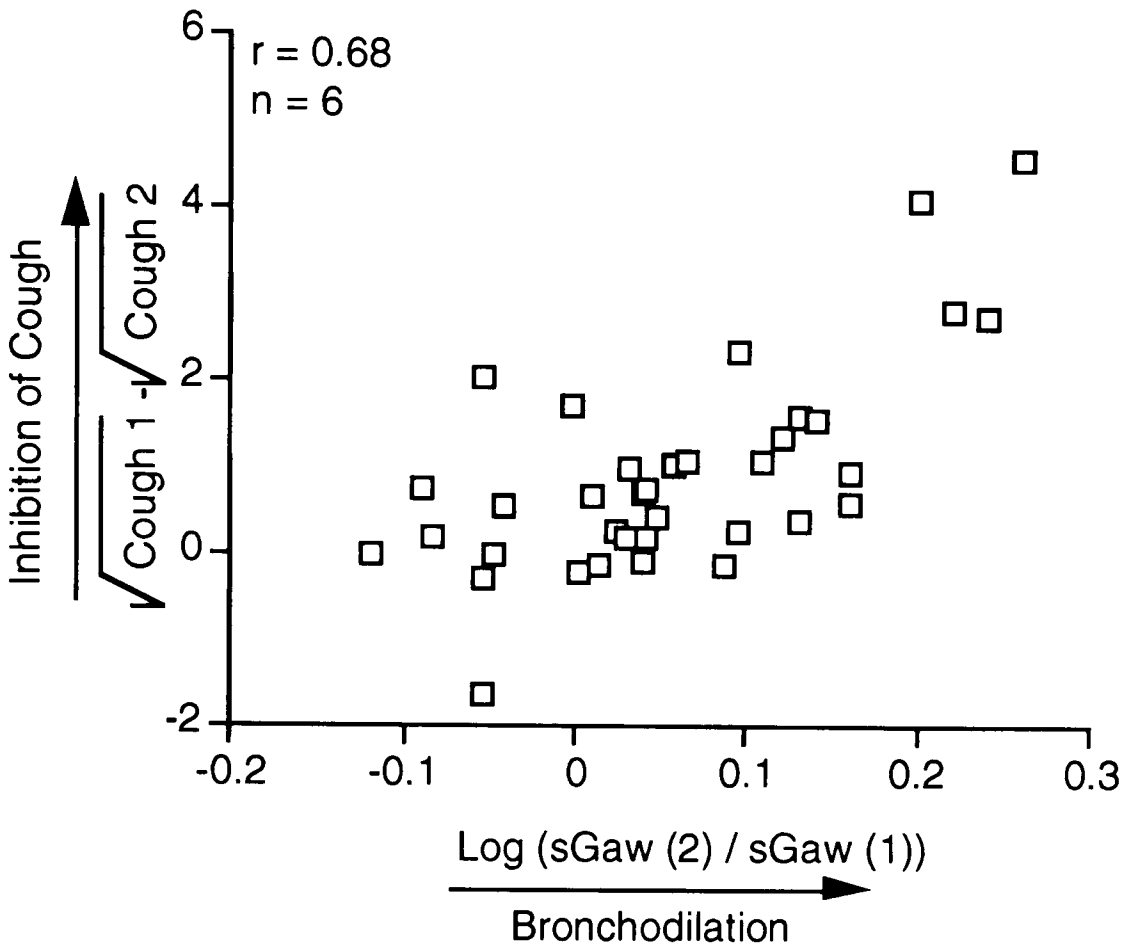
The Relationship Between Inhibition of Cough and the Increase in FEV1 in Response to Treatment



$\sqrt{\text{Cough 1}} - \sqrt{\text{Cough 2}}$ is the difference between the pre and post treatment values of transformed cough frequencies and the log (FEV1 (2) / FEV1 (1)) is the transformed ratio of pre to post treatment FEV1.

FIGURE 4.13

The Relationship Between Inhibition of Cough and the Increase in sGaw in Response to Treatment



$\sqrt{\text{Cough 1}} - \sqrt{\text{Cough 2}}$ is the difference between the pre and post treatment values of transformed cough frequencies and the log (sGaw (2) / sGaw (1)) is the transformed ratio of pre to post treatment sGaw.

4.4.4 The Effect of Bronchodilators in Asthmatics

(a) Inhaled Salbutamol and Procaterol Hydrochloride

Twenty-seven asthmatic volunteers were screened for the study; 5 subjects failed to cough sufficiently to UNDW and 2 withdrew their consent prior to entry. Ten volunteers required UNDW to elicit more than 10 coughs during a 1 minute inhalation, 9 required saline containing 31 mmol/l chloride and 1 required 75 mmol/l chloride indicating a lower threshold for cough than the healthy volunteers.

The ANOVA was performed in conjunction with experiment 4.2.1 (c) which was performed in healthy subjects. This revealed a highly significant difference between treatments ($p<0.001$). Cough was inhibited by procaterol (10 and 20 μg) and salbutamol compared with placebo ($p<0.001$) with no statistically significant difference detected between the 3 treatments ($p>0.05$). The back-transformed mean cough frequencies and 95% confidence limits are presented in Figure 4.14. ANOVA of FEV_1 data revealed significant differences between treatments ($p<0.001$) and time of measurement (baseline, 15 min post treatment and post challenge) ($p<0.001$). However, interactions between the 2 factors indicated that these effects were not consistent. Further analysis revealed that all 3 active treatments, but not placebo, induced a clinically small but statistically significant increase in FEV_1 above baseline by an average of 6% ($p<0.001$). Cough challenge induced a fall in FEV_1 on the placebo day of 5% ($p<0.001$) reflecting the initial stages of the dose-dependent bronchoconstrictor response to UNDW which occurs in asthmatics after exposures greater than 1 minute (Chadha *et al.*, 1984). Treatment with the 3 active drugs prevented the fall in FEV_1 with cough challenge ($p<0.05$). The mean FEV_1 values are presented in Table 4.4 and Figure 4.15.

ANOVA performed on log-transformed R_{aw} data revealed significant differences between treatments ($p<0.001$) and time of measurement ($p<0.001$). Again, interactions between the 2 factors indicated that the responses were not consistent. All 3 active treatments resulted in a clinically small but statistically significant reduction in R_{aw} compared with baseline values ($p<0.001$). Cough challenge increased R_{aw} on the placebo day ($p<0.001$). The mean R_{aw} values are presented in Table 4.4 and Figure 4.16.

The degree of bronchodilation as measured by the % increase in FEV_1 post- treatment compared with placebo did not correlate with the %

reduction in cough with treatment compared with placebo (r = -0.04, -0.05 and 0.23 for procaterol 10 and 20 μg and salbutamol respectively) ($p>0.05$) as shown in Figure 4.17.

Four asthmatics reported mild transient hand tremor after procaterol 20 μg, 2 of whom also reported tremor after procaterol 10 μg. Two asthmatics reported mild wheezing on the placebo day after cough challenge reflecting the known bronchoconstrictor response to UNDW in asthmatics (Schoeffel *et al.*, 1981), 1 of whom also felt wheezy on the day procaterol 20 μg was given.

FIGURE 4. 14

The Antitussive Effect of Procaterol and Salbutamol in Asthmatics

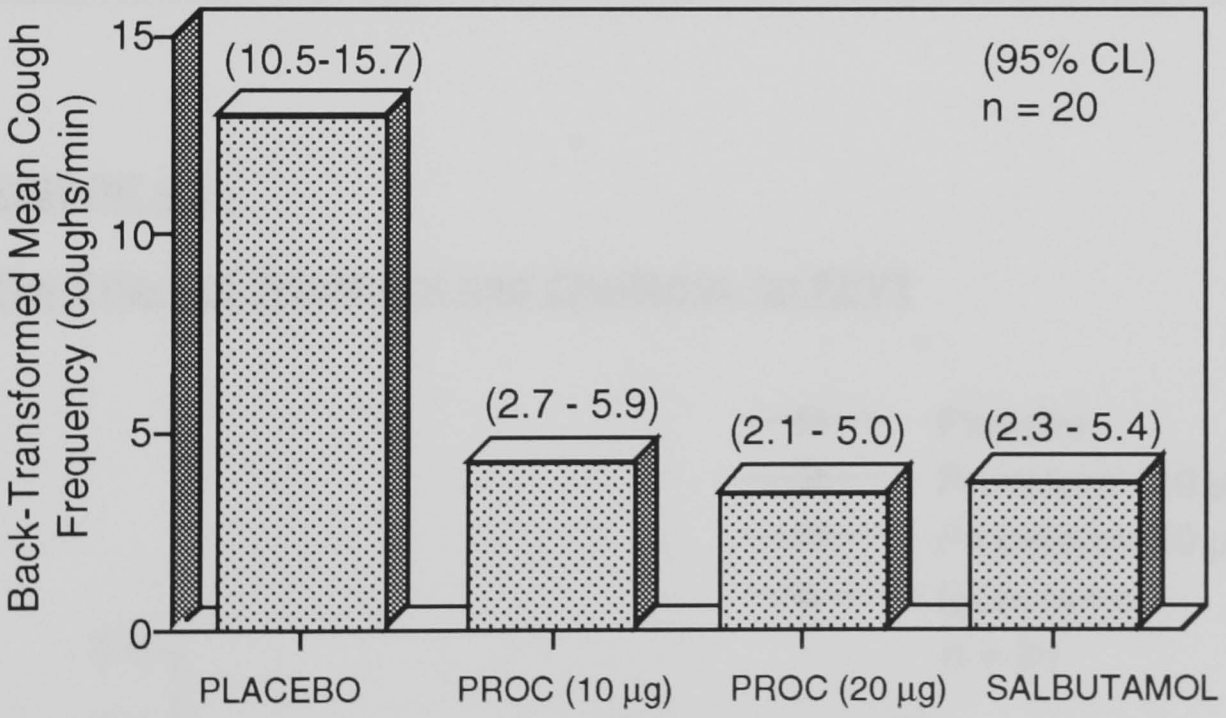


TABLE 4.4

The Effect of Treatment and Challenge on FEV_1 and R_{aw}

	MEAN FEV_1 (l)			BACK-TRANSFORMED MEAN R_{aw} (kPa/l/s)		
	Base	Post Treat	Post Chall	Base	Post Treat	Post Chall
Placebo	3.42	3.40	3.22	0.34	0.31	0.37
Procaterol 10 µg	3.45	3.63	3.67	0.33	0.27	0.27
Procaterol 20 µg	3.45	3.67	3.70	0.30	0.25	0.26
Salbutamol	3.45	3.70	3.69	0.32	0.25	0.27

FEV_1 SE = 0.021

R_{aw} SE (transformed data) = 0.012

FIGURE 4.15

The Effect of Treatment and Challenge on FEV1

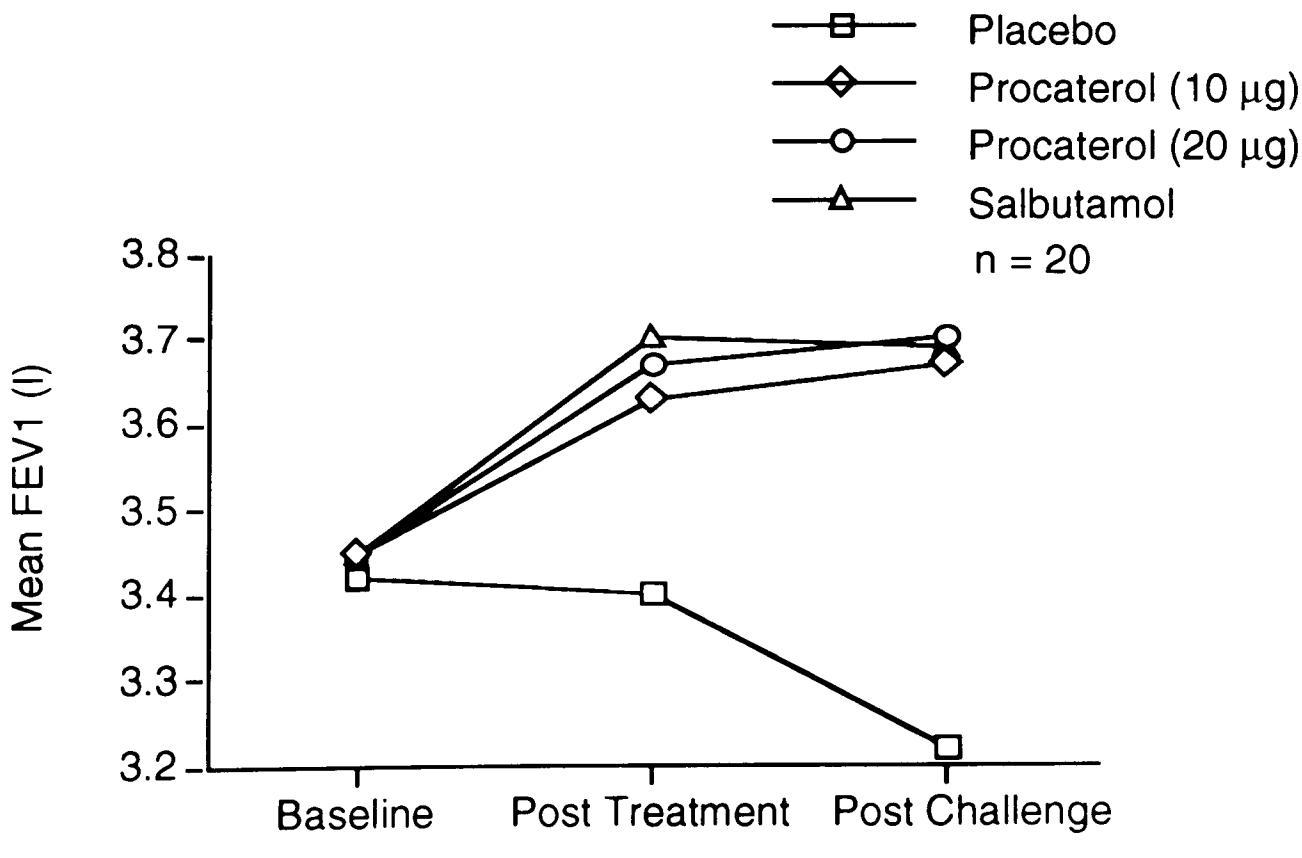


FIGURE 4.16

The Effect of Treatment and Challenge on Raw

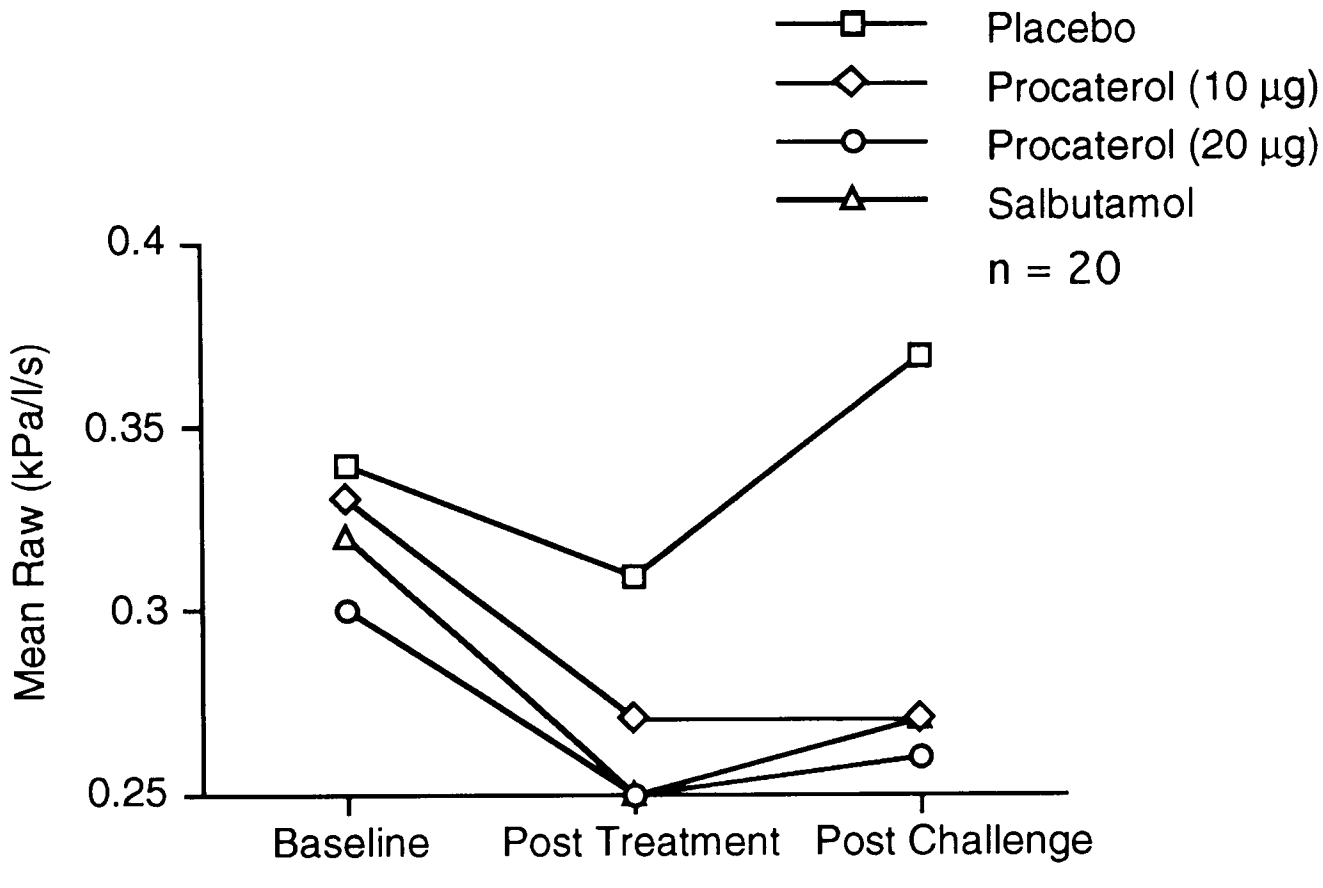
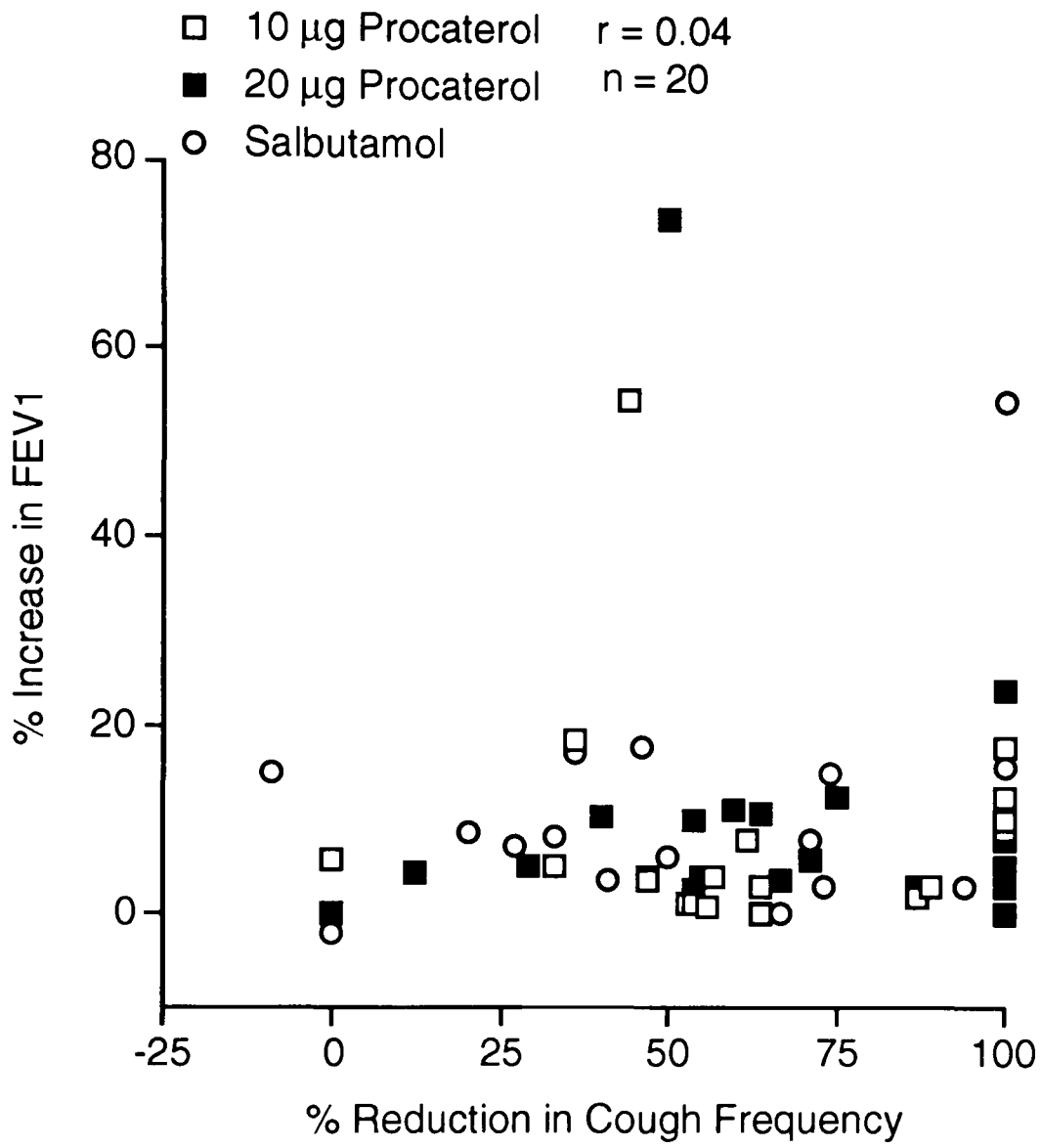


FIGURE 4.17

The Association Between Bronchodilation and Inhibition of Cough in Asthmatics



(b) Inhaled Oxitropium Bromide, Ipratropium Bromide and a Combination of Ipratropium and Fenoterol Hydrobromide.

ANOVA was performed in conjunction with experiment 4.2.2 (c) which studied healthy subjects. This showed a difference between the 2 groups of subjects with healthy subjects coughing more than asthmatics in response to placebo ($p<0.05$) in contrast to the previous experiment (Section 4.4.4 (a)) where the asthmatics exhibited a lower threshold for cough. However, there was no significant statistical interaction between

the groups of subjects (healthy and asthmatic) and treatment. This suggests that both groups of subjects responded similarly to treatment, although with the lower cough frequencies encountered with the asthmatic subjects, the effect is less evident and did not reach statistical significance ($p>0.05$).

Analysis of the combined data revealed that cough frequency in response to UNDW was significantly higher than to either 31 or 53 mmol/l chloride ($p<0.005$) for all treatments. There was no significant difference between the cough response to 31 and 53 mmol/l chloride ($p>0.05$).

Oxitropium bromide, ipratropium bromide and the combination preparation all resulted in fewer coughs than placebo in response to UNDW ($p<0.001$). There was no difference between oxitropium bromide or ipratropium bromide ($p>0.05$) but the combination preparation reduced cough more than either oxitropium ($p<0.05$) or ipratropium ($p<0.025$). No important residual effects of treatment between visits were found.

Back-transformed mean cough frequencies and 95% confidence limits for the asthmatics and the combined data with the healthy subjects are shown in Table 4.5 and presented graphically in Figure 4.18.

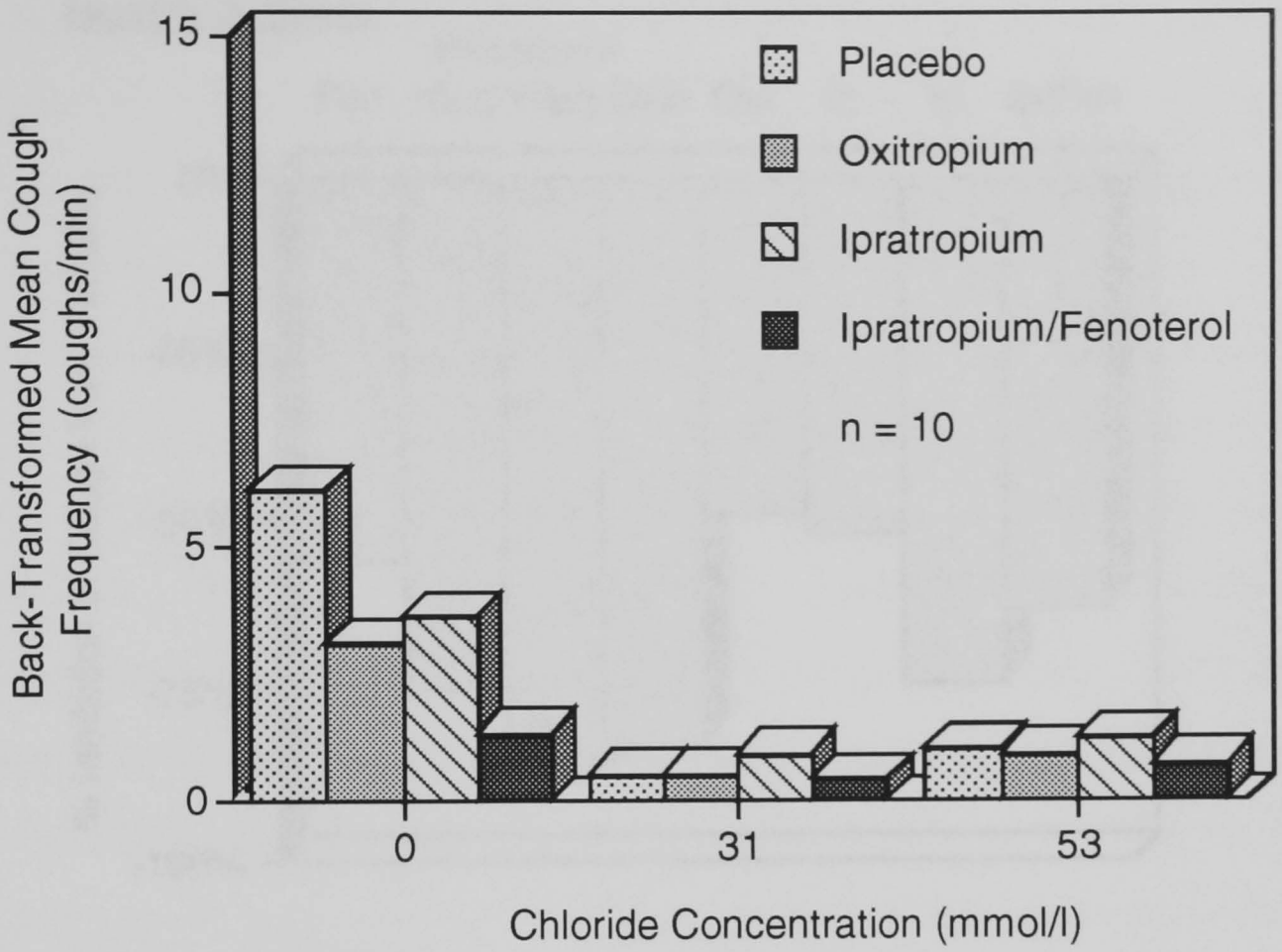
TABLE 4.5

The Antitussive Effects of Inhaled Anticholinergics in Asthmatics and Healthy Subjects

	MCF (95% CL) (coughs/min) Chloride Concentration (mmol/l)		
	0	31	53
Asthmatics n = 10			
Placebo	6.1 (3.8-8.9)	0.5 (0.0-1.9)	1.0 (0.0-2.6)
Oxitropium	3.1 (1.5-5.3)	0.5 (0.0-1.8)	0.9 (0.0-2.4)
Ipratropium	3.6 (1.8-5.9)	0.9 (0.0-2.4)	1.2 (0.0-2.8)
Ipratropium/ Fenoterol	1.3 (0.1-2.9)	0.4 (0.0-1.7)	0.7 (0.0-2.2)
Combined Data of Asthmatics and Healthy Subjects n = 26			
Placebo	10.0 (8.1-11.9)	2.1 (1.1-3.2)	2.6 (1.5-3.7)
Oxitropium	5.1 (3.7-6.6)	1.3 (0.5-2.3)	1.1 (0.3-2.0)
Ipratropium	5.2 (3.8-6.7)	1.4 (0.6-2.5)	1.5 (0.6-2.5)
Ipratropium/ Fenoterol	3.2 (2.1-4.5)	1.1 (0.3-2.0)	0.8 (0.0-1.7)

FIGURE 4.18

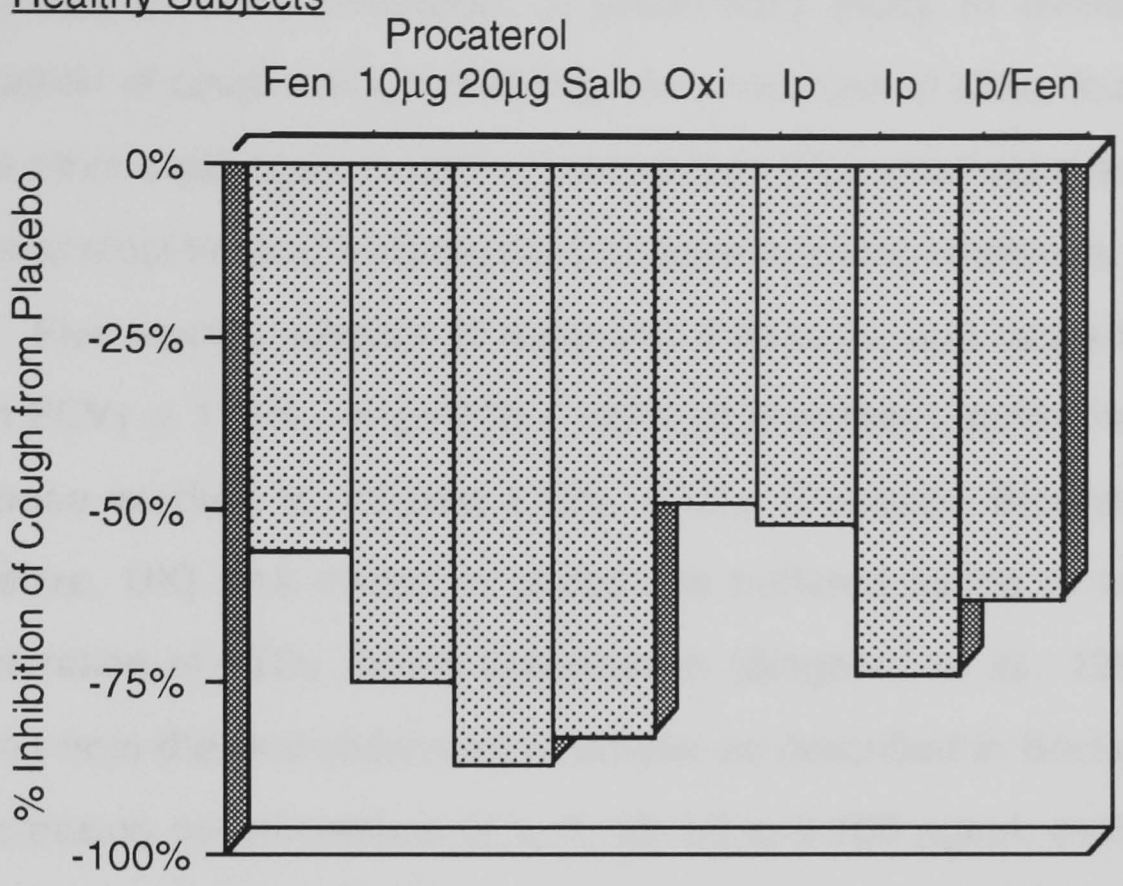
The Antitussive Effect of Anticholinergics in Asthmatics

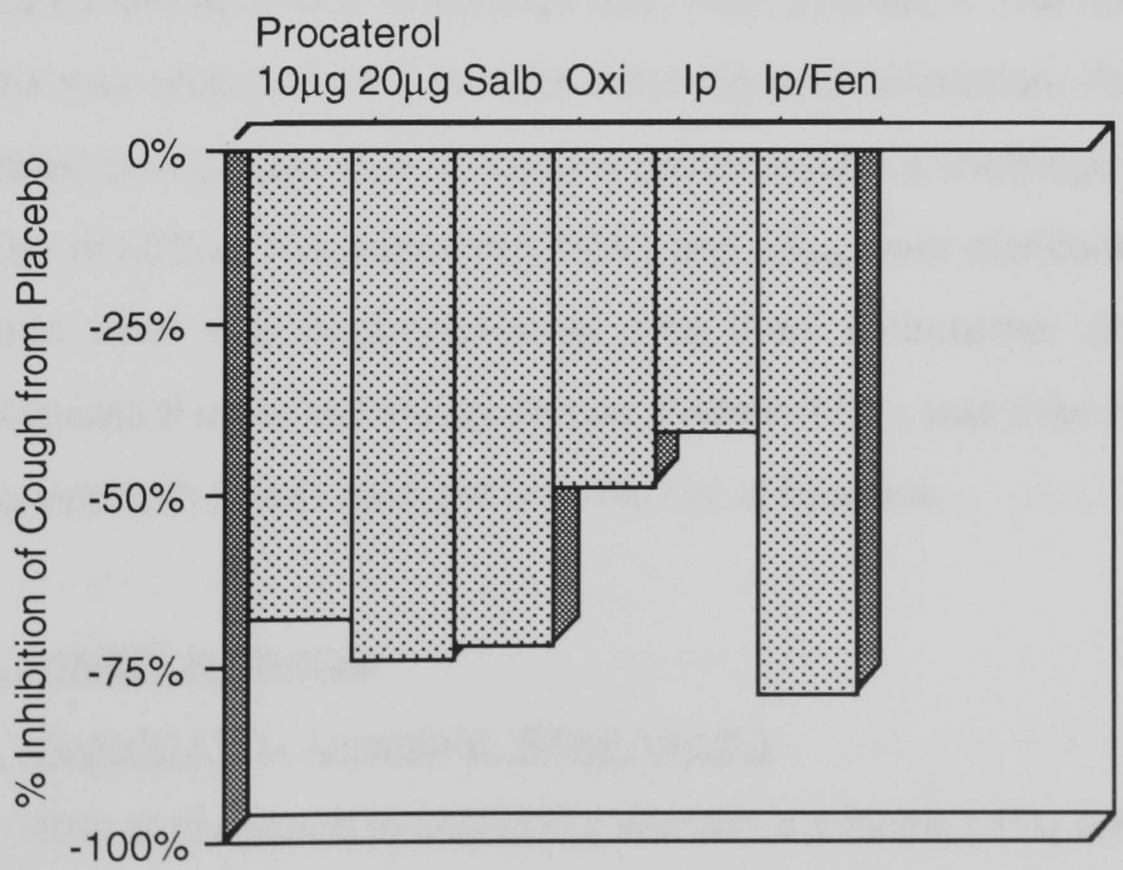


A summary of the antitussive efficacy of all the inhaled bronchodilators studied in this Chapter in healthy and asthmatic volunteers is presented in Figure 4.19.

FIGURE 4.19

The Antitussive Effects of Inhaled Bronchodilators





4.5 BRONCHOCONSTRICTION

4.5.1 Cough and Bronchoconstrictor Responses to Leukotriene D_4

This study aimed to determine the cough and bronchoconstrictor responses to LTD_4 inhalation. A preliminary study to evaluate the production of cough using increasing concentrations of LTD_4 found that, unlike other challenges, coughing started after inhalation had ceased and occurred most frequently during the 5 minutes following challenge.

Five healthy subjects (1 male and 4 females, age range 27 - 60, mean FEV_1 = 116%, range 108 - 127% of predicted), performed dose response studies to inhaled LTD_4. LTD_4 (Cascade Biochem Ltd., Berkshire, UK) was diluted in phosphate buffered saline to minimise deterioration of LTD_4 during nebulization (Bisgaard *et al.*, 1987) and inhaled from the Bronchoscreen dosimeter as described in Section 2.3.2 at increasing concentrations of 1, 5, 10, 50 and 100 μg/ml, each for 15 doses and at approximately 10 minute intervals. FEV_1 and sG_{aw} were measured before, 5 and 10 minutes after each inhalation. The number of coughs was recorded for 5 minutes following each inhalation. The study continued until greater than 10 coughs occurred after a challenge or a fall in FEV_1 of 20% or more occurred. FEV_1 and sG_{aw} were monitored for 15 minutes after the final inhalation and then salbutamol (200 μg) administered if bronchoconstriction was evident. FEV_1 was then recorded at intervals until values returned to within 5% of baseline.

4.5.2 Inhibition Studies

(a) Specific LTD_4 Antagonist, SK&F 104353

This study aimed to determine whether a specific LTD_4 antagonist inhibited both the cough and bronchoconstrictor responses to LTD_4 inhalation. Six healthy volunteers (1 male and 5 females, age range 18 - 25, mean FEV_1 = 113%, range 100 - 122% of predicted) completed this

study. Each subject was studied on 4 occasions 1 week apart. On visit 1, a baseline dose response study was performed to determine a dose for each subject causing greater than 10 coughs during the 5 minutes post challenge. LTD_4 (Miles Laboratories, Slough, UK) (3 x 100 µg ampoules) was delivered on dry ice on the morning of the challenge, diluted in 3 ml phosphate buffered saline and used immediately. All 6 subjects were completed within 3 hours and were tested in the same order each week to minimise the effect of any deterioration of LTD_4 with nebulization. A 2 x 10^{-4} mol/l (100 µg/ml) solution of LTD_4 was inhaled from the Bronchoscreen with increasing numbers of active breaths over the minute challenge. The challenge was stopped when greater than 10 coughs occurred during the 5 minutes post challenge. Coughs were recorded using a tie microphone connected to a tape recorder. FEV_1 was recorded pre challenge and 0, 5 and 10 minutes post challenge. R_{aw} was recorded using the Bronchoscreen as the average of the final 5 measurements of 1 minute air breathing pre-, during and 5 minutes post- challenge.

On visits 2 to 4, SK&F 104343 50 µg, 400 µg or matched placebo (diluent alone) was inhaled from the Bronchoscreen in random order determined by two Latin squares of order 3, balanced for 1st order residual effects, 20 minutes prior to LTD_4 cough challenge. FEV_1 was recorded in duplicate pre, immediately and 5 minutes post treatment and 5 min post LTD_4 challenge. R_{aw} was measured pre-, during and 5 min post-treatment and during and 5 min post LTD_4 challenge.

(b) Non-Specific Inhibition with Salbutamol

This study aimed to determine whether a non-specific bronchodilator prevented both the cough and bronchoconstrictor response to LTD_4 inhalation. Five healthy subjects (1 male and 4 females, age range 18 - 30 yrs, mean FEV_1 = 108%, range 95 - 120% of predicted), who had previously performed a dose response study to inhaled LTD_4

completed this two day study. After baseline measurements of FEV_1 and sG_{aw}, treatment with salbutamol mdi 200 μg (2 x 100 μg/puff) or matched placebo (2 puffs) was administered in random order and double-blind. 15 minutes later, FEV_1 and sG_{aw} were repeated followed by a 1 minute challenge with LTD_4 delivered from the Bronchoscreen at a dose causing greater than 10 coughs in the 5 minutes following challenge. Cough frequency was recorded for the following 5 minutes. FEV_1 and sG_{aw} were recorded 5 and 15 minutes post LTD_4 challenge. Salbutamol (200 μg) was then administered if bronchoconstriction was evident and FEV_1 monitored until baseline values had returned.

4.6 RESULTS AND STATISTICAL ANALYSIS

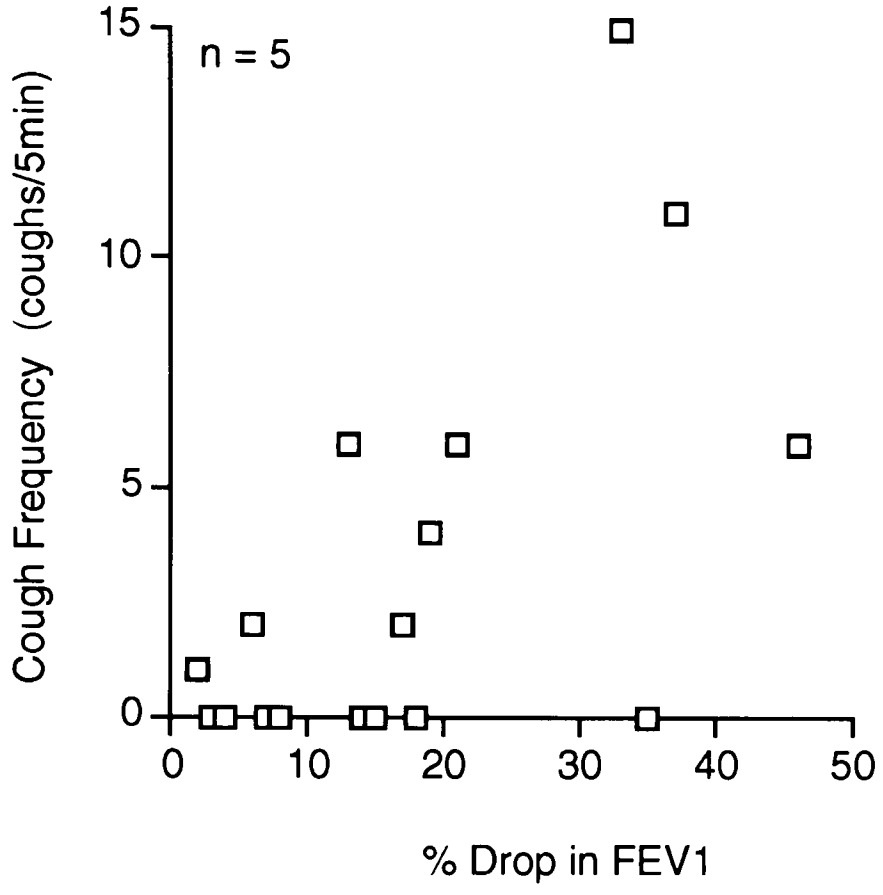
The raw data and ANOVA tables for the following studies are presented in Appendix 2.

4.6.1 Cough and Bronchoconstrictor Responses to Leukotriene D_4

LTD_4 did not cause cough during the minute inhalations. Cough occurred only at doses of LTD_4 that caused marked bronchoconstriction and occurred with most frequency during the 5 minutes following inhalation in line with the time course of bronchoconstriction. Cough and bronchoconstriction followed a dose-dependent manner. The association between cough and bronchoconstriction as measured by a fall in FEV_1 is presented in Figure 4.20.

FIGURE 4.20

The Cough and Bronchoconstrictor Responses to LTD4



4.6.2 Inhibition Studies

(a) Specific LTD_4 Antagonist, SK&F 104353

ANOVA was performed on square-root transformed cough frequencies and log transformed values of FEV_1 and R_{aw}. SK&F 104353 50 and 400 µg did not alter FEV_1 or cause cough but inhibited LTD_4-induced cough ($p<0.05$) and bronchoconstriction ($p<0.05$) in a dose dependent manner The mean % fall in FEV_1 after LTD_4 was 20%, 16% and 6% for placebo, 50 and 400 µg SK&F 104353 respectively. The inhibition of cough correlated with the inhibition of bronchoconstriction ($r = 0.65$; $p<0.02$). The back-transformed mean cough frequencies, FEV_1 and R_{aw} values are presented in Table 4.6 and Figures 4.21, 4.22 and 4.23

respectively. The association between inhibition of cough and inhibition of bronchoconstriction is presented in Figure 4.24.

FIGURE 4.21

The Effect of SK&F 104353 on LTD4-Induced Cough

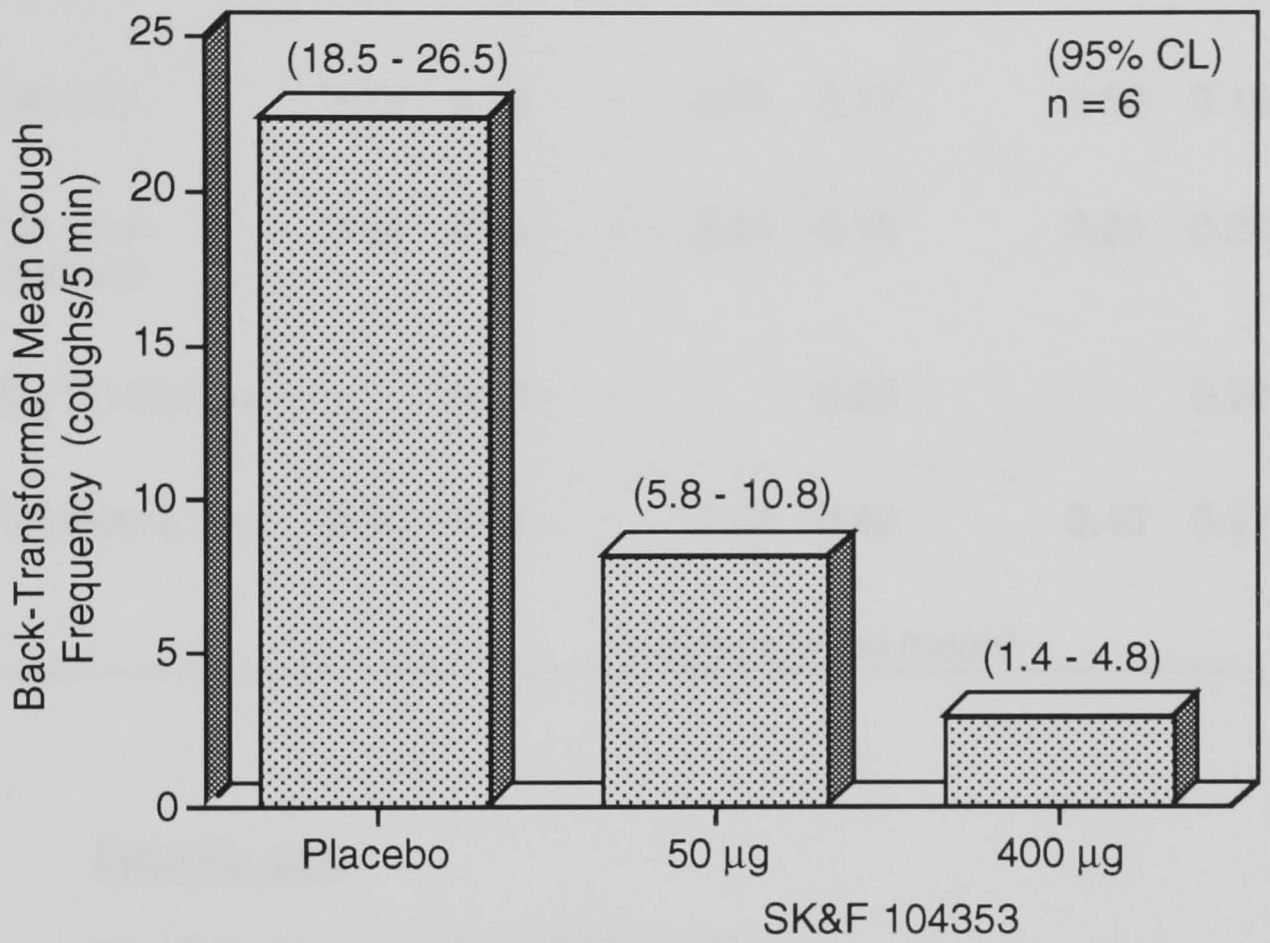


TABLE 4.6

The Effect of SK&F 104353 on FEV_1 and R_{aw}

			SK&F 104353			
	PLACEBO		50 µg		400 µg	
	FEV_1	R_{aw}	FEV_1	R_{aw}	FEV_1	R_{aw}
Baseline	3.67	0.27	3.65	0.23	3.64	0.20
Treatment	3.58	0.18	3.61	0.17	3.58	0.16
5 min Post-Treatment	3.64	0.20	3.61	0.18	3.61	0.20
LTD_4 Challenge		0.25		0.23		0.20
5 min Post LTD_4	2.90	0.54	3.02	0.42	3.40	0.27

(values are the mean)

FIGURE 4.22

The Effect of SK&F 104353 on FEV1

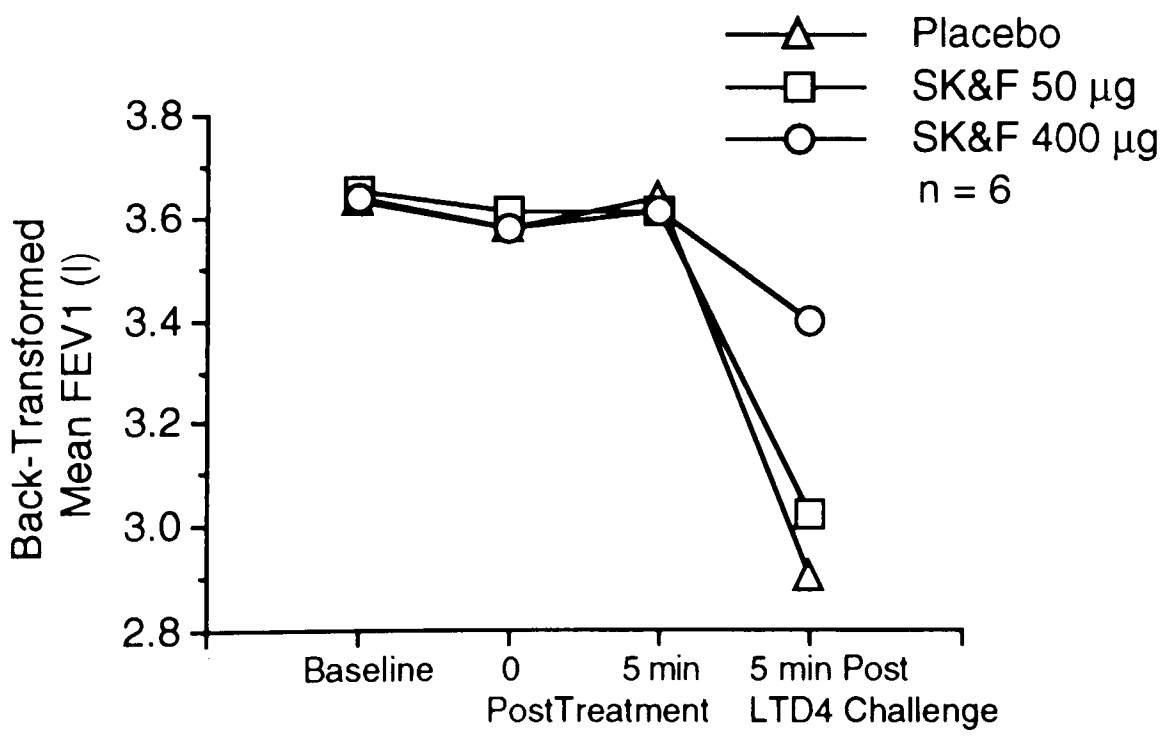


FIGURE 4.23

The Effect of SK&F 104353 on Raw

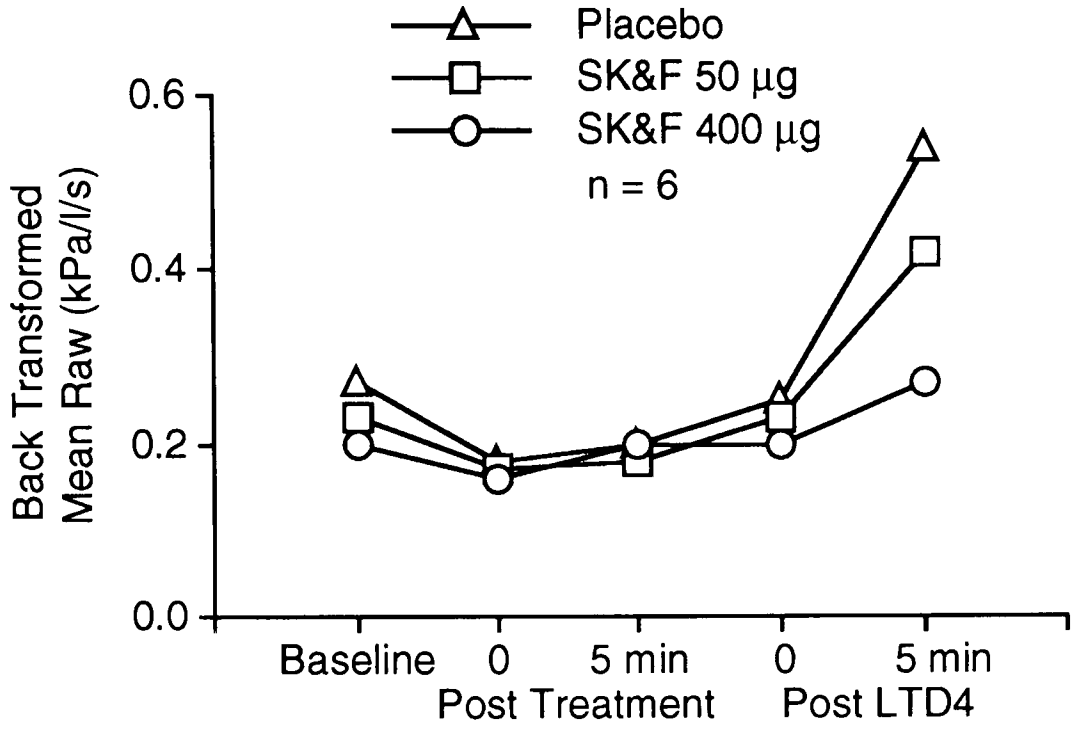
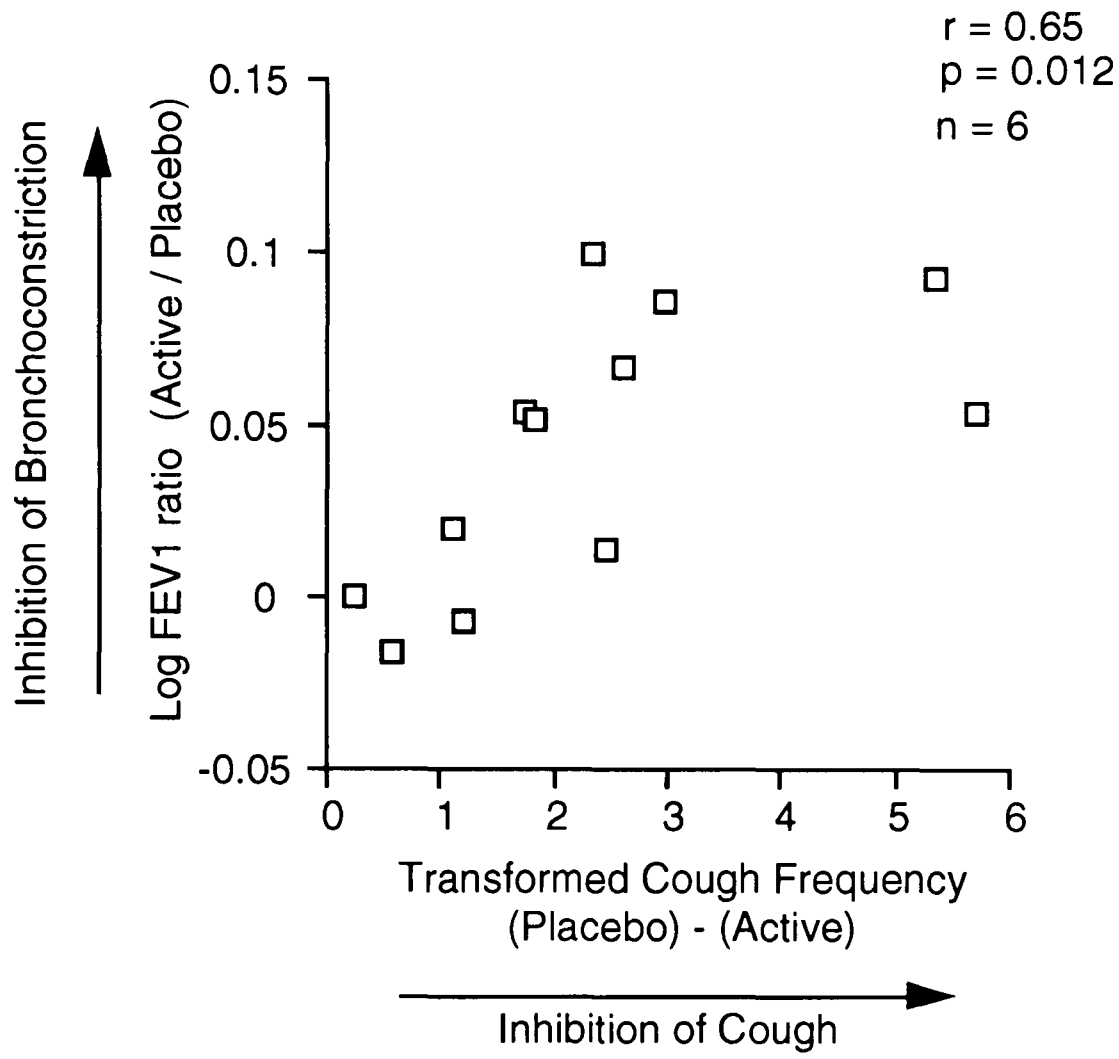


FIGURE 4.24

The Inhibition of Cough Associated with the Inhibition of Bronchoconstriction



(b) Non-Specific Inhibition with Salbutamol

ANOVA was performed on transformed data for cough frequency, FEV_1 and R_{aw}. This revealed that pre-treatment with salbutamol inhibited both the cough ($p<0.05$) and bronchoconstriction ($p<0.02$) induced by inhalation of LTD_4 (mean % fall in FEV_1 after LTD_4 = 15% and 2% for placebo and salbutamol respectively). Salbutamol caused a mean 2% increase in FEV_1 and 66% increase in sG_{aw} which was reversed by LTD_4 inhalation. The back-transformed mean cough frequencies and 95% confidence limits are presented in Figure 4.25 and the back-transformed

means for FEV_1 and sG_{aw} in Table 4.7 and Figures 4.26 and 4.27 respectively.

FIGURE 4.25

The Effect of Salbutamol on LTD4-Induced Cough

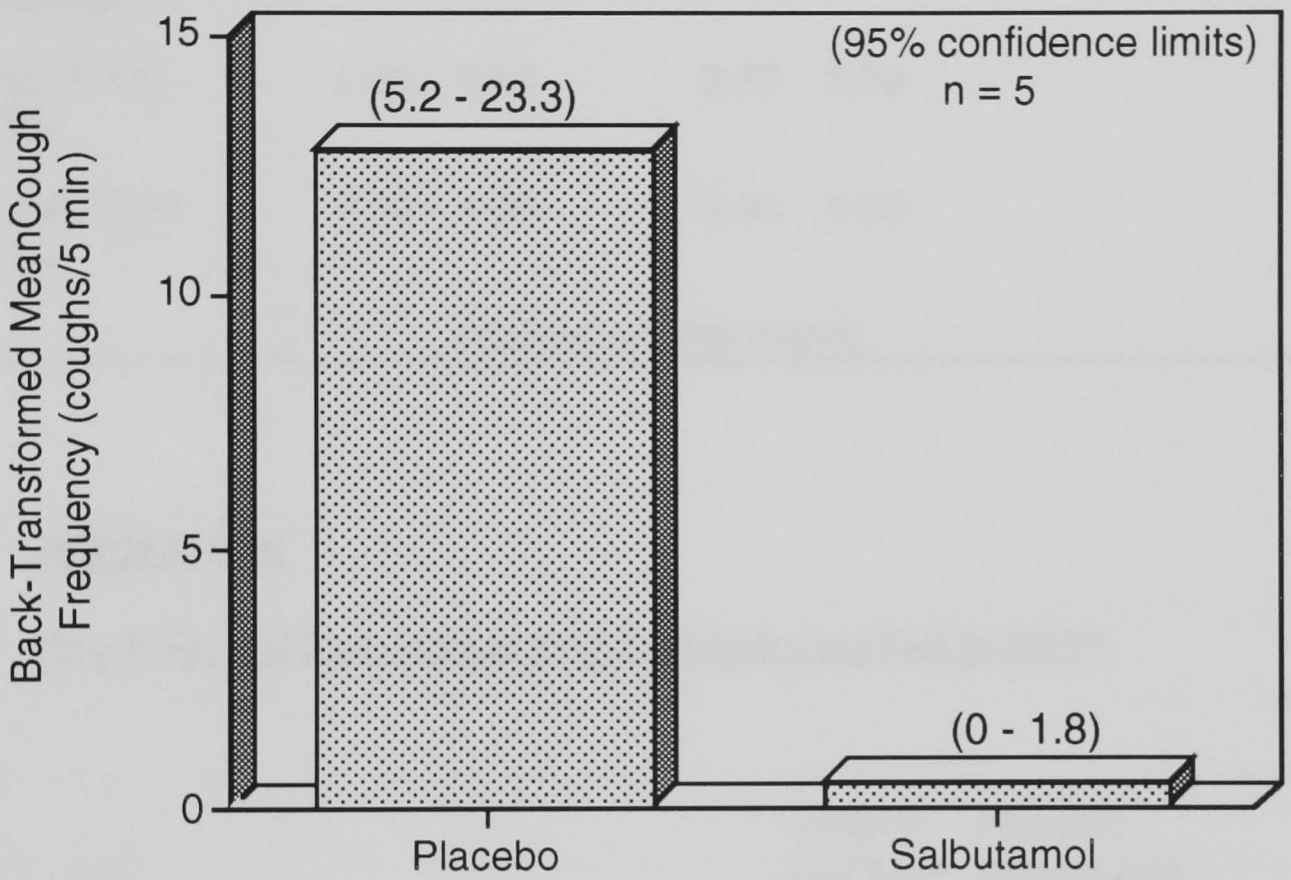


TABLE 4.7

Effect of Salbutamol on LTD_4-Induced Changes in FEV_1 and sG_{aw}

	PLACEBO		SALBUTAMOL	
	FEV_1	sG_{aw}	FEV_1	sG_{aw}
Baseline	3.36	2.24	3.38	2.14
15 min Post-Treatment	3.37	2.24	3.45	3.55
5 min Post-LTD_4	2.85	0.66	3.37	2.29
15 min Post-LTD_4	3.02	1.00	3.33	2.88

(Values are the mean)

FIGURE 4.26

The Effect of Salbutamol on LTD4-Induced Fall in FEV1

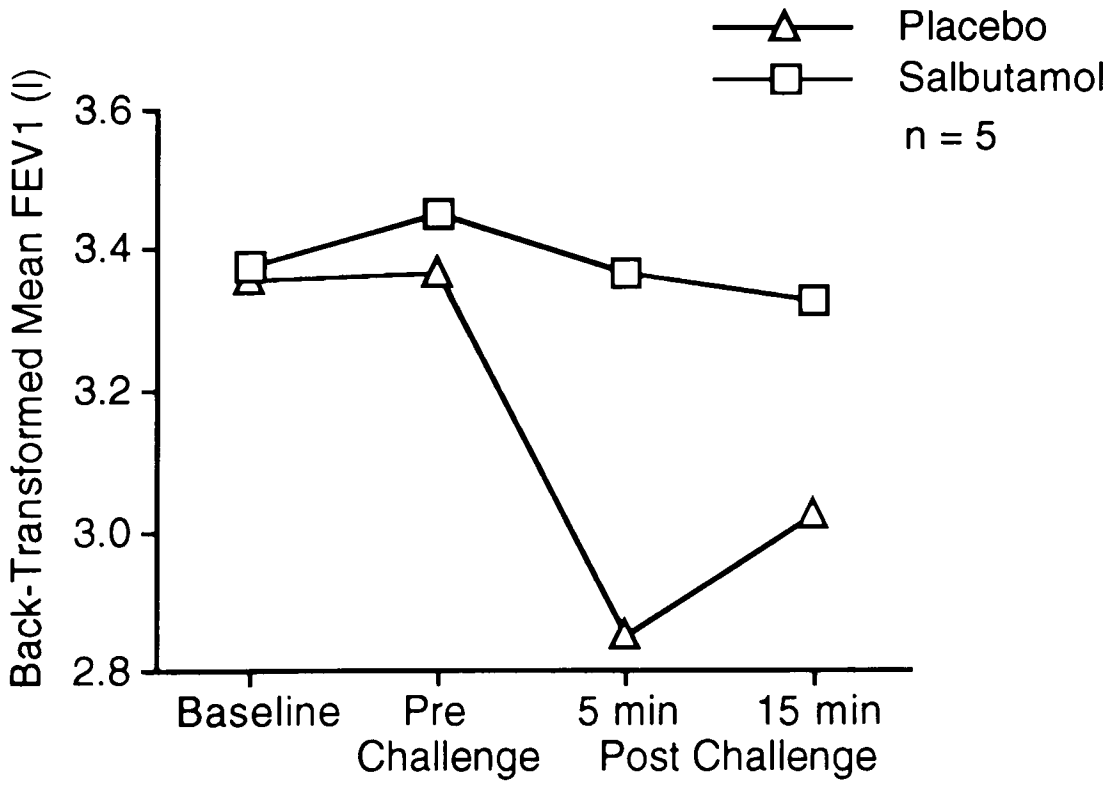
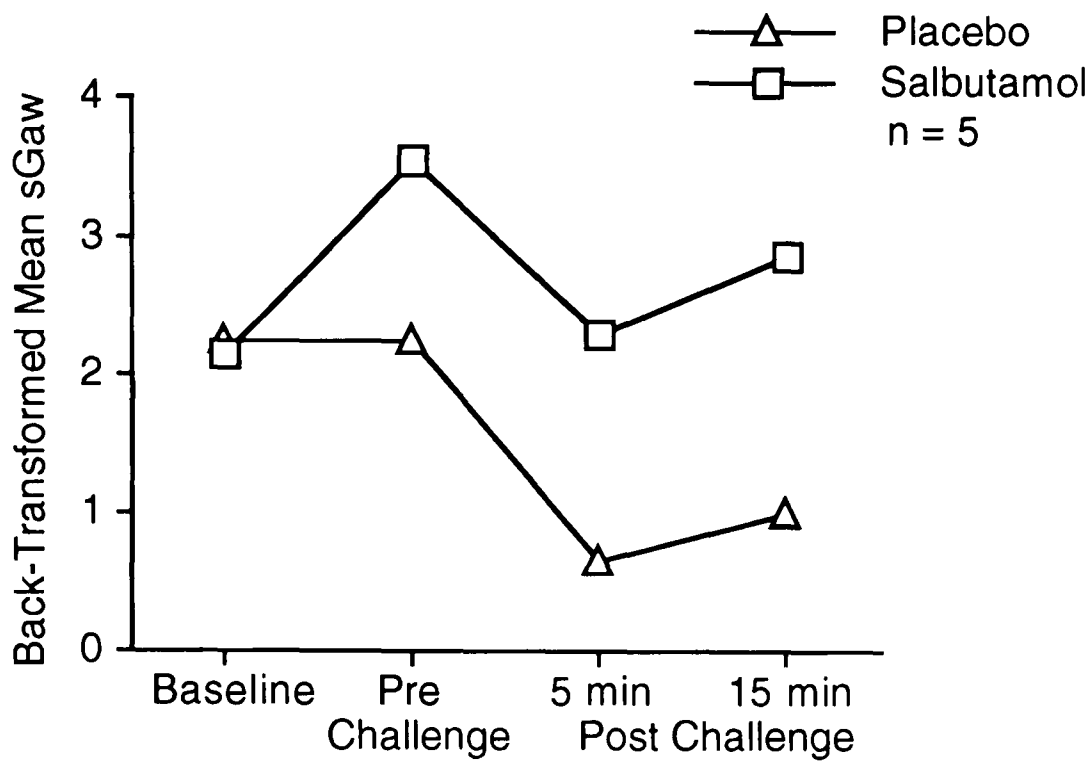


FIGURE 4.27

The Effect of Salbutamol on LTD4-Induced Fall in sGaw



4.7 DISCUSSION

UNDW induces cough but not bronchoconstriction in healthy subjects. However, the studies described in this chapter show convincingly that inhaled bronchodilators inhibit this UNDW-induced cough. Coughing was reduced by both beta-adrenergic agonists and anticholinergics in healthy and asthmatic subjects by between 41 - 87% compared with placebo. Investigation of the association between alteration in airway tone and inhibition of cough (Section 4.3.3) found that a very small bronchodilation occurred in conjunction with the inhibition of cough suggesting an association between the two variables. However, the later studies of procaterol and salbutamol which employed large numbers of subjects failed to detect a correlation between this bronchodilation, as measured by an increase in FEV_1, and the inhibition of cough. In addition, although oral salbutamol induced a similar increase in FEV_1 as the inhaled preparation (both mean of 3%), their effects on UNDW-induced cough

were very different. Oral salbutamol resulted in a mean 16% reduction in cough compared to placebo while inhaled salbutamol resulted in an 83% reduction. Furthermore, the asthmatic subjects studied (Section 4.4.4), despite having a greater bronchodilator response than the healthy subjects to the drugs tested, did not exhibit a greater antitussive response.

The observation that non-asthmatics develop bronchial hyperresponsiveness and an increased cough response to citric acid during URTI (Empey *et al.*, 1976) suggests that asthmatics may be more sensitive to tussive stimuli. However, the asthmatics studied in this Chapter, although they were mild asthmatics without significant airways obstruction, did not generally appear to exhibit a lower threshold for cough in response to 'low chloride' aerosols than the healthy subjects. This is in agreement with other workers who found that citric acid (Pounsford *et al.*, 1985), tartaric acid (Fujimura *et al.*, 1992 a) and capsaicin (Collier & Fuller, 1984) cough thresholds were similar in asthmatic and non-asthmatic subjects. Furthermore, no correlation could be found between tartaric acid cough threshold and the concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) (Fujimura *et al.*, 1992 a). Therefore cough can be induced independently of bronchoconstriction and is not affected by bronchial hyperreactivity.

These results suggest that small changes in airway tone are not associated with the inhibition of cough. Therefore, the antitussive action of bronchodilators on UNDW-induced cough appears to result from a topical effect on airway epithelium, separate from their action on airway smooth muscle. This is supported by the greater efficacy of bronchodilators given by inhalation rather than orally.

UNDW-induced cough appears to be mediated by stimulation of RARs located within the paracellular spaces of the laryngeal and tracheobronchial epithelium by the reduced concentration of chloride in

ASL. One mechanism of inhibiting cough would be to reduce the access of ASL to the RARs. Cyclic AMP appears to control the paracellular spaces of 'leaky' epithelium (Moreno *et al.*, 1986). Beta-adrenergic agonists increase intraepithelial levels of cyclic AMP (Al-Bazzaz, 1981) and may reduce the volume of the paracellular spaces (Rose & Loewenstein, 1975) by an action on epithelial beta-adrenoceptors (Morrison *et al.*, 1993). Similarly, cholinergic drugs increase permeability of the gastric mucosa (Shoemaker *et al.*, 1970) suggesting that anticholinergics may decrease permeability. Therefore, topical bronchodilators may inhibit UNDW-induced cough by decreasing the permeability of the paracellular spaces and thereby reducing access of the 'low chloride' ASL to the RARs.

Another mechanism of action of bronchodilators is their effect on epithelial ion transport. However, whilst beta-adrenergic agonists are thought to increase passive transepithelial chloride secretion towards the lumen by increasing intracellular cAMP in both animal and human airways (Al-Bazzaz & Cheng, 1979; Davis *et al.*, 1979; Knowles *et al.*, 1982) which could dilute the effect of 'low chloride' aerosols on the ASL, anticholinergics appear to have no effect on basal secretory rates (Joris *et al.*, 1993), but rather prevent increases that occur during vagal reflex stimulation (Marin *et al.*, 1976; German *et al.*, 1980). Beta-adrenergic agonists also increase mucus secretion from epithelial glands and increase mucociliary clearance (Foster *et al.*, 1976; Phipps *et al.*, 1982) but anticholinergics are thought to have little effect (Foster *et al.*, 1976).

Another mechanism of inhibition of UNDW-induced cough would be a direct action on RARs but so far, only the existence of $beta_2$ -adrenoceptors on vagal efferent terminals in humans has been postulated (Aizawa *et al.*, 1991).

In contrast to the inhibition of UNDW-induced cough by bronchodilators described in this Chapter, other investigators have found

no effect using a similar anticholinergic to ipratropium, atropine (Sheppard *et al.*, 1983; Fuller & Collier, 1984). Other studies of the antitussive effects of bronchodilators on cough induced by other stimuli have produced variable results. Nebulized bronchodilators (salbutamol and ipratropium) decreased citric acid-induced cough in asthmatics but not healthy subjects (Pounsford *et al.*, 1985) although others have found that inhaled (Karttunen *et al.*, 1987) but not oral (Belcher & Rees, 1986) salbutamol is effective in healthy subjects. Bronchodilators also appear to be ineffective against capsaicin-induced cough (Nichol *et al.*, 1990 a; Smith *et al.*, 1991; Fujimura *et al.*, 1992 b). This suggests that coughing in response to these stimuli are mediated by different mechanisms than UNDW-induced cough.

The ability of bronchoconstriction to promote cough was studied using inhaled LTD_4 (See Section 4.6.1). LTD_4 is not irritant during inhalation, unlike UNDW which causes cough immediately on inhalation and ceases on removing the stimulus. However, coughing occurred most frequently during the 5 minutes following inhalation coinciding with the onset of bronchoconstriction which was also most pronounced within 5 minutes of inhalation. However, cough only occurred with LTD_4 at high doses which caused marked bronchoconstriction associated with a 20% or more fall in FEV_1. Inhibition of bronchoconstriction either by the specific LTD_4 antagonist, SK&F 104353, or with salbutamol, which inhibits bronchoconstriction regardless of the stimulus, also inhibited cough. The degree of inhibition of cough correlated with the degree of inhibition of bronchoconstriction. These results suggest that LTD_4 does not cause cough directly but rather, indirectly through marked bronchoconstriction. LTD_4-induced bronchoconstriction is thought to result from direct stimulation of airway smooth muscle rather than via a vagal reflex or from release of other inflammatory mediators (Smith *et al.*, 1987; Ayala *et al.*, 1988) The anaesthetic, lidocaine does not inhibit the bronchoconstrictor

response to LTD_4 suggesting that RARs do not mediate the response (Smith *et al.*, 1987) and LTD_4 does not directly stimulate RARs in cats and dogs (Holroyde & Jackson, 1983). This suggests that RAR stimulation by LTD_4 leading to cough, is indirect and a result of bronchoconstriction. Similarly, standard bronchial challenge testing in asthmatics with histamine is only associated with coughing at concentrations causing a similar degree of bronchoconstriction (Chausow & Banner, 1983). Bronchoconstriction may stimulate RARs (Widdicombe & Sterling, 1970) and sensitise SARs (Widdicombe, 1961) which could lead to cough by altering the threshold of the medullary 'cough' neurones (Hanacek *et al.*, 1984; Sant'Ambrogio *et al.*, 1984). However, participation of C-fibres in these responses cannot be excluded.

In summary, coughing may be induced either by direct stimulation of RARs or indirectly through marked bronchoconstriction which could stimulate RARs as a result of the physical changes in airway tone. Cough associated with bronchoconstriction may explain the apparent efficacy of bronchodilators in treating chronic cough in asthmatics (Ellul-Micallef, 1983). Small changes in airway tone are not responsible for cough production or inhibition. Rather, the inhibitory action of bronchodilators on UNDW-induced cough appears to result from a mechanism separate from their action on airway smooth muscle. The role of bronchodilators in treating pathological cough in the absence of marked bronchoconstriction is unclear.

CHAPTER 5: ANTITUSSIVE STUDIES

5.1 INTRODUCTION

This chapter describes studies of UNDW-induced cough with a variety of centrally and peripherally acting drugs with possible antitussive properties. Opiates are the most commonly prescribed group of antitussives in the UK and in particular, codeine has been used for many years as a positive control with which to test other antitussives. This was based on an extensive review by Eddy *et al.* (1969), and early studies of cough, many of which were performed on animal models. With the advent of experimentally induced cough with citric acid aerosols in humans, the effectiveness of many opiates, including codeine, noscapine and dextromethorphan was confirmed (Bickerman *et al.*, 1957). Opiates are thought to inhibit the central integration of the cough reflex (Eddy *et al.*, 1969) and would therefore be expected to inhibit cough regardless of the stimulus or afferent pathway. However, the clinical efficacy of opiates as antitussives has been questioned by their lack of effect on cough associated with chronic obstructive airways disease (Edwards *et al.*, 1977) and upper respiratory tract infection (Eccles *et al.*, 1992) and their potency is questioned in the British National Formulary (1992).

Nedocromil sodium, however, has been reported to inhibit citric acid-induced cough in dogs (Jackson, 1988) possibly by a peripheral action on airway sensory nerves (Barnes, 1986) and may reduce cough severity in asthmatics (Grief *et al.*, 1989; Cherniack *et al.*, 1990). Nedocromil sodium is a non-steroidal prophylactic treatment for asthma having no bronchodilator action. It is an anti-inflammatory agent with actions in humans similar to sodium cromoglycate including stabilisation of mast cells (Leung *et al.*, 1988) and inhibition of reflex bronchoconstriction (Shaw & Kay, 1985).

Diuretics alter the ion transport mechanisms of epithelial cells which may be important in 'low chloride' stimulation of cough. Amiloride hydrochloride is a potassium sparing diuretic which acts on the apical surface of cells blocking sodium absorption (Benos, 1982) which stimulates chloride secretion (Knowles *et al.*, 1984). The loop diuretic frusemide acts on the basolateral surface (Knowles *et al.*, 1984) inhibiting sodium, potassium and chloride co-transport and inhibits chloride secretion in dog tracheal epithelium (Widdicombe *et al.*, 1983). Amiloride and frusemide inhibit the fall in nasal potential difference (a measure of epithelial resistance) induced by UNDW inhaled intranasally (Wood *et al.*, 1989) and frusemide is reported to inhibit exercise and UNDW-induced bronchoconstriction (Robuschi *et al.*, 1989; Bianco *et al,* 1988).

5.2 METHODS

5.2.1 Opiates

This study examined the antitussive efficacy of the commonly prescribed opiate, codeine phosphate, with another opiate, noscapine hydrochloride (Loder, 1969), administered in therapeutic doses, compared with placebo on cough induced by UNDW and ultrasonically nebulized citric acid (UNCA). Citric acid challenge was included to allow comparison with previous published reports.

Twenty-four healthy volunteers completed the study (8 males and 16 females, age range 20-40 yrs, of whom 5 were smokers). All coughed in response to UNDW and an isotonic mixture of 0.68% citric acid in saline and all had normal spirometric values (mean FEV_1 = 112% of predicted) prior to study.

Subjects attended the laboratory on 12 occasions; 3 consecutive days per week for 4 weeks. Treatments were administered on separate weeks, the order of administration randomised using 6 Latin squares of

order 4 balanced for residual (carryover) effects. Placebo was given on day 1 of each week to minimise learning effects on cough frequency. Data from this open placebo day were not included in the analysis. On days 2 and 3 of each week, subjects were given either noscapine hydrochloride 50 mg (1 x 50 mg noscapine + 2 placebo tablets), noscapine 150 mg (3 x 50 mg noscapine tablets), codeine phosphate 60mg (1 x 60mg codeine + 2 placebo tablets) or placebo (3 x placebo tablets).

Ninety minutes after treatment, a cough challenge was performed to either UNDW or UNCA. These were inhaled for 1 minute periods during which cough frequency was recorded. Distilled water was always given on the placebo control day of each week. Twelve subjects were challenged with UNDW on days 2 and UNCA on days 3 while the remaining 12 subjects were challenged in the reverse order.

5.2.2 Nedocromil Sodium

The aims of this study were to compare the antitussive properties of inhaled nedocromil sodium with the beta-adrenergic agonist, fenoterol hydrobromide, and placebo on cough induced by UNDW, UNCA and capsaicin, the hot extract of red pepper, which may induce cough by stimulation of C-fibres (Collier & Fuller, 1984). The purpose of using the 3 challenges was to determine whether antitussive efficacy was stimulus-dependent and therefore to elucidate the afferent pathways involved and the mechanisms of drug action.

Eighteen healthy volunteers (7 males and 11 females, age range 19 - 31 yrs, mean FEV_1 = 113% of predicted, range 88 - 145%) completed this 4 day study. On visit 1, baseline cough challenges were performed to familiarise subjects with the procedure and to determine a dose of capsaicin causing greater than 10 coughs during a 1 minute inhalation. Capsaicin (Sigma Chemicals Ltd., Poole, Dorset, UK) was dissolved in

ethanol before dilution in isotonic saline and delivered by jet nebulizer (Section 2.3.1) in increasing concentrations from 1 to 5 μmol/l. On visits 2 to 4, treatment by mdi with nedocromil sodium 4 mg (2 x 2 mg/puff), fenoterol hydrobromide 0.36 mg (2 x 0.18 mg/puff) or placebo (2 puffs) was administered in random order determined by 6 Latin squares of order 3, 30 minutes before cough challenges. UNDW, UNCA (0.68% citric acid in 0.79% saline) and capsaicin were delivered for 1 minute periods and at 5 minute intervals respectively. Adaptation of cough during challenges was also assessed by recording the number of coughs over the 3 consecutive 20 second periods of each challenge. FEV_1 was recorded post-treatment.

5.2.3 Diuretics

Inhalation of UNDW results in both cough and a dose-dependent bronchoconstriction in asthmatics (Chadha *et al.*, 1984). Similar to other standard bronchial reactivity tests, for example, using histamine or methacholine, the severity of asthma is related to the duration of exposure to UNDW inhalation; the more severe the asthma, the lower the exposure to UNDW required to produce a 20% fall in FEV_1. The aims of this study were to determine whether amiloride and frusemide, administered at doses which inhibit the UNDW-induced fall in nasal potential difference, inhibit both the cough and bronchoconstrictor responses to inhaled UNDW in asthmatics.

Eight volunteers from hospital staff with stable bronchial asthma requiring daily bronchodilator therapy (2 males and 6 females, age range 18 -34 yrs, mean FEV_1 = 98%, range 77 - 118% of predicted) completed this 4 day study. None were taking oral steroids and the previous 3 months had been free of acute exacerbations of their asthma. All bronchodilators and inhaled corticosteroids were withheld for 12 hours prior to each study day. On visit 1, a baseline UNDW challenge was

performed. This consisted of inhaling increasing amounts of UNDW for 30, 60, 90 and thereafter 120 seconds until a 20% or more fall in FEV_1 was recorded (Chadha *et al.*, 1984). FEV_1 was recorded prior to challenge and then 30 and 90 seconds after each inhalation. The total duration of exposure to inhalation required to cause a 20% fall in FEV_1 was recorded as the PD_{20}. Cough frequency during the first 30 second inhalation was recorded for analysis as adaptation occurred to subsequent inhalations. On visits 2 to 4, at the same time of day, treatment with amiloride hydrochloride 16 ml (2×10^{-4} mol/l diluted in isotonic saline), frusemide 16 ml (9×10^{-3} mol/l diluted in isotonic saline) or placebo 16 ml (isotonic saline) was inhaled from the ultrasonic nebulizer in random order, 5 minutes prior to PD_{20} UNDW challenge. FEV_1 was recorded pre- and post- treatment and post UNDW as described above. Again, cough frequency was recorded during the first 30 second UNDW inhalation.

5.3 RESULTS AND STATISTICAL ANALYSIS

The raw data and ANOVA tables relating to these experiments are presented in Appendix 3.

5.3.1 Opiates

After transformation, three-way analysis of variance was performed on all data, the factors being subjects, treatments and challenge. The resultant means and 95% confidence limits were then back-transformed. ANOVA revealed no significant reduction in cough frequency in response to either UNDW or UNCA with either doses of noscapine or codeine when compared with placebo ($p>0.05$). Back-transformed mean cough frequencies and 95% confidence limits are shown in Table 5.1 and presented graphically in Figure 5.1.

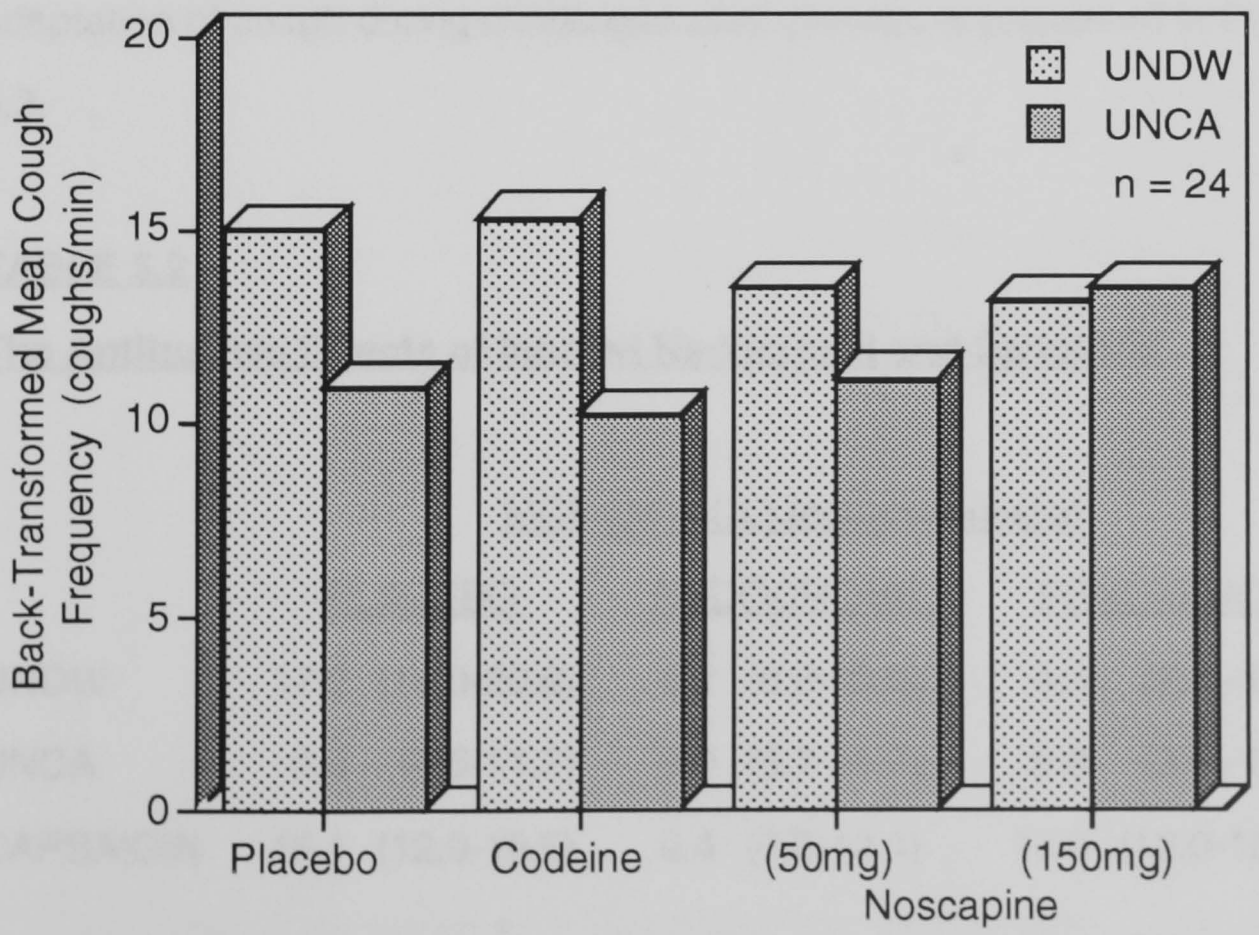
TABLE 5.1

The Effect of Opiates on UNDW- and UNCA-Induced Cough

	Back-transformed mean cough frequency (coughs/min) (95% confidence limits)	
	UNDW	UNCA
Placebo	15.0 (12.7-17.3)	10.9 (9.0-13.0)
Codeine	15.3 (13.0-17.7)	10.2 (8.3-12.2)
Noscapine (50mg)	13.5 (11.4-15.8)	11.1 (9.1-13.1)
Noscapine (150mg)	13.1 (11.1-15.4)	13.5 (11.4-15.8)

FIGURE 5.1

The Effect of Opiates on UNDW and UNCA-Induced Cough



5.3.2 Nedocromil Sodium

ANOVA was performed on transformed cough frequencies allowing analysis of the effects of subjects, visit, treatment, challenge and period of challenge.

Fenoterol hydrobromide inhibited UNDW-induced cough ($p<0.001$) confirming the previous observation (Section 4.4.1(a)), but had no effect on capsaicin-induced cough ($p>0.05$). A small reduction in cough induced by UNCA occurred but this did not reach statistical significance. However, it is possible that the prior administration of UNDW may have reduced the response to UNCA. Nedocromil sodium inhibited cough induced by UNDW ($p<0.001$) and capsaicin ($p<0.05$) but had no effect on UNCA-induced cough ($p>0.05$). Adaptation of cough occurred during all challenges irrespective of treatment ($p<0.001$). FEV_1 did not alter with any of the treatments (mean FEV_1 = 3.80, 3.84 and 3.74 after placebo, fenoterol and nedocromil respectively). The back-transformed mean cough frequencies and 95% confidence limits are presented in Table 5.2 and Figure 5.2. The adaptation of cough during challenges after placebo is presented in Figure 5.3.

TABLE 5.2

The Antitussive Effects of Inhaled Nedocromil and Fenoterol

	MCF (95% CL) (coughs/minute)		
	PLACEBO	NEDOCROMIL	FENOTEROL
UNDW	17.2 (14.0-20.8)	8.2 (6.0-10.8)	4.3 (2.6 - 6.3)
UNCA	9.0 (6.6-11.7)	9.0 (6.7-11.7)	5.8 (3.9 - 8.1)
CAPSAICIN	16.1 (12.9-19.5)	9.4 (7.0-12.1)	15.0 (12.0-18.4)

FIGURE 5.2

The Antitussive Effects of Nedocromil and Fenoterol

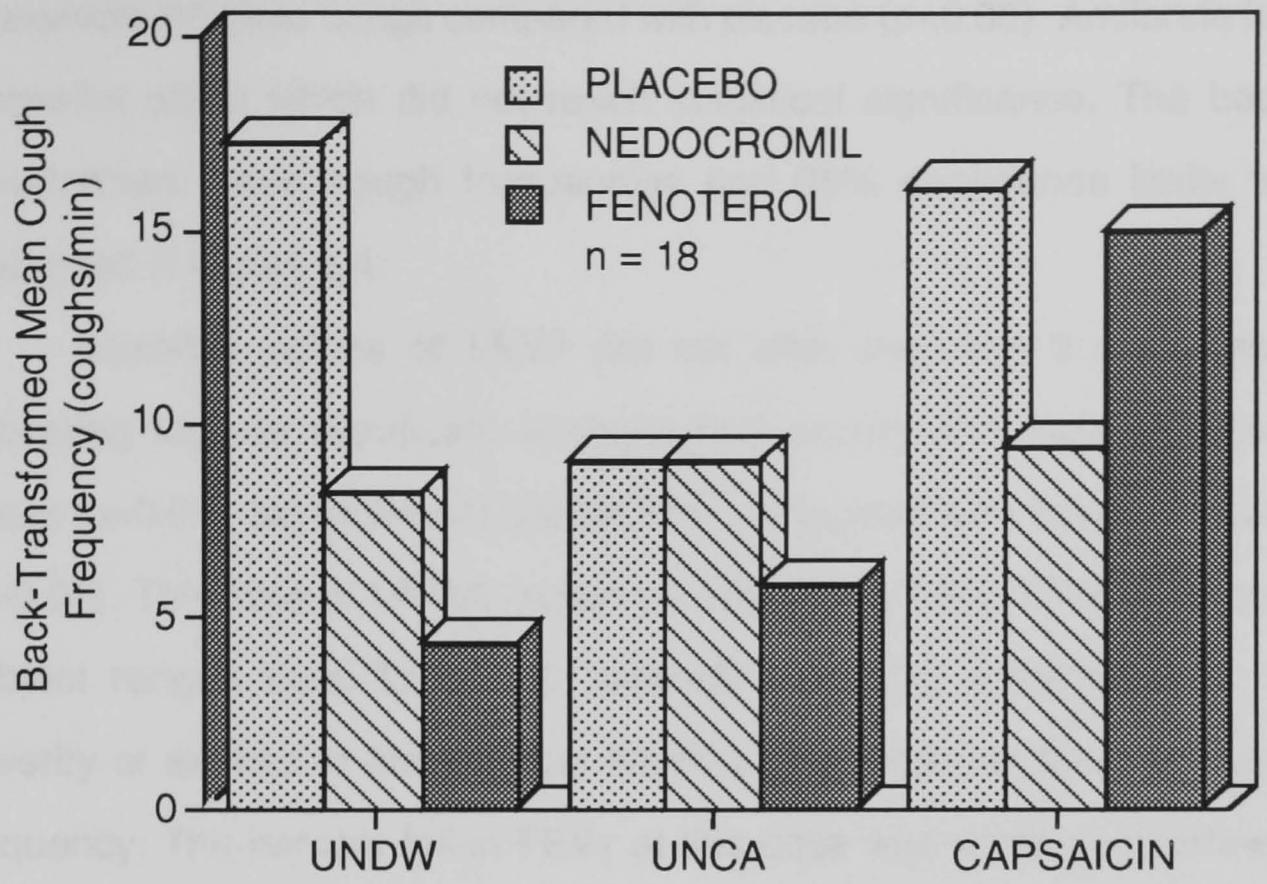
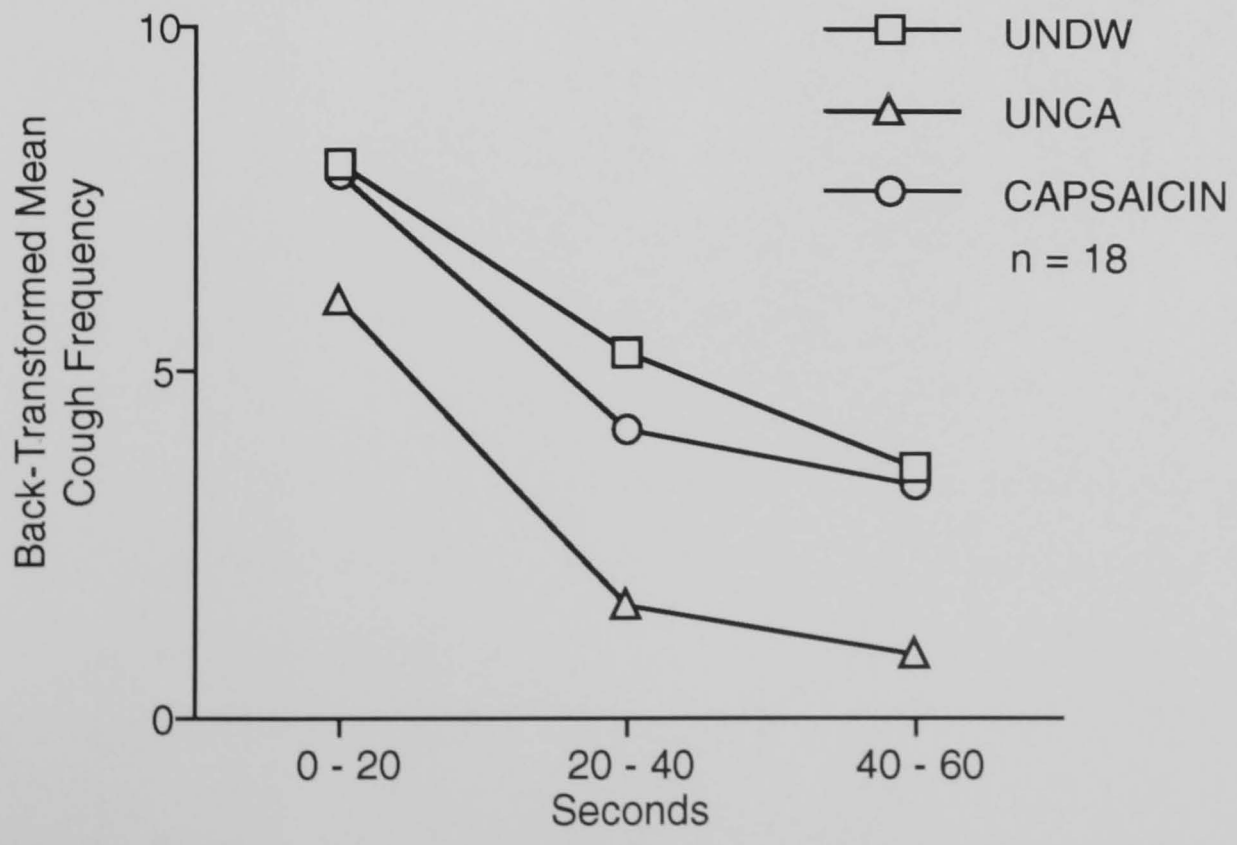


FIGURE 5.3

Adaptation of Cough During Challenge on Placebo



5.3.3 Diuretics

ANOVA was performed on transformed cough frequency data. Two asthmatics failed to cough during the first 30 second exposure to UNDW. Frusemide inhibited cough compared with placebo ($p<0.05$). Amiloride had a smaller effect which did not reach statistical significance. The back-transformed mean cough frequencies and 95% confidence limits are presented in Figure 5.4.

Baseline values of FEV1 did not alter over the 3 study days indicating that no significant changes had occurred in their asthmatic status ($p>0.05$). No treatment altered FEV_1 compared with baseline values ($p>0.05$). The dose of UNDW causing a 20% fall in FEV_1 (PD_{20}) for each subject ranged from 30 to 300 seconds indicating a variation in the severity of asthma in the subjects studied. This was unrelated to cough frequency. The percent fall in FEV_1 at this dose was compared between the 3 treatments. Both amiloride ($p<0.01$) and frusemide ($p<0.001$) inhibited UNDW-induced bronchoconstriction compared with placebo (mean % fall in FEV_1 (SE) = 24.0 (1.8), 14.6 (1.7) and 5.9 (1.8) % for placebo, amiloride and frusemide respectively). The mean values for FEV_1 are presented in Figure 5.5.

FIGURE 5.4

The Antitussive Effect of Amiloride and Frusemide on UNDW-Induced Cough in Asthmatics

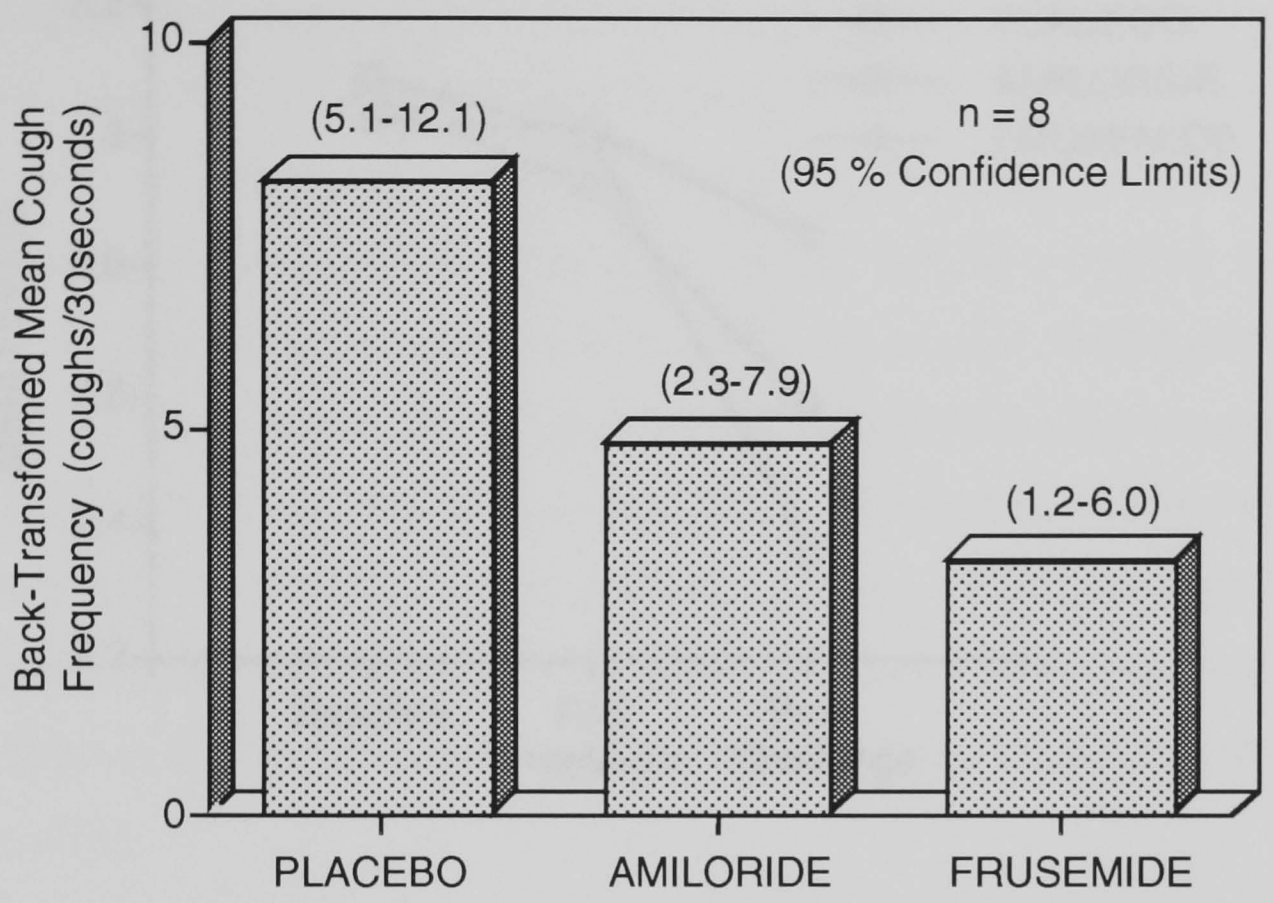
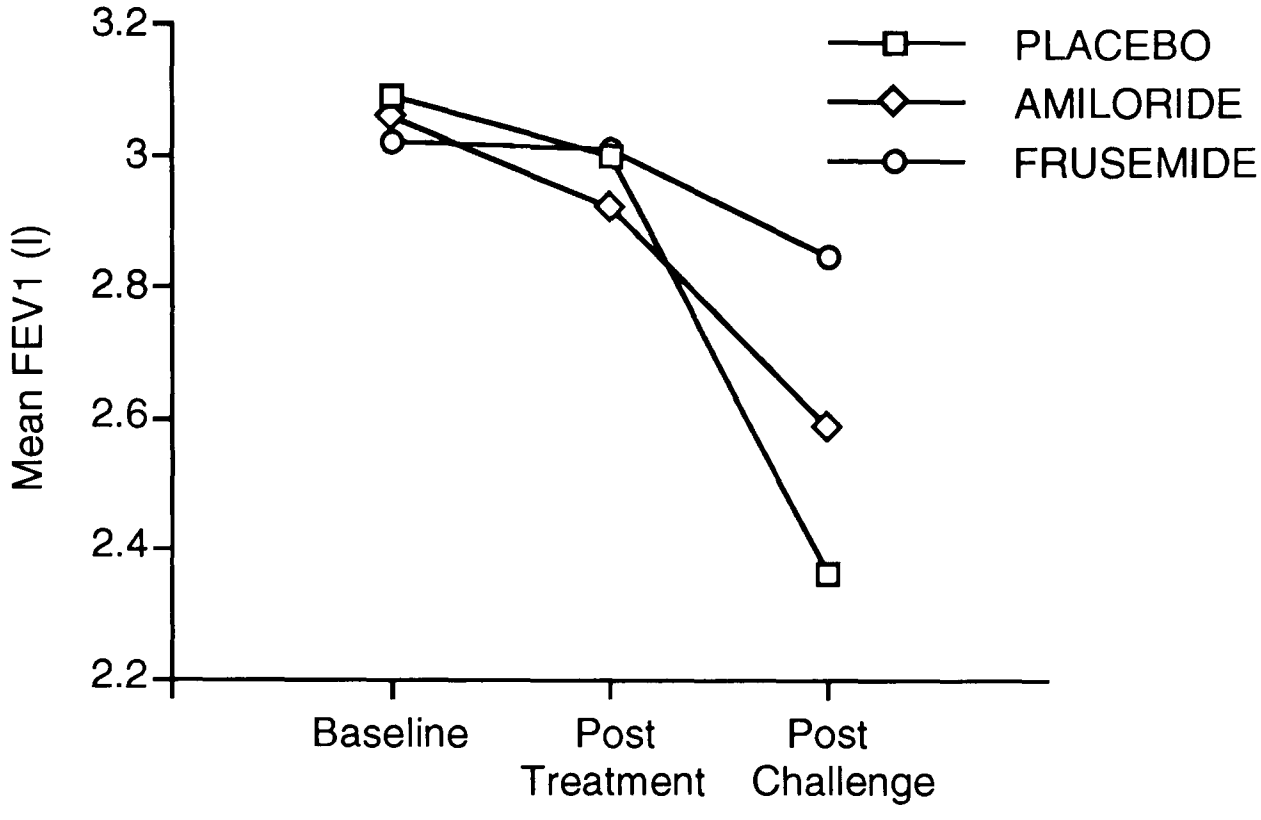


FIGURE 5.5

The Effect of Amiloride and Frusemide on UNDW-Induced Bronchoconstriction in Asthmatics



5.4 DISCUSSION

Using UNDW and UNCA aerosols to stimulate RARs, no apparent antitussive activity of the opiates, codeine and noscapine was found. Similar studies have found codeine (Rees & Clark, 1983; Cox *et al.*, 1984), noscapine (Bickerman *et al.*, 1957), pholcodine (Belcher & Rees, 1986) and dextromethorphan (Karttunen *et al.*, 1987) to be effective in reducing citric acid-induced cough in normal subjects, although others have failed to obtain significant results (Empey *et al.*, 1979). This discrepancy may lie in the specificity of the challenge. Citric acid used in previous studies has been hypertonic and lacking in chloride. This combination of hypertonicity, low pH and lack of chloride may have a disruptive effect upon the integrity of the epithelial lining (Erlij & Martinez-Palomo, 1972; Wade *et al.*, 1973;

Ferreira & Hill, 1982), and such an intense stimulus may affect C-fibre nociceptors. Although the antitussive activity of opiates is thought to result from depression of the medullary cough centre, recent evidence suggests a possible site of action at peripheral opioid receptors on vagal sensory nerves where they may inhibit substance P release from C-fibres (Lembeck & Donnerer, 1985). This inhibitory effect of opiates on the release of sensory neuropeptides from airway C-fibres has also been observed by other workers (Frossard & Barnes, 1987; Belvisi *et al.*, 1988; Belvisi *et al.*, 1989; Rogers & Barnes, 1989). The lack of effect of codeine and noscapine on UNDW- and UNCA-induced cough supports a mechanism of action for opiates other than central inhibition as this would be expected to inhibit cough regardless of the afferent pathway. The observation that codeine inhibits capsaicin-induced cough (Fuller *et al.*, 1988) which may be mediated by C-fibres also supports a peripheral site of action.

Capsaicin-induced cough was therefore included in the studies of nedocromil sodium, which together with UNDW and UNCA provided a comprehensive range of cough challenges. This study confirmed that differences in antitussive efficacy could be detected by using these various challenges. Whilst fenoterol only inhibited UNDW-induced cough, nedocromil inhibited both UNDW- and capsaicin-induced cough with neither drug significantly affecting UNCA-induced cough. The lack of effect of bronchodilators on capsaicin-induced cough has also been observed by other investigators (Smith *et al.*, 1991). Thus all 3 challenges appear to be mediated by different mechanisms. As discussed above, the low pH of UNCA may be injurious to epithelium which could block the mechanism of antitussive action. The antitussive efficacy of nedocromil on both UNDW and capsaicin-induced cough suggests that it may have a direct inhibitory action on vagal sensory nerves including both C-fibres and RARs. Recent

reports suggest that nedocromil may inhibit C-fibres in guinea-pigs (Verleden *et al.*, 1991) and in human subjects, SO_2-induced broncho-constriction which may be mediated by C-fibres, is inhibited by nedocromil (Altounyan *et al.*, 1986; Dixon *et al.*, 1987).

Despite the previous observations that marked bronchoconstriction can be associated with coughing (Section 4.6.1), UNDW-induced cough and bronchoconstriction in asthmatics are not related. UNDW induces a rapidly adapting cough response immediately on inhalation which is thought to be mediated by RARs. The bronchoconstrictor response follows inhalations of over 1 minute duration and increases with increasing doses of UNDW. This effect is thought to be mediated by the low osmolarity of water which may stimulate a sub-group of RARs and SARs (Sant'Ambrogio *et al.*, 1993). The antitussive action of frusemide (Section 5.3.3) was not due to inhibition of bronchoconstriction since coughing was assessed during the first 30 second exposure prior to the bronchoconstrictor response. However, the relative potencies of amiloride and frusemide in inhibiting cough and bronchoconstriction were similar. The mechanism of the antitussive action of frusemide is unclear. Frusemide does not inhibit capsaicin-induced cough (Ventresca *et al.*, 1990; Karlsson *et al.*, 1992) suggesting that frusemide may have a direct inhibitory action on RARs. In support of this, Sant'Ambrogio *et al.* (1993) found that frusemide inhibited the response of laryngeal RARs to low chloride solutions. If the low concentration of chloride in ASL results in loss of chloride from the nervous endings leading to depolarisation, then inhibition of chloride transport by frusemide could reduce this effect. Frusemide does not affect baseline activity of RARs (Sant'Ambrogio *et al.*, 1993; Mohammed *et al.*, 1992) but appears to only inhibit activity when the receptors are stimulated. The reason for the reduced efficacy of amiloride is unclear. Both amiloride and frusemide inhibited UNDW-induced

bronchoconstriction confirming previous reports (Robuschi *et al.*, 1989). The mechanism for this inhibition of bronchoconstriction is unlikely to be a direct effect on airway smooth muscle since frusemide does not inhibit methacholine-induced bronchoconstriction (Nichol *et al.*, 1990 b; Vaghi *et al.*, 1988). Frusemide also has no effect on the stimulation of laryngeal SARs by water (Sant'Ambrogio *et al.*, 1993) which is mediated by the low osmolarity. This could have been due to reduced access of frusemide to the SARs which are located deep in the airway wall. Alternatively, RARs in the intrapulmonary bronchi of dogs are responsive to alterations in osmolarity rather than low chloride concentrations (Pisarri *et al.*, 1992). The inhibitory action of frusemide on these endings is not known. It is possible that these mediate UNDW-induced bronchoconstriction and that frusemide may inhibit these RAR endings.

CHAPTER 6: AFFERENT LUNG C-FIBRE STIMULANTS AND COUGH

6.1 INTRODUCTION

The afferent vagal innervation of the lungs involves three distinct groups of nerve fibres; RARs, SARs and C-fibres. Whilst these are morphologically distinct, the functional difference in terms of the relative sensitivity of their receptors to humoral and pharmacological stimuli is unclear. All types of vagal afferent nerves appear to be involved in the cough reflex. The RARs are, as previously discussed, an integral part of the cough reflex while SARs may facilitate cough since inhibition of SARs results in inhibition of RAR-mediated cough (Sant'Ambrogio *et al.*, 1984; Hanacek *et al.*, 1984). Pulmonary and bronchial C-fibres may also cause coughing (Collier & Fuller, 1984). They are the most numerous of the afferent vagal nerves accounting for up to 80% of vagal afferent fibres in the cat (Jammes *et al*, 1982). C-fibres contain a variety of sensory peptides including substance P which are released from afferent terminals by local axon reflexes (Barnes, 1986).

The product of arachidonic acid metabolism, prostaglandin E_2 (PGE_2) is also thought to stimulate C-fibre receptors (Coleridge *et al.*, 1976). In the skin (Ferreira, 1981), it can cause a long lasting hyperalgesia, i.e. a persistent lowering of polymodal nociceptor threshold to both mechanical and chemical stimulation which lasts several hours. This hyperalgesia of the skin also potentiates the response to capsaicin (Otsuka & Yanagisawa, 1987). By contrast, repeated application of capsaicin to the skin causes hypoalgesia or anaesthesia (Hayes *et al.*, 1984). It can also inhibit the production of PGE_2 in the epithelium (Flynn *et al.*, 1986). Both aerosols of capsaicin and PGE_2 when inhaled by humans cause coughing (Collier & Fuller, 1984; Costello *et al.*, 1985), but the

afferent nerves involved remain unconfirmed. Indeed, it has also been argued that stimulation of C-fibres can inhibit cough (Tatar *et al.*, 1988). Part of the difficulty in interpreting experimental data is the knowledge that whilst C-fibres appear particularly sensitive to substances such as capsaicin, RARs may also be stimulated by these agents (Tatar *et al.*, 1988; Karlsson *et al.*, 1988).

The aims of the following studies were to characterise the cough responses to the two putative C-fibre stimulants, capsaicin and PGE_2.

6.2 METHODS

6.2.1 Cough Responses To Inhaled Capsaicin And Prostaglandin E_2

Twelve healthy volunteers (3 males and 9 females, age range 19 - 59 yrs) performed dose response studies to inhaled PGE_2. Initial studies revealed that the cough response was subject to marked and prolonged tachyphylaxis and therefore challenges were performed on separate days. PGE_2 (Prostin E_2, Upjohn Ltd., Crawley, West Sussex, UK) was diluted in isotonic saline and administered in random order from the Bronchoscreen in a ratio of 1:5 with placebo (isotonic saline) for 18 breaths i.e. a total of 3 doses of active stimulant being inhaled, in concentrations ranging from 0.028 to 28 μmol/l. Cough frequency was recorded over each 18 breath challenge.

Dose response studies for capsaicin were performed as above by 10 of the subjects. Capsaicin was dissolved in ethanol and diluted in isotonic saline and then inhaled in increasing concentrations of 1, 2 and 5 μmol/l at 5 minute intervals until greater than 10 coughs occurred during a single challenge.

6.2.2 Adaptation And Cross-Adaptation Of Cough

The aims of this study were to determine whether the stimulants UNDW, capsaicin and PGE_2 act on the same afferent pathways by determining the adaptation and cross-adaptation of cough in response to these agents. Adaptation of reflex activity occurs when repetition of sensory stimuli induces a lowering of the frequency of impulses and this response is also known as tachyphylaxis. By contrast, cross-adaptation occurs between 2 or more stimuli when a common afferent pathway is involved.

Five healthy volunteers (1 male and 4 females, age range 19 -59 yrs, mean FEV_1 = 145% of predicted) were studied. All subjects had previously performed baseline dose response curves in Experiment 6.2.1 for PGE_2 and capsaicin to determine a dose causing greater than 10 coughs. A baseline challenge to UNDW was also performed. An ultrasonic nebulizer had to be used for this challenge as coughing does not occur when water is inhaled from a jet nebulizer (See Section 3.3.6).

Subjects then attended the laboratory on 9 separate days at the same time of day. On each visit, subjects received 3 challenges; the first was followed 5 minutes later by a second challenge which was then repeated 3 hours after the first challenge. Challenge 1 consisted of inhalation of one of the 3 stimulants UNDW, capsaicin or PGE_2 administered in random order and on 3 occasions. Challenges 2 and 3 consisted again of one of the 3 stimulants, thus each stimulant was followed by itself and the other 2 stimulants so giving the 9 possible combinations.

Cough frequency was recorded as the total number of coughs during a challenge. This total was also divided into the number of coughs occurring during 3 equal and consecutive periods of the challenge, i.e. for

capsaicin and PGE_2, the number of coughs following each of the 3 active breaths and for UNDW, the number of coughs during each 20 second period of challenge. This allowed assessment of any adaptation of cough occurring immediately during the challenge.

R_{aw}, which was measured breath by breath during both air and challenge breathing from the Bronchoscreen was recorded as the average of the final 5 measurements from 1 minute air breathing pre- and post-challenge and during challenge with capsaicin and PGE_2.

6.2.3 The Antitussive Efficacy Of Nedocromil Sodium

To determine whether the choice of stimulus as well as the method of aerosol delivery are important variants when testing antitussives, the efficacy of nedocromil sodium, which inhibited UNDW-induced cough and jet nebulized capsaicin-induced cough, has been studied on cough induced by capsaicin and PGE_2 delivered by ultrasonic and jet nebulizers.

Sixteen healthy volunteers (6 males and 10 females, age range 19-59, mean FEV_1 = 113%, range 85-133%) were recruited, 8 to each trial. Trial 1 used ultrasonically nebulized stimuli, while trial 2 used the Bronchoscreen. For trial 1, 0.4 μmol/l capsaicin and 1.4 μmol/l PGE_2 were used which elicited greater than 10 coughs for all subjects. For trial 2, subjects had previously performed dose response curves for capsaicin and PGE_2 and concentrations causing greater than 10 coughs were used.

On each of 4 visits, nedocromil sodium 4 mg (2 x 2 mg/puff) or placebo (2 puffs) were administered 30 minutes prior to challenge with either capsaicin or PGE_2. Treatment and challenge were randomised by 2 Latin squares of order 4. Cough frequency during each challenge and during the 5 minutes following ultrasonic challenge was recorded. FEV_1 was recorded pre- and post-treatment and post-ultrasonic challenge. R_{aw} was recorded pre- and post- treatment and during jet challenge.

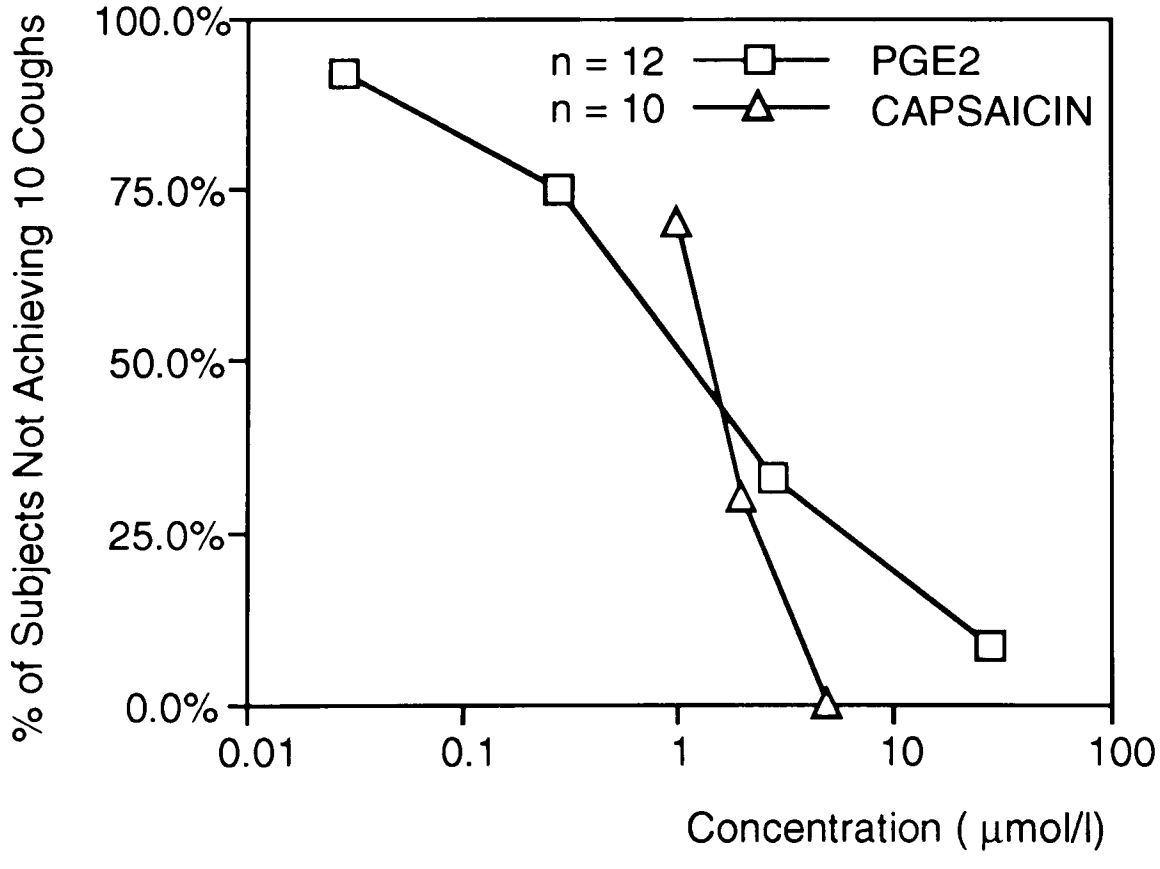
6.3 RESULTS AND STATISTICAL ANALYSIS

6.3.1 Cough Responses to Capsaicin and Prostaglandin E_2

The cough response to PGE_2 was found to exhibit marked adaptation (tachyphylaxis) requiring the different concentrations to be inhaled on separate days. Some coughing following challenge was also noted confirming the observations of Costello *et al.* (1985). Capsaicin produced a steeper gradient of dose response curve and did not produce cough following inhalation. Both challenges were however associated with transient retrosternal soreness. The dose response curves are presented in Figure 6.1.

FIGURE 6.1

Concentration / Cough Response to PGE2 and Capsaicin

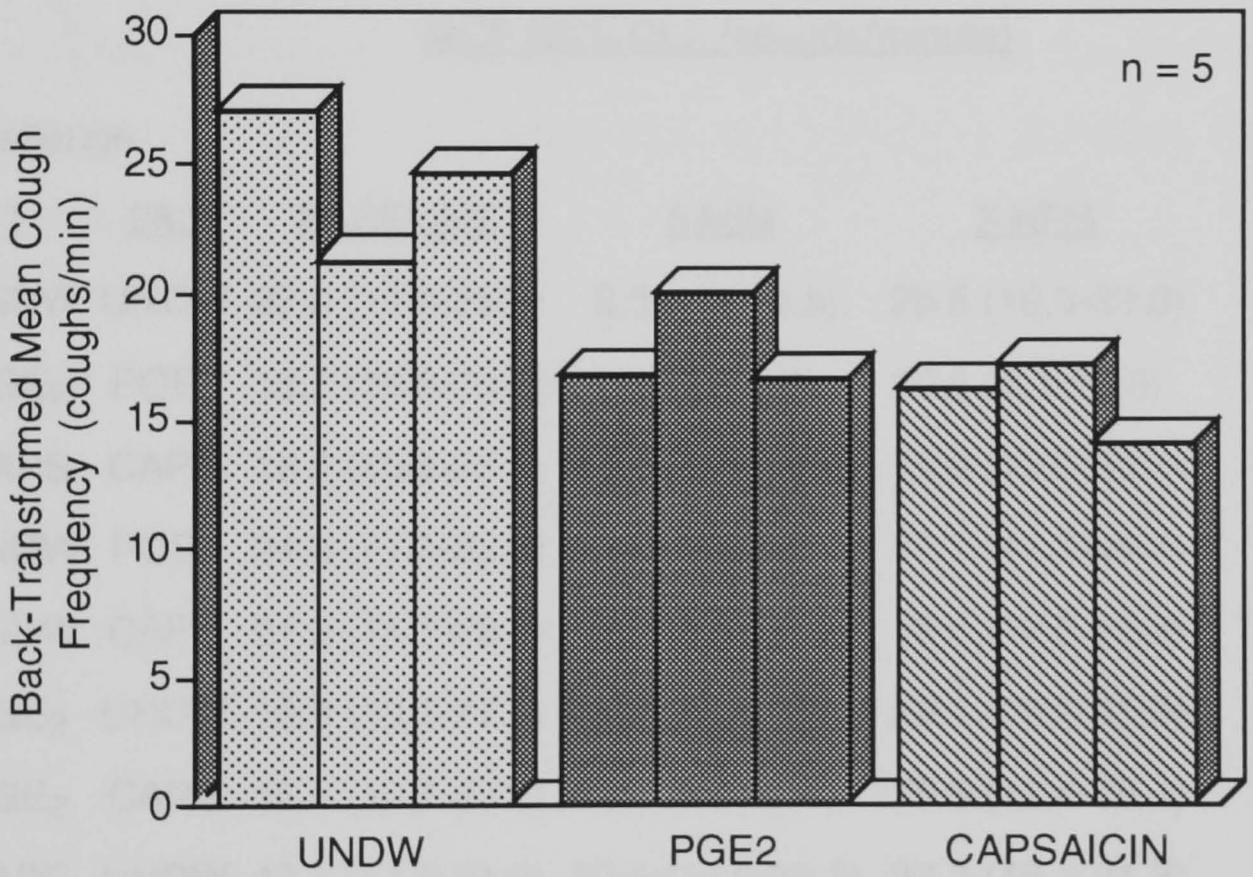


6.3.2 Adaptation and Cross-Adaptation of Cough

ANOVA was first performed on the transformed baseline (Challenge 1) cough frequencies to assess repeatability of the measurements and comparability of the 3 challenges. This found no differences between stimulants ($p>0.05$) or days of challenge ($p>0.05$) as presented in Figure 6.2.

FIGURE 6.2

Baseline Cough Challenges



Subsequent analyses examined carryover effects from one challenge to the next and within challenges and revealed significant carryover effects from one challenge to the next ($p<0.01$). Examining this further found no difference in the cough response from Challenge 1 (baseline) and Challenge 3 (3 hours) ($p>0.05$) but significant carryover effects were found from Challenge 1 to Challenge 2 (5 minutes) ($p<0.01$).

Adaptation of cough from Challenge 1 to Challenge 2 occurred with both UNDW ($p<0.001$) and PGE_2 ($p<0.001$) but not with capsaicin ($p>0.05$) as presented in Figure 6.3. Cross-adaptation of cough occurred after PGE_2 which inhibited subsequent cough at 5 minutes to UNDW ($p<0.001$) as presented in Figure 6.4. Back-transformed mean cough frequencies and 95% confidence limits are shown in Table 6.1.

TABLE 6.1

Adaptation and Cross-Adaptation of Cough

		MCF (95% CL) (coughs/minute)		
Challenge				
1	2&3	BASELINE	5 MIN	3 HRS
UNDW	UNDW	27.0 (19.6-35.5)	8.3 (1.3-13.5)	25.5 (18.3-33.8)
PGE_2	PGE_2	16.7 (10.9-23.6)	3.1 (0.6-6.7)	10.6 (6.1-16.3)
CAPS	CAPS	16.2 (10.5-23.1)	16.3 (10.6-23.2)	18.0 (12.0-25.1)
UNDW	PGE_2	24.6 (17.5-32.8)	21.2 (14.6-28.8)	17.7 (11.7-24.7)
UNDW	CAPS	21.2 (14.7-28.9)	9.9 (5.5-15.5)	18.6 (12.5-25.9)
PGE_2	UNDW	19.9 (13.6-27.3)	6.3 (2.8-10.9)	23.1 (16.2-31.0)
PGE_2	CAPS	16.6 (10.8-23.5)	11.7 (6.9-17.7)	18.5 (12.4-25.7)
CAPS	UNDW	17.1 (11.2-24.0)	20.5 (14.0-28.0)	23.3 (16.4-31.3)
CAPS	PGE_2	14.1 (8.8-20.6)	20.7 (14.3-28.3)	19.7 (13.4-27.1)

To assess any immediate adaptation of coughing during a challenge, ANOVA was performed on the data obtained by dividing the cough frequencies to Challenge 1 into 3 equal and successive time periods. This showed a significant difference between periods ($p<0.05$) and an interaction between challenge and periods ($p<0.01$) which

suggests that not all challenges adapted in the same way. Further analysis revealed that adaptation of cough during baseline challenges occurred with UNDW ($p<0.001$) and with PGE_2 ($p<0.05$), but the cough response to capsaicin increased from period 1 to 2 ($p<0.001$) as presented in Figure 6.5.

Values of R_{aw} were log transformed prior to ANOVA which compared values pre- and post-challenges and pre- and during challenge (excluding UNDW). ANOVA revealed no difference between pre- and post- challenge measurements ($p>0.05$). However, R_{aw}, increased slightly during the first challenge with both PGE_2 and capsaicin compared with pre-challenge values ($p<0.05$). This airway constriction was reduced when the stimuli were given at Challenges 2 and 3 ($p>0.05$) as presented in Figure 6.6.

FIGURE 6.3

Adaptation of Cough

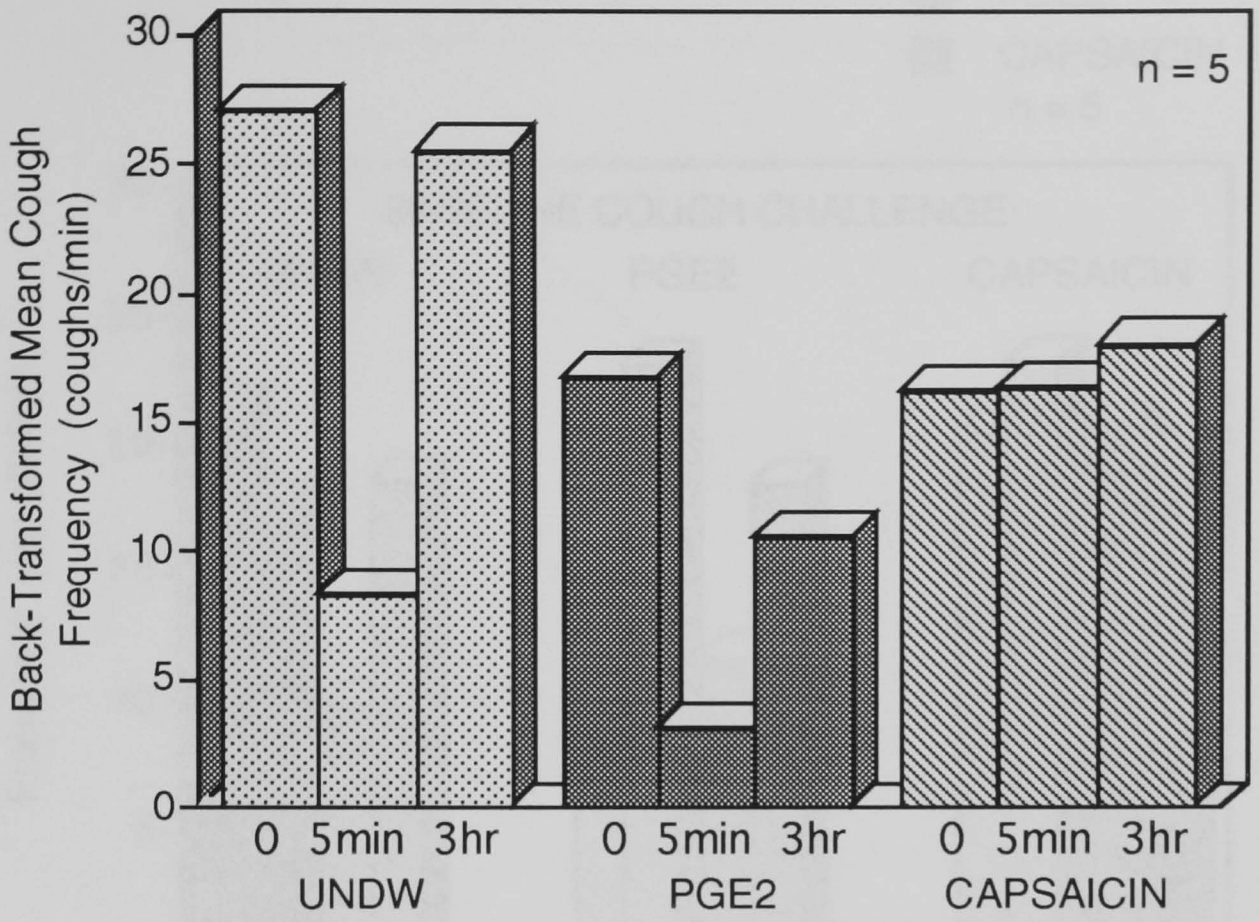
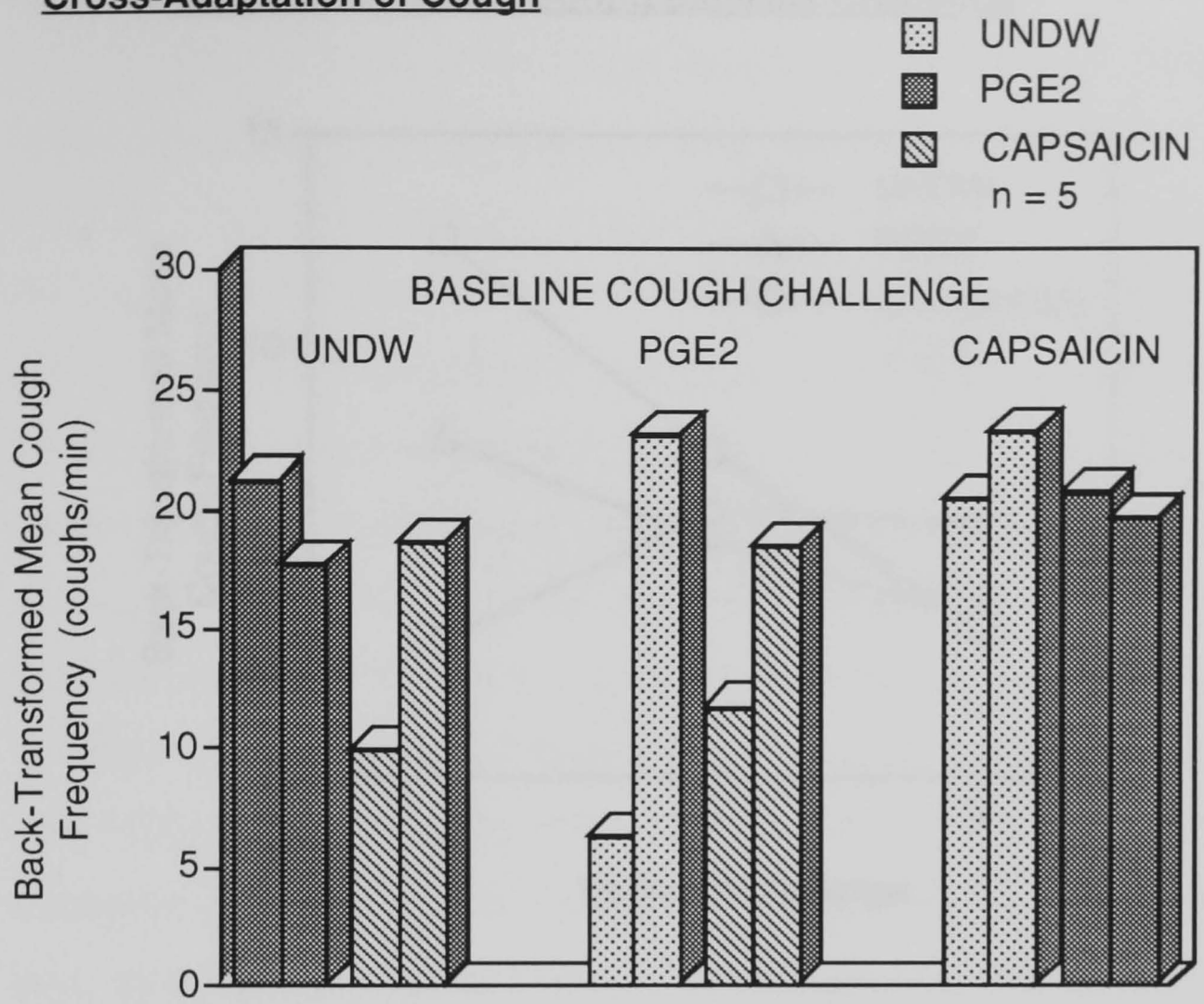


FIGURE 6.4

Cross-Adaptation of Cough



The 1st column of each group represents the cough response to challenge at 5 min; the 2nd column at 3 hrs.

FIGURE 6.5

Adaptation of Cough During Baseline Challenge

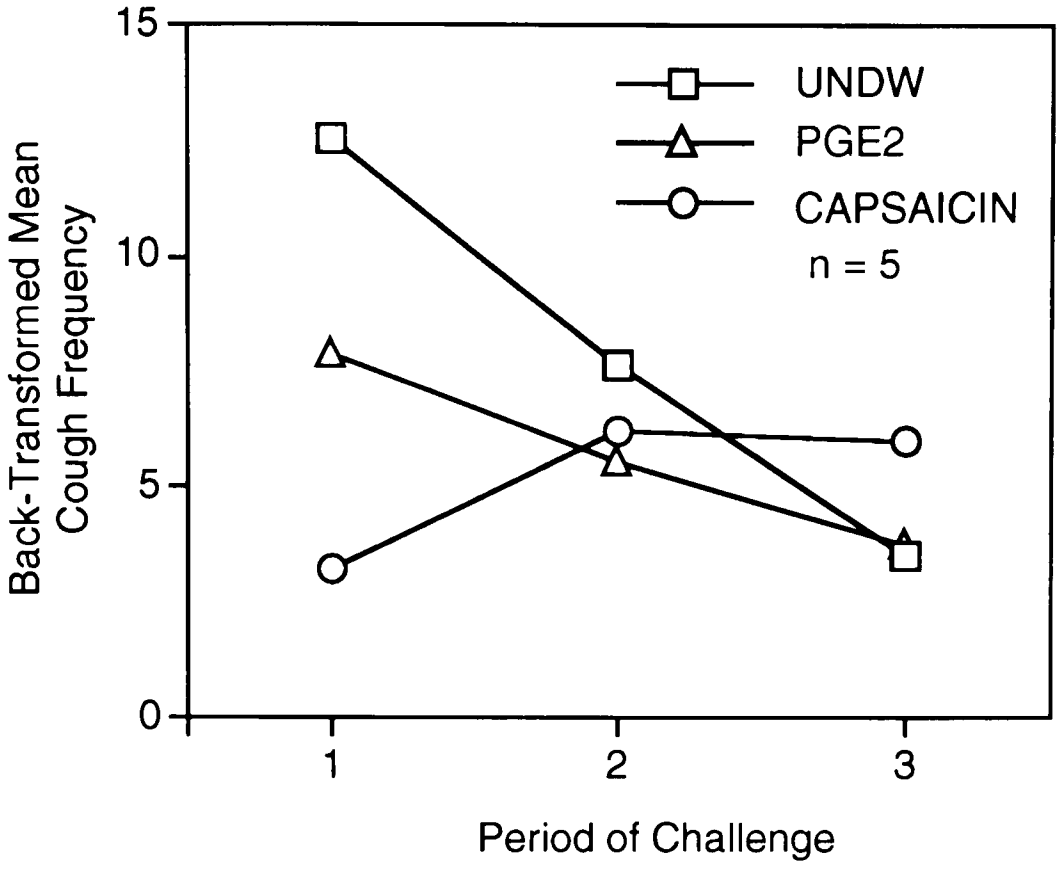
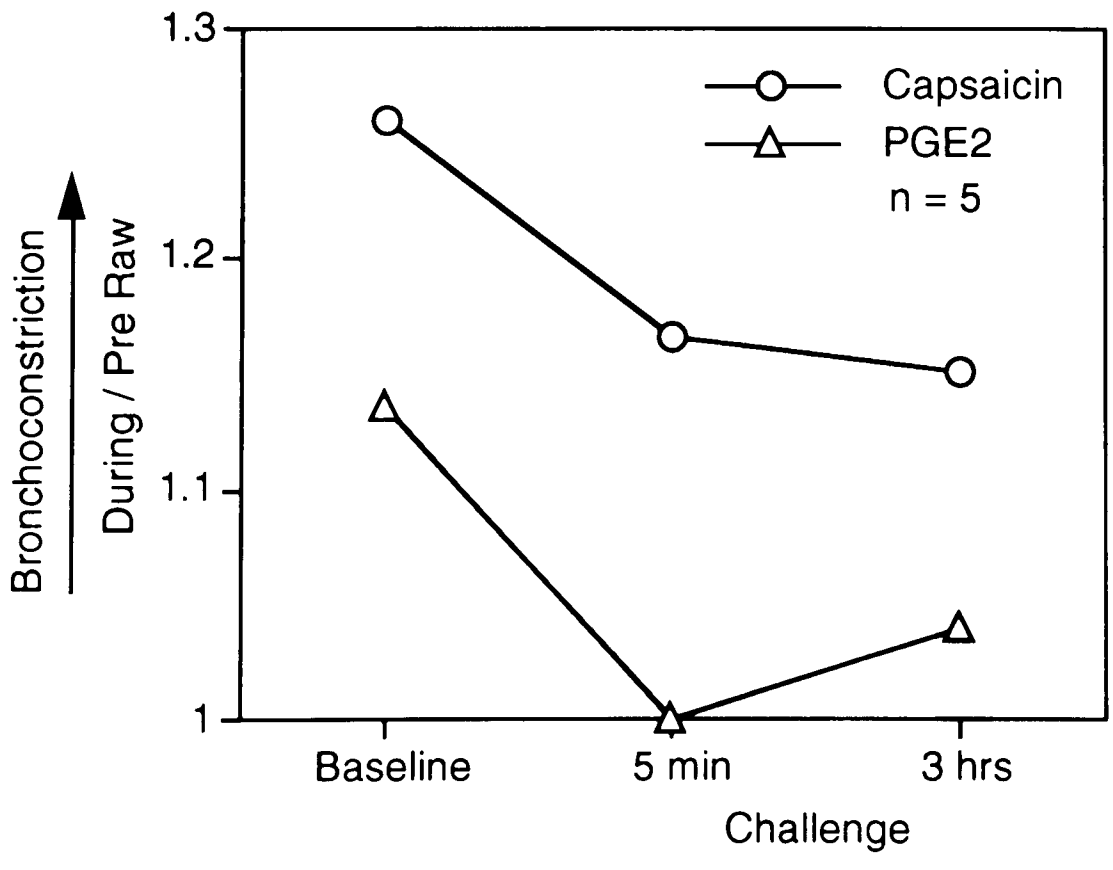


FIGURE 6.6

Alteration of During / Pre Ratios of Raw with Challenge



6.3.3 The Antitussive Efficacy of Nedocromil Sodium

ANOVA of transformed cough frequencies revealed no effect of nedocromil sodium on PGE_2-induced cough delivered by ultrasonic or jet nebulizer ($p>0.05$). However, nedocromil sodium inhibited capsaicin-induced cough delivered by the jet nebulizer Bronchoscreen ($p<0.02$) but not by ultrasonic nebulizer ($p>0.05$). Adaptation of cough was assessed by breaking down cough frequencies into 3 equal and successive periods of challenge. This revealed adaptation of cough with PGE_2 regardless of the delivery system ($p<0.01$), but adaptation of capsaicin-induced cough only occurred when delivered from the ultrasonic nebulizer ($p<0.01$). Cough recorded over the 5 minutes following ultrasonic challenge was higher after PGE_2 than capsaicin ($p<0.01$). FEV_1 did not alter with treatment or ultrasonic challenge ($p>0.05$). However, analysis of log transformed R_{aw} data revealed an increase during challenge ($p<0.01$) which was independent of treatment or challenge stimulus.

TABLE 6.2

The Antitussive Effect of Nedocromil Sodium

		MCF (95% CL) (coughs/minute)	
		Ultrasonic Nebulizer	Jet Nebulizer
PGE_2	Placebo	12.6 (6.5-20.4)	11.7 (9.3-14.4)
	Nedocromil	12.1 (6.2-19.9)	10.3 (8.0-12.9)
CAPSAICIN	Placebo	15.9 (9.1-24.6)	15.0 (12.2-18.0)
	Nedocromil	19.6 (11.9-29.0)	10.2 (7.9 -12.8)

FIGURE 6.7

The Effect of Nedocromil on Ultrasonic Challenge

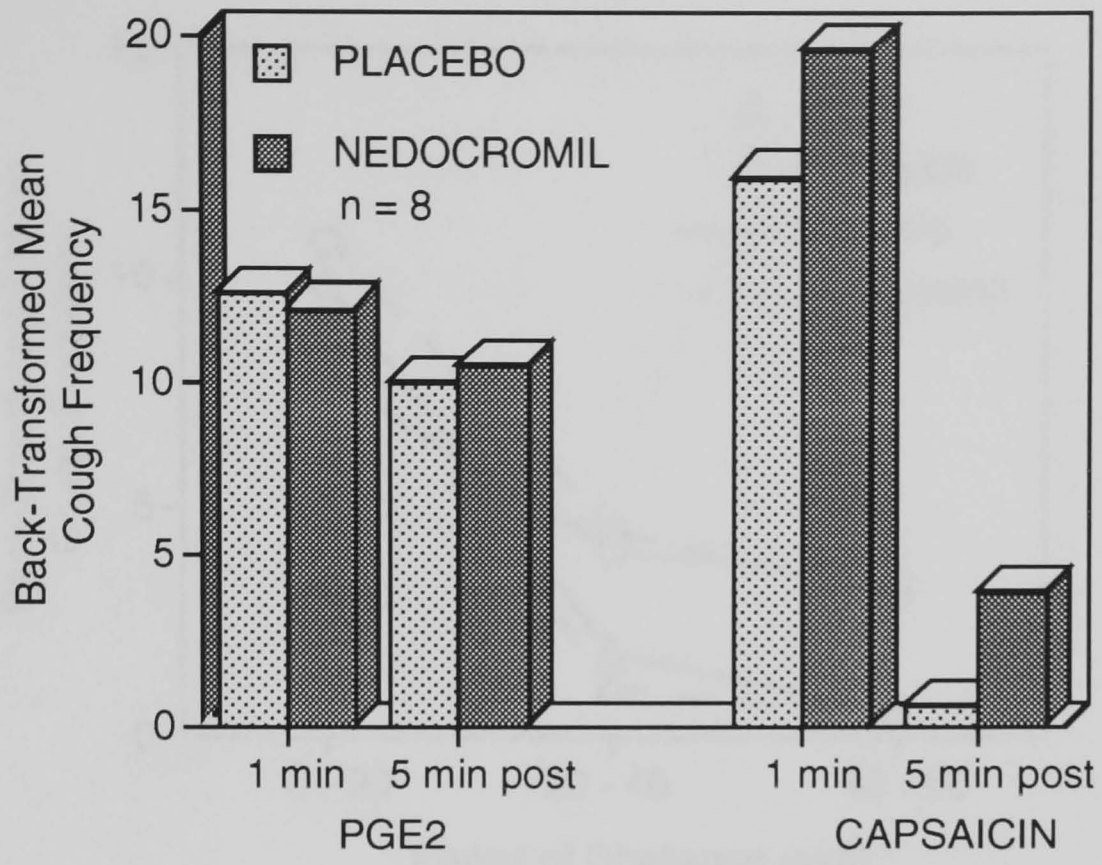


FIGURE 6.8

The Effect of Nedocromil on Jet Challenge

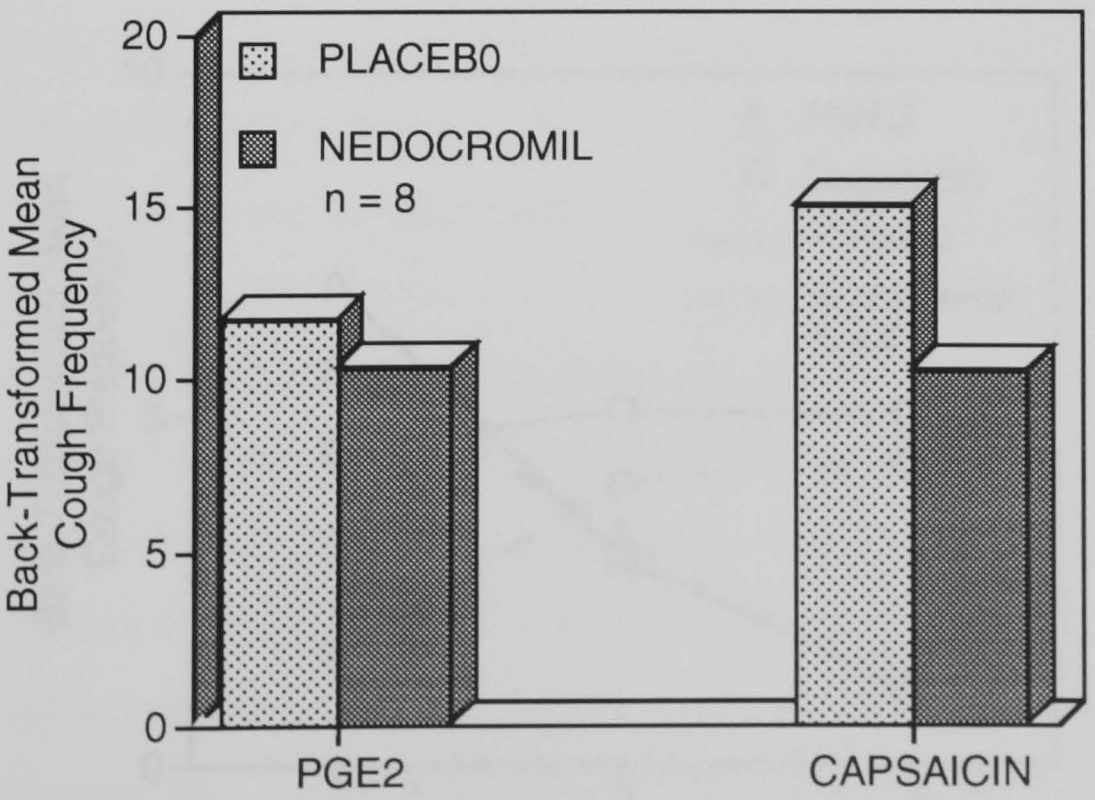


FIGURE 6.9

Adaptation of Cough During Ultrasonic Challenge

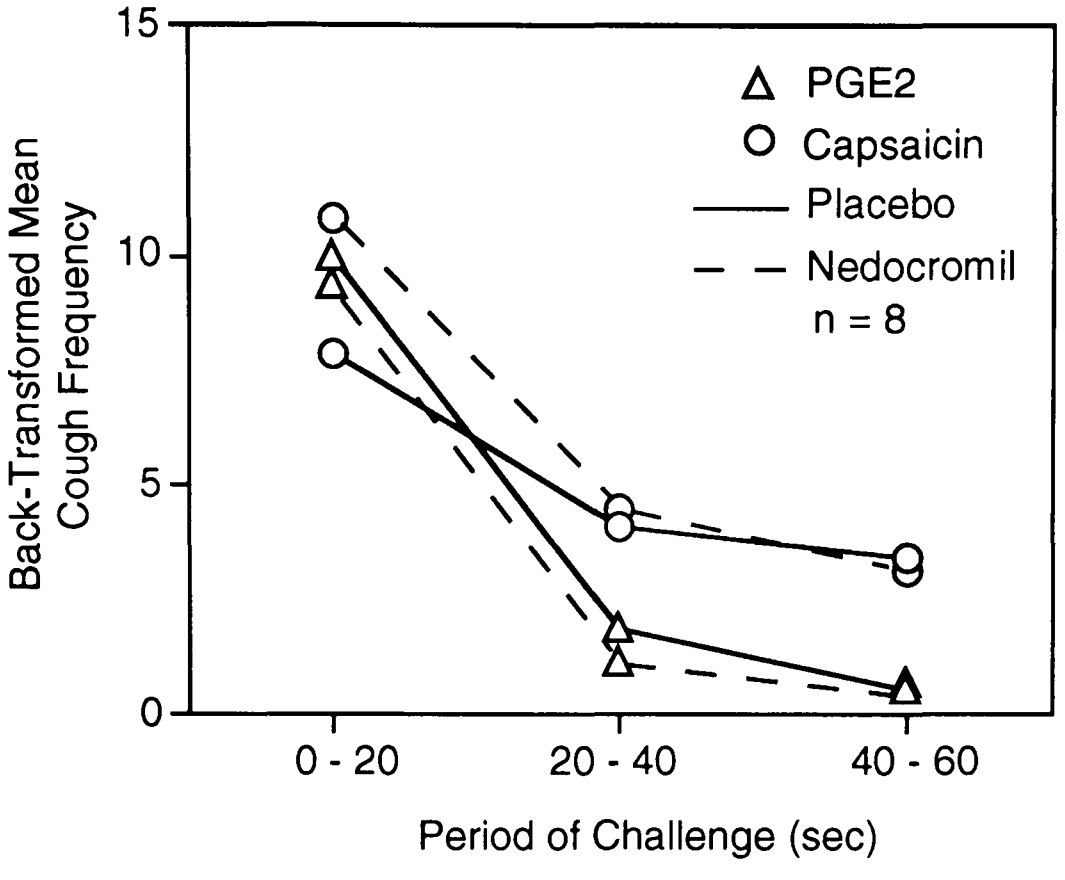


FIGURE 6.10

Adaptation of Cough During Jet Challenge

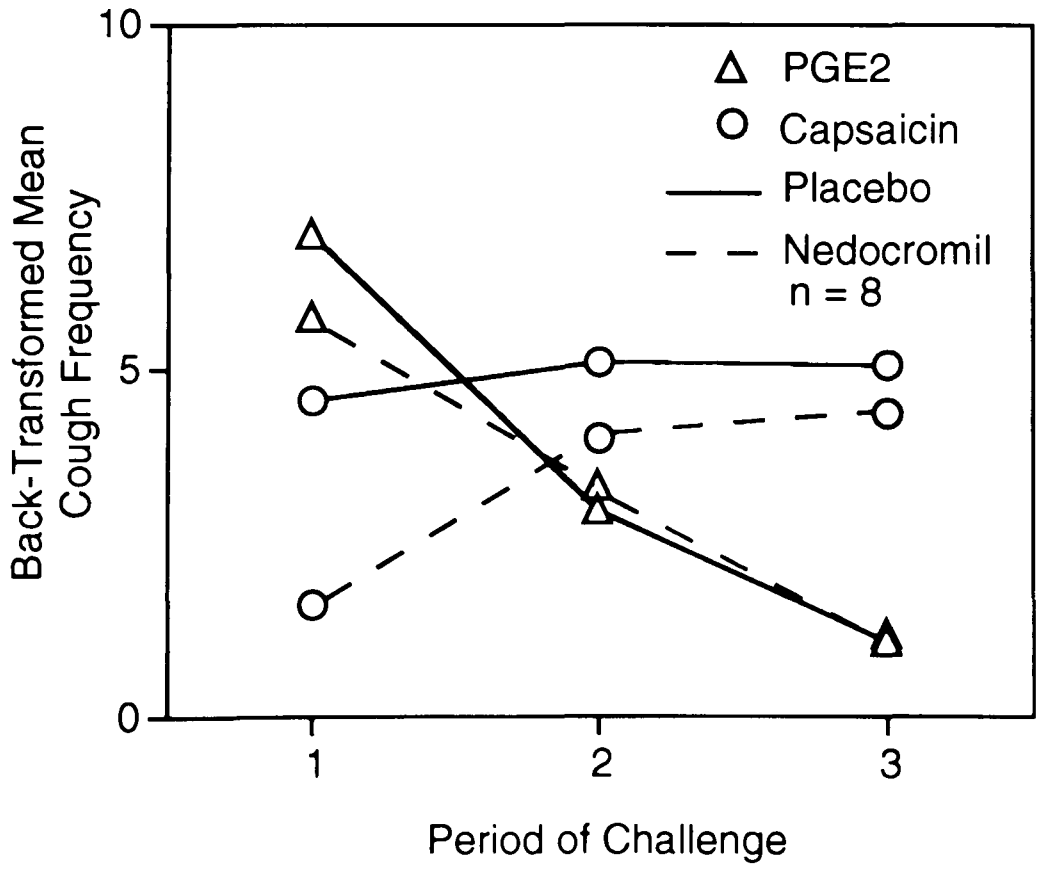
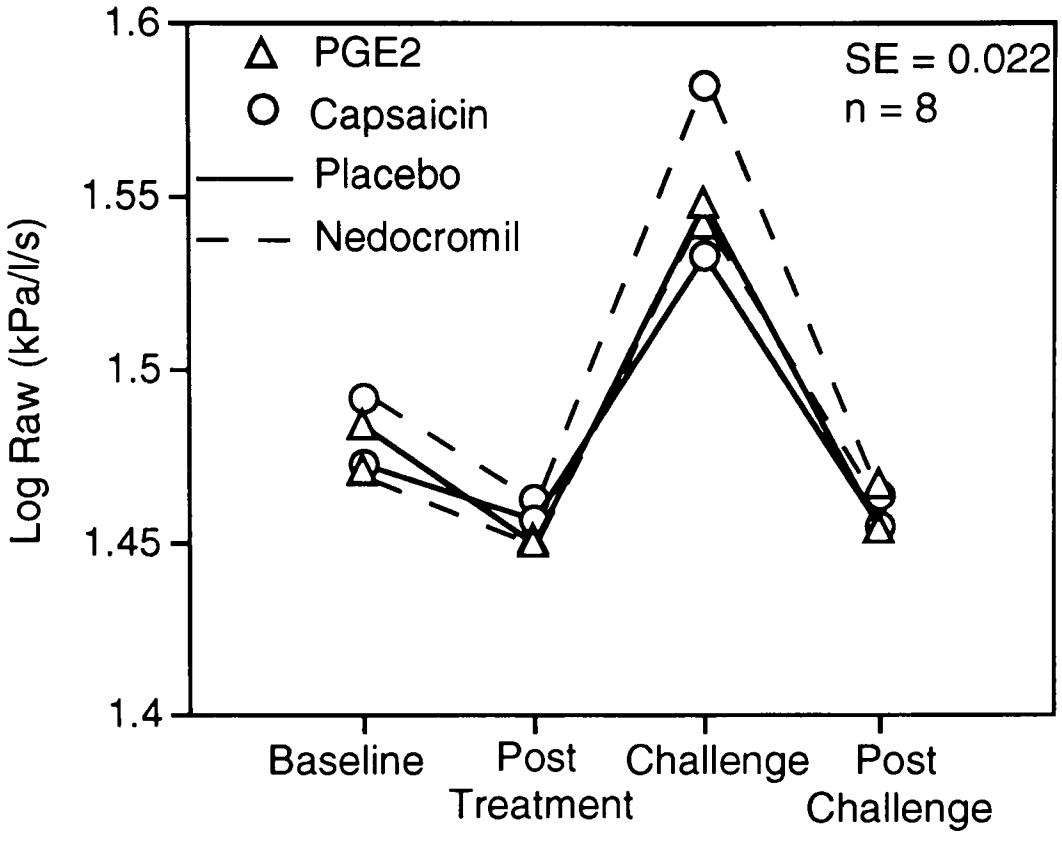


FIGURE 6.11

Change in Raw During Jet Challenge



6.4 DISCUSSION

Inhalation of UNDW, PGE_2 and capsaicin appear to evoke cough by different mechanisms. UNDW-induced cough exhibited a rapid adaptation which recovered within 3 hours, consistent with an effect on RARs. No effect on the cough response to capsaicin or PGE_2 following UNDW was observed.

Capsaicin-induced cough, however, did not exhibit adaptation of response when delivered by the Bronchoscreen or cross-adaptation with either of the other 2 challenges suggesting mediation by a different receptor. It has been suggested that capsaicin has dual actions depending on the dose administered. Low doses of capsaicin stimulate C-fibre receptors while high doses may stimulate RARs (Karlsson *et al.*, 1988; Tatar *et al,* 1988). This dual action of capsaicin has been observed by

other workers. Hua & Lundberg, (1986), found that low doses inhibited guinea-pig ureter motility which was mediated by calcitonin gene related peptide while high doses stimulated motility and this was effected by tachykinins. Inhalation of capsaicin in humans produces a small transient bronchoconstriction (Collier & Fuller, 1984) which could reflect a laryngeal response rather than airway smooth muscle bronchoconstriction, but in certain circumstances, for example, in heart-lung transplant patients, capsaicin can initiate a bronchodilator reflex (Lammers *et al*, 1989; Hathaway *et al,* 1993). Capsaicin-induced cough may be related to the tachykinin release at sensory C-fibre endings as cough is augmented by the angiotensin converting enzyme inhibitor, captopril (Morice *et al*, 1987) and by neutral endopeptidase inhibitors (Kohrogi *et al*, 1988) which delay the breakdown of tachykinins released into the airways by C-fibre stimulation. The nature of tachykinins released appears to depend on the dose of capsaicin administered and these in turn determine the effect on target cells (Lundberg *et al*, 1985).

PGE_2-induced cough, unlike capsaicin, exhibited rapid adaptation, similar to UNDW-induced cough, but which had not fully recovered after 3 hours. PGE_2 inhibited subsequent UNDW-induced cough, but not vice versa, disproving a common receptor. However, UNDW was administered from a different nebulizer than PGE_2 and capsaicin, and despite evoking comparable cough frequencies during baseline challenges, may have resulted in a different pattern of aerosol deposition.

The different characteristics of cough induced by capsaicin and PGE_2 oppose the view that both stimulate the same C-fibres. It is possible that PGE_2 and capsaicin stimulate functionally distinct sub-groups of C-fibres. The afferent innervation of the lower respiratory tract appears to resemble that of the skin (Coleridge & Coleridge, 1985). Cutaneous C-fibres can be classified into four major groups according to their response

profiles; warm and cold thermoreceptors, mechanoreceptors and polymodal nociceptors. Polymodal nociceptors account for 80% of afferent nerve fibres from rat skin (Lynn & Hunt, 1984). Nociceptors can be further sub-divided. One sub-group, for example, responds to itching powder (cowhage) (Tuckett, 1980). In addition, 15-30% of cutaneous C-fibres contain neuropeptide tachykinins such as substance P (Hokfelt *et al*, 1976) which is released at sensory nerve endings, experimentally, by capsaicin. A similar functional heterogeneity of pulmonary C-fibres may exist. Indeed, phenyldiguanide, another C-fibre stimulant appears to act on a different population of airway C-fibres than capsaicin (Skofitsch *et al*, 1983).

No potentiation of cough, suggesting a hyperalgesic effect, was found with any of the cough stimuli used in contrast to other studies which have found a small potentiation of capsaicin-induced cough after $PGF_{2\alpha}$ (Nichol *et al*, 1990 a) and PGE_2 (Choudry *et al*, 1989). Indeed, PGE_2 inhibited water-induced cough suggesting an inhibitory effect on RARs.

Thus all three stimuli evoke cough by different mechanisms. Capsaicin and PGE_2, once thought of as specific C-fibre stimulants, may selectively stimulate sub-populations of vagal airway C-fibre receptors.

The differential effects of nedocromil sodium on capsaicin and PGE_2-induced cough also supports different mediation of cough. Nedocromil inhibited capsaicin- but not PGE_2-induced cough. However, this effect was only apparent when the stimulus was delivered by jet nebulizer and not by ultrasonic nebulizer. Also, adaptation of capsaicin-induced cough only occurred during continuous challenge with the ultrasonic nebulizer but not with the dosimeter. PGE_2, however, exhibited adaptation during challenge from both nebulizers. The inhibitory effect of nedocromil on UNDW- and jet-nebulized capsaicin suggests that

nedocromil may inhibit RARs and a sub-population of capsaicin-sensitive C-fibres located in the intrapulmonary airways.

In conclusion, capsaicin and PGE_2 evoke cough by different mechanisms which are distinct from UNDW-induced cough. Differences between stimuli can be detected by studying their differential responses to adaptation and antitussives and this may also be influenced by the mode of aerosol delivery which can affect deposition. This variability may need to be taken into account when assessing the clinical application of antitussives based on efficacy studies using induced cough.

CHAPTER 7: THE EFFECT OF BRONCHODILATOR THERAPY ON COUGH ASSOCIATED WITH VIRAL INFECTION

7.1 INTRODUCTION

Cough is a common symptom of upper respiratory tract infection (URTI), often developing within a few days of the initial symptoms and sometimes lasting for several weeks or even months after infection. When cough is troublesome, serving no useful function and when no effective treatment for the underlying disease exists, symptomatic treatment is indicated. The need for such treatment of URTI-associated cough is apparent from the wide range of medications available over-the-counter and by prescription and from the fact that cough may be responsible for up to 50% of visits by patients to their General Practitioner during the winter months (Korpas & Tomori, 1979). However, an effective symptomatic treatment for cough associated with URTI has yet to be identified. Many therapies contain no known antitussives and act simply as demulcents; others contain doses of antitussives too low to be effective. However, objective studies in volunteers with URTI have demonstrated antitussive activity of an antihistamine-decongestant therapy (Curley *et al.*, 1988) and a dextromethorphan-beta$_2$-agonist combination (Tukiainen *et al.*, 1986), but not of the expectorant guaifenesin (Kuhn *et al.*, 1982) or of the commonly prescribed opiate, codeine (Eccles *et al.*, 1992).

As URTI is associated with a transient bronchial hyper-responsiveness in non-asthmatics (Empey *et al.*, 1976) and as bronchodilators inhibit cough in asthma (Ellul-Micallef, 1983), a possible role of bronchodilators in treating cough during URTI is implied.

The aim of the following study was to evaluate the role of oxitropium bromide, an anticholinergic bronchodilator, in the management

of those who acquired a community based URTI including cough during the winter season. Oxitropium bromide inhibits UNDW-induced cough (See Section 4.4.2(c)) and therefore this study was also designed to ascertain the relationship between induced cough and naturally occurring cough.

7.2 METHODS

Fifty-six non-asthmatic volunteers (age range 18 - 60 years) from hospital staff entered the study, 2 of whom withdrew before completion of the study. The study design was randomised, double-blind, parallel group and placebo-controlled.

Volunteers were asked to attend the laboratory as soon as possible after onset of the symptom of cough and not later than 72 hours. Volunteers who had a cough and at least 2 other symptoms of URTI undertook a cough challenge consisting of a one minute inhalation of UNDW during which cough frequency was recorded. Nine volunteers were unable to complete the challenge because of the severity of their cough, and in these cases, the duration of UNDW inhalation was recorded for use during the second visit. FEV_1, FVC and PEFR were recorded. Subjects were then randomly assigned to treatment with either oxitropium bromide (200 μg) or identical placebo aerosols delivered by metered dose inhalers. They were also provided with a mini-Wright peak flow meter to record PEFR at home and a diary card. All volunteers were trained in the use of the inhaler and peak flow meter. The trial treatment was taken 8-hourly after recording PEFR (best of 3 attempts) at approximately 7am, 3pm and 11pm for 10 days. Frequency of cough, severity of cough, nocturnal symptoms and general malaise were assessed using 5cm Visual Analogue Scales (VAS) in the diary card at the end of each day. After the 10 day period, subjects returned to the laboratory for repeat tests. No

treatment was taken on the day of this 2nd visit to allow comparison of measurements with those from Visit 1.

7.3 STATISTICAL ANALYSIS

ANOVA was performed to assess the changes in FEV_1, FVC and PEFR data collected during Visits 1 and 2.

The diary PEFR data were analysed by fitting 3 regression lines (for 7am, 3pm and 11pm) for each subject and the mean slopes and intercepts analysed using ANOVA. The slope represented the change over the 10 day period and the intercept (fitted across the middle of the treatment period) the average over the 10 day period. The factors fitted were drug, subject, time of day and the interaction between time of day and drug.

Cough frequencies, in response to UNDW, obtained during Visits 1 and 2, were transformed as previously described to the square-root prior to ANOVA. Where a full minute challenge was not completed, the number of seconds of challenge was recorded and used for the 2nd challenge.

Non-parametric summary statistics of median and 25th and 75th percentiles were produced for the VAS scores.

7.4 RESULTS

The principal raw data and ANOVA tables for this study are presented in Appendix 5.

Fifty-four completed records were collected for analysis. The subjects within each of the two groups, placebo (28 subjects) and oxitropium (26 subjects), were comparable for age (mean placebo = 31yrs; oxitropium = 30yrs) and sex (8 of 15 males and 18 of 39 females were assigned oxitropium). Seven of 12 smokers and 4 of 9 ex-smokers were assigned oxitropium. Baseline measurements of lung function (Table 7.1), VAS scores (Table 7.2) and UNDW-induced cough frequency (Table 7.3)

taken at Visit 1 were also comparable. Mean predicted PEFR was 480 l/min for both groups.

TABLE 7.1

The Change In Lung Function During URTI And The Effect Of Treatment

	PLACEBO (n = 28)		OXITROPIUM (n = 26)	
	Visit 1	Visit 2	Visit 1	Visit 2
FEV_1 (l)	3.8 (0.13)	3.8 (0.14)	3.7 (0.18)	3.7 (0.18)
FVC (l)	4.5 (0.16)	4.5 (0.17)	4.3 (0.22)	4.3 (0.22)
PEFR (l/min)	480 (16)	500 (14)	460 (17)	490 (16)

Values are the mean (with standard error)

TABLE 7.2

The Change In VAS Scores From Baseline To Recovery Visit

	PLACEBO (n = 28)		OXITROPIUM (n = 26)	
	Visit 1	Visit 2	Visit 1	Visit 2
Cough frequency	24	1	24	4
Cough severity	24	1	26	1
Nocturnal symptoms	5	0	9	0
General malaise	25	3	24	2

Values are median scores based on a scale of 0 to 50.

TABLE 7.3

The Change In Cough Response To UNDW Inhalation

	MCF (95% CL)	
	PLACEBO (n = 28)	OXITROPIUM (n = 26)
VISIT 1	20.4 (17.1 - 24.1)	22.0 (18.0 - 26.4)
VISIT 2	12.3 (9.7 - 15.2)	12.4 (9.4 - 15.8)

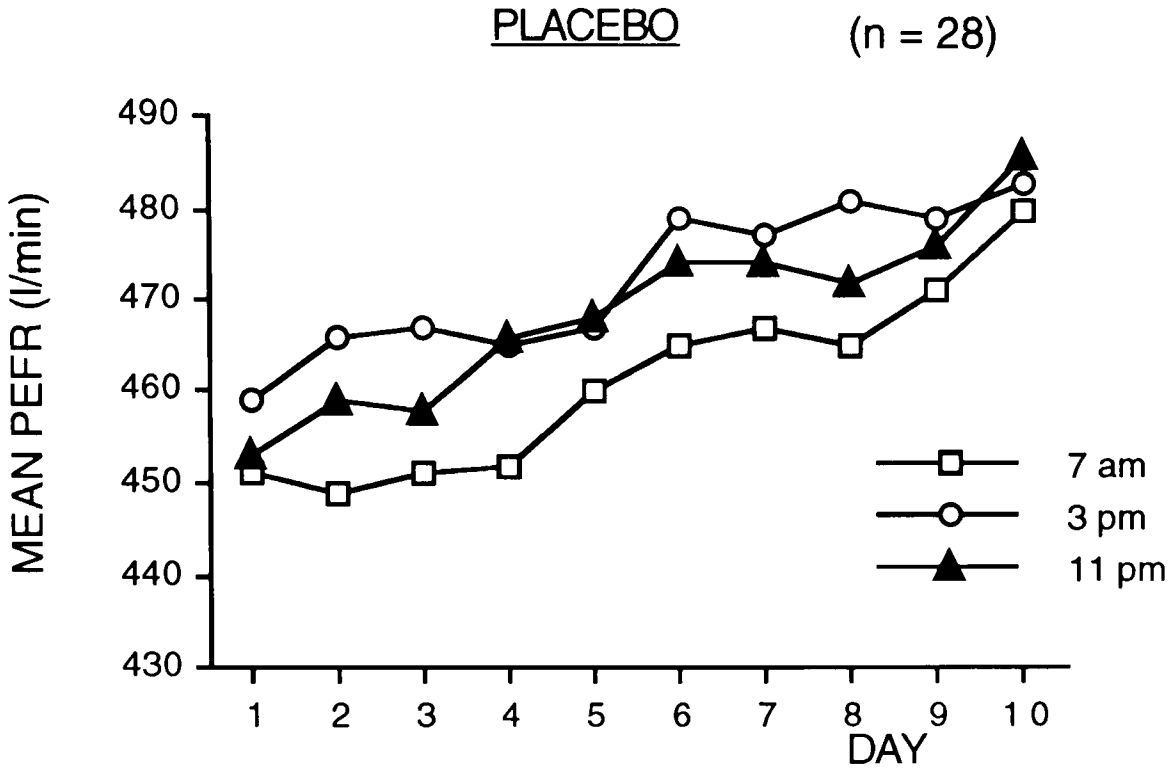
FEV_1 and FVC did not alter during the trial from Visits 1 to 2 and there was no statistically significant difference between placebo and oxitropium groups ($p>0.05$) but PEFR improved by an overall mean of 23 l/min, (SE 5.5 l/min) from Visit 1 to 2 ($p<0.05$). This increase occurred regardless of treatment.

PEFR data from the subject diaries revealed an overall improvement over the 10 days of study. In addition, ANOVA revealed that values were on average, highest in the afternoon and lowest on waking ($p<0.001$) similar to a 'morning dip' usually found in asthma. The average variation between morning and afternoon recordings with 95% confidence limits was 15.0 l/min (7.9, 22.0). There was no treatment with time interaction in the ANOVA suggesting that treatment with oxitropium did not differ from placebo in respect of this circadian rhythm ($p>0.05$). The PEFR data is represented graphically in Figure 7.1.

VAS scores of frequency of cough, severity of cough, nocturnal symptoms and general malaise became less severe over the 10 day treatment period as shown in Figure 7.2 but again, oxitropium fared no better than placebo.

The cough response to UNDW was similar for both the placebo and oxitropium groups. A similar reduction in cough frequency occurred in both groups from Visit 1 to 2 ($p<0.001$) as shown in Table 7.2 which probably reflects the combination of a learning effect on cough which occurs after the first challenge (See Section 4.2.2 (c)) and amelioration of symptoms. The 10 day treatment regime with oxitropium did not alter the sensitivity of the cough reflex to a greater extent than placebo. Treatment was not given on the day of the challenge so no comparison can be made with our previous study (See Section 4.4.2 (c)) where a single dose of oxitropium administered 1 hour before challenge inhibited water-induced coughing.

FIGURE 7.1 The Diary Recordings of PEFR



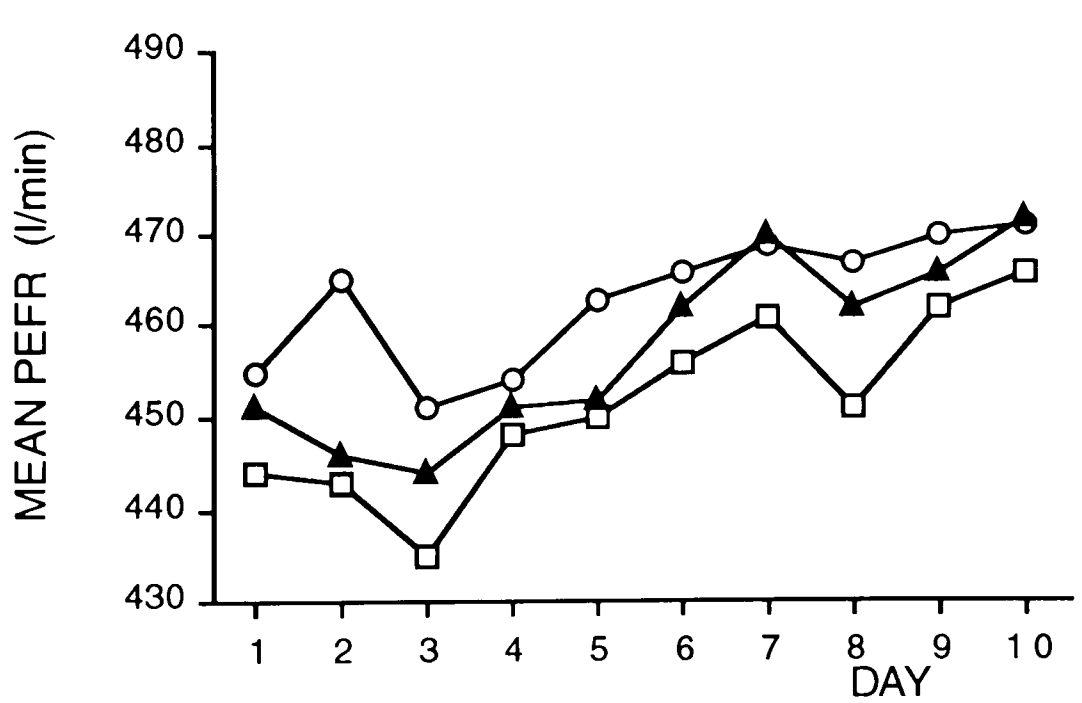
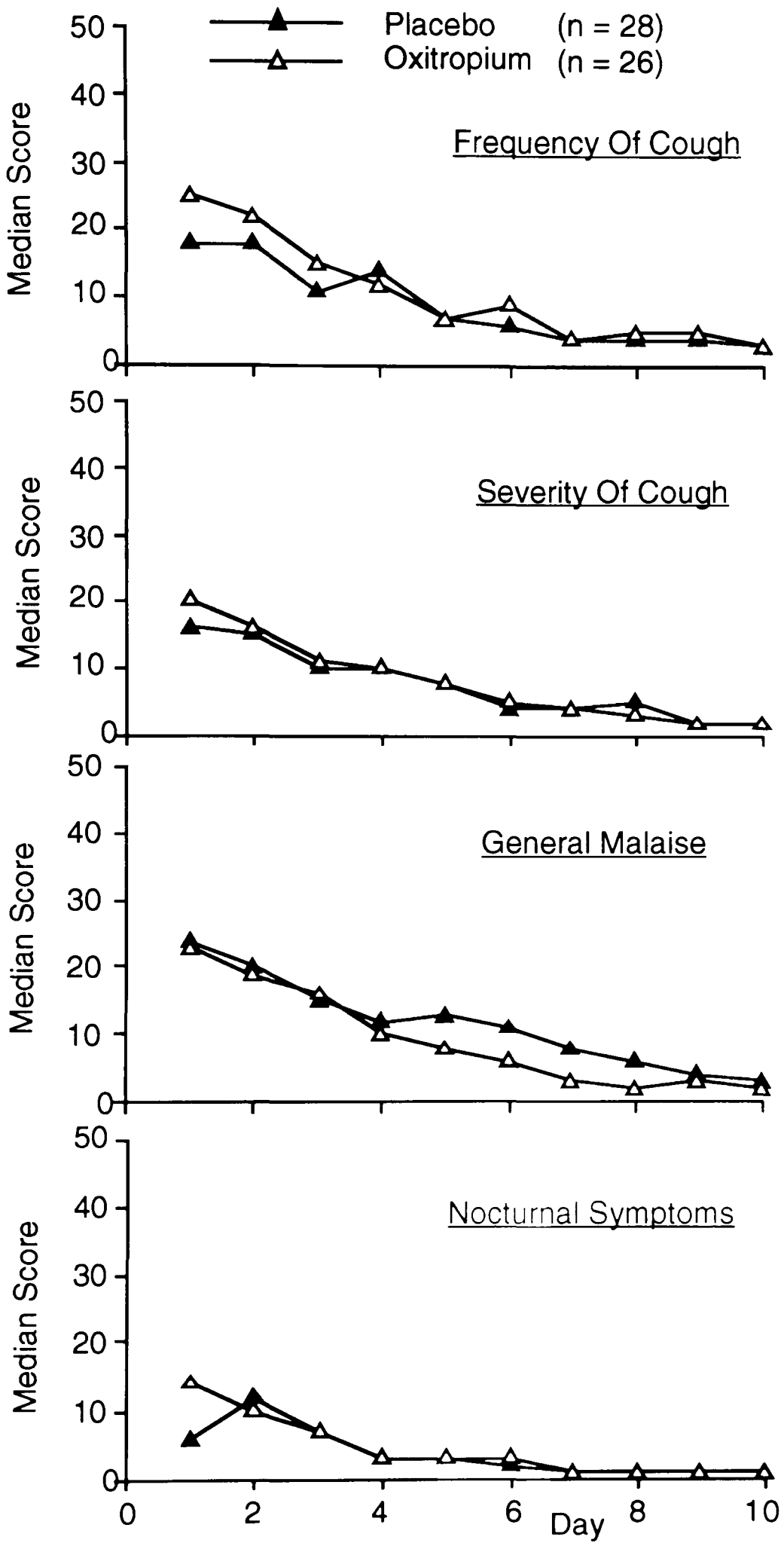


FIGURE 7.2: The Diary Recordings of VAS



The median VAS score recorded daily on a scale from 0 to 50. The higher the score, the more severe the symptom was. Although the scores decrease over the study, oxitropium failed to enhance the improvement.

7.5 DISCUSSION

This study describes the daily time-course of cough and pulmonary function (as measured by PEFR) associated with an upper respiratory tract infection in a large sample of otherwise healthy volunteers. Although cough improved steadily over the 10 days of study as expected, treatment with oxitropium bromide failed to enhance the reduction in either the frequency or severity of cough. A similar study also found no effect on cough during URTI with another anticholinergic therapy, ipratropium bromide (Salzman *et al.*, 1990). This lack of effect of oxitropium on naturally occurring cough differs from the study of UNDW-induced cough, where oxitropium was highly effective in inhibiting cough in healthy and asthmatic volunteers (Section 4.4.2 (c) & 4.4.4 (b)). The reason for this may be that different afferent pathways are involved in cough induced by UNDW and URTI or that the mechanism of bronchodilator inhibition of UNDW-induced cough in ineffective against URTI-associated cough.

Whilst UNDW-induced cough is thought to be mediated by 'low chloride' stimulation of RARs, the stimulus for URTI-induced cough may be mechanical deformation of the airway epithelium by mucus or postnasal drip (Curley *et al.*, 1988) and/or exposure and sensitisation of RARs as a result of epithelial damage (Empey *et al.*, 1976). This could lead to a transient mild airway hyperreactivity (Empey *et al.*, 1976; Hall & Hall, 1979). This study found that PEFR appeared to be reduced during infection, with a clinically small but statistically significant diurnal variation. This could reflect hyperresponsiveness, although the overall increase in PEFR over the treatment period may be due to a learning effect on the measurement. The fact that oxitropium, a long-acting bronchodilator, failed to affect PEFR, supports this view. An alternative explanation, is that a reduction in PEFR without a corresponding decrease in FEV1 may

suggest an extra-thoracic airway obstruction, which has been previously observed during URTI (Curley *et al.*, 1988), and this would not be expected to respond to bronchodilator therapy.

However, recent theories of cough and hyperresponsiveness during URTI have focused on the possible decreased degradation of sensory neuropeptides during URTI (Borson *et al.*, 1989; Jacoby *et al.*, 1988). Levels of neutral endopeptidase (NEP), which degrades neuropeptides can be reduced by 40% in the airway mucosa during experimental viral infections (Dusser *et al.*, 1989) and inhibitors of endopeptidase potentiate coughing in response to capsaicin (Kohrogi *et al.*, 1988). Similarly, viral infection appears to enhance airway responses to substance P and capsaicin (Dusser *et al.*, 1989). These results suggest that cough associated with URTI may be due to stimulation of unmyelinated C-fibre afferents, rather than RARs by increasing the levels of sensory neuropeptides in the airways. This would explain the lack of effect of oxitropium on cough associated with URTI since bronchodilators appear to inhibit cough mediated by RARs but not by C-fibres (Section 5.3.2).

An alternative explanation for the difference in UNDW- and URTI-induced cough is that exposure and sensitisation of RARs during URTI may mask an inhibitory action of bronchodilators. A topical effect of bronchodilators on airway epithelium inhibiting access to paracellular RARs through the 'tight' junctions would be ineffective in the presence of epithelial damage.

This study demonstrates that antitussive efficacy demonstrated using induced cough studies may not be indicative of efficacy against naturally occurring cough.

CHAPTER 8: DISCUSSION

The use of 'low chloride' aerosols such as UNDW to evoke cough represents a novel method of studying the human cough reflex *in vivo*. The cough response is prone, however, to intersubject variation, with cough being evoked in approximately 80% of subjects. The reason for this variation may lie within the central control mechanisms which may modify cough (Banner, 1988). A small learning effect on cough frequency after subjects are first exposed to the stimulus may also occur but thereafter cough appears to be reproducible within subjects between challenges performed on separate days. Adaptation of cough occurs during a one minute challenge which can reduce the response to successive challenges. These findings have implications for the design of studies investigating alterations in cough response. The intersubject variation suggests the use of crossover rather than parallel group trials to identify differences within subjects, while the possibility of a learning effect warrants the use of 'trained' subjects. The adaptation of cough that occurs suggests the use of a single challenge to evoke cough rather than performing dose response challenges and that the treatments be administered on separate days. The use of similarly designed protocols in this thesis has demonstrated that the model is capable of identifying antitussive activity of drugs compared with placebo.

The model has also been used successfully to investigate the afferent pathway of cough and has found that UNDW-induced cough results from the lack of chloride rather than the low osmolarity of water. Extremes of pH and marked hyperosmolarity of inhaled aerosols were further stimuli for cough provocation. The rapid adaptation of UNDW-induced cough implies an involvement of RARs, while the preferential induction of cough with large particle aerosols suggests that these RARs

are located primarily within the central airways. A comparison of these results with animal studies (described in detail in Section 3.4), in particular, the apnoeic reflex in neonatal puppies (Boggs & Bartlett, 1982), suggests that a group of RARs responsive primarily to a low chloride concentration of the ASL may exist within human large airway epithelium which mediate cough.

On the other hand, the bronchoconstrictor response to UNDW in asthmatics is thought to result from the low osmolarity (Schoeffel *et al.*, 1981; Eschenbacher *et al.*, 1984) and may be mediated by another sub-group of RARs or C-fibres that are sensitive to changes in osmolarity of the ASL (Sant'Ambrogio *et al.*, 1991; Pisarri *et al.*, 1992). Marked bronchoconstriction induced by LTD_4 (Section 4.6) appears to be associated with coughing possibly by mechanical distortion of the airway epithelium. Histamine is reported to stimulate RARs in this way (Mills *et al.*, 1969) but may also have an additional direct effect on RARs (Vidruk *et al.*, 1977). Thus RAR-mediated cough appears to be induced by two mechanisms, direct stimulation of RARs and indirectly through marked bronchoconstriction.

Despite the fact that UNDW is not associated with bronchoconstriction in normal subjects, inhaled bronchodilators were remarkably effective at inhibiting UNDW-induced cough. That their effect was independent of the small degree of bronchodilation achieved in both healthy and asthmatic volunteers, suggests that their antitussive efficacy is independent of their action on airway smooth muscle. A comparison of bronchodilators on cough induced by other stimuli (citric acid, capsaicin and URTI), however, failed to corroborate their antitussive efficacy. This suggests that antitussive activity may be stimulus dependent and may explain the variable efficacy of antitussives on induced cough observed by other investigators described previously. The specificity of bronchodilators

for UNDW-induced cough implies that their mechanism of action is dependent on the mechanism of cough induction with UNDW, i.e. the low concentration of chloride in the ASL. The protective effect of bronchodilators on 'low chloride' sensitive RARs could result from a reduced permeability of the paracellular spaces where the RARs are located or from a direct inhibitory action on RARs. Although there is little evidence to support a direct inhibition of RARs, inhibition of ion co-transport in afferent nerves has been suggested as a possible mechanism of action for frusemide (Sant'Ambrogio *et al.*, 1993), which also inhibits UNDW-induced cough. A reduction in the permeability of the paracellular spaces could be ineffective against citric acid and URTI associated cough where disruption of the epithelium may occur. However, against this hypothesis is the observation that the inhibition of UNDW-induced cough by bronchodilators was still apparent in mild asthmatics, where the epithelium may be damaged (Laitinen, 1985). Thus, it is possible that these stimuli may induce cough by mechanisms other than this group of RARs. Capsaicin and PGE_2-induced cough certainly differs from that induced by UNDW, being independent of the chloride concentration and may be mediated by C-fibres. Although the precise location of C-fibres is not known, they may also lie within the paracellular spaces and would also be expected to be inhibited by a reduced paracellular pathway.

Differential antitussive activity was also observed with nedocromil sodium which inhibited cough induced by UNDW and capsaicin but not by citric acid or PGE_2. This suggests that in addition to the proposed inhibition of capsaicin-sensitive C-fibres (Barnes, 1993), nedocromil may also inhibit 'low chloride' RARs. Further differences between the cough responses induced by these various stimuli were identified by studying their properties of adaptation and cross-adaptation. UNDW and PGE_2 but not capsaicin, exhibited a rapid adaptation of cough and PGE_2 inhibited

the subsequent cough response to UNDW. The lack of cross-adaptation suggests that all 3 stimuli evoke cough via distinct afferent pathways. The differential responses of capsaicin and PGE_2 to adaptation and antitussives may reflect selectivity to sub-groups of C-fibres.

A surprising finding in this thesis was the lack of effect of codeine on induced cough. This suggests that the mechanism of antitussive action of opiates is not central, but rather peripheral, perhaps inhibiting C-fibres which would explain their inhibition of capsaicin-induced cough (Fuller *et al.*, 1988). This aspect requires further investigation.

The use of induced cough provides an important tool with which to study the mechanisms of cough induction and suppression. A wide range of stimuli are now being used by several investigators to induce cough including citric, acetic and tartaric acids, capsaicin and prostaglandins (Fujimura *et al.*, 1992 a & b; Hansson *et al.*, 1988; Choudry *et al.*, 1989). However, the lack of correlation of the antitussive efficacy of bronchodilators between UNDW-induced cough and cough associated with URTI (Section 7.4) suggests that the clinical relevance of antitussive studies using induced cough may not be applicable in all cases. The significance of this is illustrated by the fact that the Food and Drug Administration in the USA accept data from such studies of induced cough, in addition to clinical trials, as supportive evidence for antitussive registration (Food & Drug Administration, 1976). Capsaicin-induced cough, which is resistant to many agents that are also clinically ineffective as antitussives (Fuller, 1991) may be more representative of pathological cough than UNDW. Although the stimuli for pathological cough are not known, release of neuropeptides which mediate neurogenic inflammation by antidromic stimulation of nonadrenergic, noncholinergic nerves, may be involved. These neuropeptides include substance P and are degraded by an enzyme, neutral endopeptidase (enkephalinase) (NEP) which is

present within airway epithelium where it may correspond to the putative 'epithelium relaxant factor' and may also be present within vagal afferent nerves (Nadel, 1991). URTI exaggerates neurogenic inflammation and is associated with reduced levels of NEP (Jacoby *et al.*, 1988; Dusser *et al.*, 1989). Furthermore, NEP inhibitors have been found to potentiate capsaicin-induced cough in guinea-pigs (Kohrogi *et al.*, 1988). This suggests that the cough associated with inflammatory processes may be mediated by neuropeptides and requires further investigation.

Since the optimum site of action for antitussives probably lies within the afferent limb of the cough reflex, the possibility of delivering peripherally acting antitussives direct to the cough sensitive airways should also be considered. Treatments for asthma have for many years been administered by inhalation to maximise their effectiveness and minimise systemic effects. Such principles can also be applied to the treatment of cough.

APPENDIX 1 - Characterisation Of The Cough Reflex

Raw Data And Anova Tables

3.3.1 Chemical Sensitivity:

Raw Data Table

Cough Frequencies (coughs/min)

	Urea	UNDW	Sodium Acetate	D-Glucose	Sodium Bicarbonate	Sodium Chloride
1	10	6	7	7	0	0
	7	14	11	6	6	0
2	11	12	22	18	17	0
	16	15	20	16	17	0
3	5	14	17	11	2	0
	3	5	7	6	3	0
4	21	16	7	18	8	0
	10	16	11	12	11	0
5	8	12	8	2	16	0
	14	9	6	6	6	0
6	0	0	0	0	0	0
	0	0	0	0	0	0
7	14	18	0	7	5	0
	7	40	22	13	4	0
8	5	20	1	18	21	0
	14	7	14	5	8	0
9	16	11	24	16	11	0
	0	11	9	6	24	0
10	0	0	0	0	0	0
	0	0	0	3	0	0

ANOVA Table

Transformed Cough Frequencies (excluding sodium chloride)

Source of Variation	df	SSq	MSq	F
Between Subjects	9	79.21	8.80	10.17 $p<0.001$
Between Challenges	4	3.36	0.84	0.97 $p>0.05$
Interaction	36	39.72	1.10	1.27 $p>0.05$
Within	50	43.27	0.87	
TOTAL	99	165.56		

SE = 0.29

95% CL = 0.59

3.3.2 Anion Sensitivity:

Raw Data Table

Cough Frequencies (coughs/min)

Chloride Concentration (mmol/l)

	147	112	75	53	31
1	0	6	17	18	30
	0	0	11	15	26
2	0	0	10	12	33
	0	0	0	10	17
3	0	0	0	6	14
	0	0	4	6	14
4	0	0	0	6	22
	0	0	9	7	8
5	0	0	0	0	21
	0	0	0	12	23

ANOVA Table

Transformed Cough Frequencies

Source of Variation	df	SSq	MSq	F
Between Subjects	4	10.85	2.71	4.87 $p<0.01$
Between Challenges	4	88.25	22.06	39.57 $p<0.001$
Interaction	16	7.48	0.47	0.84 $p>0.05$
Within	25	13.94	0.56	
TOTAL	49	120.53		

3.3.3 pH Sensitivity:

Raw Data Table

	Cough Frequencies (coughs/min)			
		pH		
Subject	2.6	4.8	8.0	10.0
1	2	0	0	5
2	5	0	0	10
3	0	0	0	2
4	4	0	0	7
5	4	0	0	0
6	2	0	0	6
7	0	0	0	0

ANOVA Table

pH 2.6 & 10.0 only

Source of Variation	df	SSq	MSq	F
Between Subjects	6	5.29	0.88	2.96 NS
Between pH	1	0.48	0.48	1.60 NS
Residual	6	1.79	0.30	
TOTAL	13	7.55		

SE = 0.206

95%CL = 0.504

3.3.4 Osmolarity Sensitivity:

Raw Data Table

Cough Frequencies (coughs/min)

Sodium Chloride					D-Glucose				
Osmolarity (mosmol/l)									
77	154	308	616	1232	77	154	308	616	1232
7	0	0	0	1	7	25	28	15	20
22	4	0	0	0	27	21	26	22	31
8	2	0	0	3	14	14	16	19	26
11	0	0	0	0	6	2	0	2	6
13	15	0	0	10	3	12	10	18	11
4	1	0	0	0	5	3	19	5	7
0	0	0	0	0	1	2	2	8	0

ANOVA Table

Transformed Cough Frequency

Source of Variation	df	SSq	MSq	F
D-Glucose				
Between Subjects	6	49.16	8.19	13.83
Between Challenges	4	2.51	0.63	1.06 p>0.05
Residual	24	14.22	0.59	
TOTAL	34	65.90		

SE = 0.29

95% CL = 0.60

Sodium Chloride (excluding 308 & 616 data which did not cause cough)				
Between Subjects	6	13.77	2.3	4.2 p<0.02
Between Challenges	2	8.81	4.40	8.1 p<0.01
Residual	12	6.51	0.54	
TOTAL	20	29.08		

SE = 0.28

95% CL = 0.61

3.3.5 Citric Acid-Induced Cough:

Raw Data Table

Cough Frequencies (coughs/min)				
0.68% Citric Acid in 0.79% NaCl	D-Glucose	Water	Sodium Citrate	Sodium Citrate in NaCl
6	30	26	22	0
8	10	10	6	0
11	26	27	28	0
12	6	6	6	0
23	18	23	17	0
18	23	6	9	0
6	20	20	7	0

ANOVA Table

Transformed Cough Frequencies (excluding sodium chloride)

Source of Variation	df	SSq	MSq	F
Between Subjects	6	14.29	2.38	3.42 $p<0.05$
Between Challenges	3	3.15	1.05	1.51 $p>0.05$
Residual	18	12.55		
TOTAL	27	29.98		

SE = 0.32

95% CL = 0.66

3.3.6 Particle Size Dependence:

ANOVA Table

Transformed Cough Frequencies (coughs/min)

Source of Variation	df	SSq	MSq	F
Between Subjects	11	14.64	1.33	1.39 p>0.05
Between U/S vs Jet	1	32.78	32.78	34.2 p<0.001
Residual	11	10.54	0.96	
TOTAL	23	57.96		

SE = 0.28

95%CL = 0.62

APPENDIX 2 - The Effect Of Altering Airway Tone On Cough Raw Data And Anova Tables

4.4.1**(a)** Inhaled Fenoterol Hydrobromide:

Raw Data Tables

Cough Frequencies (coughs/min)

	Chloride Concentration (mmol/l)									
	0		31		75		112		150	
	P	A	P	A	P	A	P	A	P	A
1	9	0	0	0	0	0	0	0	0	0
2	14	12	10	12	2	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	14	4	11	4	6	2	0	0	0	0
5	17	10	11	0	0	0	0	0	0	0
6	13	0	0	0	0	0	0	0	0	0
7	15	10	8	0	0	0	0	0	0	0
8	1	0	2	0	0	0	0	0	0	0
9	15	0	13	0	1	0	0	0	0	0
10	9	5	6	1	0	0	0	0	0	0
11	42	12	23	10	3	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	28	24	18	9	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	2	0	1	0	0	0	0	0	0	0
16	27	13	4	24	15	0	0	0	0	0
17	25	17	7	18	0	0	0	0	0	0
18	12	8	11	5	0	0	0	0	0	0
19	12	6	1	5	3	0	0	0	0	0
20	27	17	19	2	8	0	2	0	0	0

4.4.1**(a)** Inhaled Fenoterol Hydrobromide:

ANOVA Table

Transformed Cough Frequencies (excluding 112 & 150 data)

Source of Variation	df	SSq	MSq	F
Between Subjects	19	92.1	4.85	8.49 p<0.01
Between Challenges	2	61.6	30.82	31.2 p<0.01
Between Treatments	1	15.0	15.00	23.2 p<0.01
Subjects x Challenges	38	37.5	0.99	1.73 NS
Subjects x Treatment	19	12.3	0.65	1.13 NS
Challenge x Treatments	2	2.19	1.10	1.92 NS
Residual	38	21.7	0.57	
TOTAL	119	242.4		

SE = 0.17

95% CL = 0.34

4.4.1**(b)** Oral Salbutamol Sulphate:

Cough Frequencies (coughs/min)

	Chloride Concentration (mmol/l)							
	0		31		75		150	
	P	A	P	A	P	A	P	A
1	13	35	32	0	0	1	0	0
2	2	18	2	3	0	0	0	0
3	16	13	9	9	0	0	0	0
4	18	19	14	8	9	4	0	0
5	12	9	0	0	0	0	0	0
6	42	37	24	25	0	0	0	0
7	3	1	0	0	0	0	0	0
8	6	0	0	0	0	0	0	0
9	12	5	7	14	0	0	0	0
10	29	4	16	3	0	0	0	0
11	0	0	0	0	0	0	0	0

ANOVA Table

Transformed Cough Frequencies (excluding 150 mmol/l data)

Source of Variation	df	SSq	MSq	F
Between Subjects	10	60.51	6.05	6.07 p<0.01
Between Challenges	2	55.20	27.60	18.16 p<0.01
Between Treatments	1	1.42	1.42	2.18 NS
Subjects x Challenges	20	30.38	1.52	1.52 NS
Subjects x Treatment	10	6.54	0.65	<1 NS
Challenges x Treatments	2	0.78	0.39	<1 NS
Residual	20	19.95	1.00	
TOTAL	65	174.78		

SE = 0.30

95% CL = 0.63

4.4.2 **(a)** Inhaled Ipratropium Bromide:

Raw Data Table

Cough Frequencies (coughs/min)

	Chloride Concentration (mmol/l)							
	0		31		75		150	
	P	A	P	A	P	A	P	A
1	26	6	8	0	4	0	0	0
2	32	0	6	0	0	0	0	0
3	15	4	4	2	0	0	0	0
4	17	8	15	8	0	0	0	0
5	29	7	16	9	0	0	0	0
6	0	0	0	0	0	0	0	0
7	26	7	20	0	5	0	0	0
8	22	4	0	3	0	0	0	0
9	0	0	0	0	0	0	0	0
10	21	0	0	0	0	0	0	0
11	5	8	2	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	8	1	0	0	0	0	0	0
14	11	15	0	5	0	0	0	0

ANOVA Table

Transformed Cough Frequencies (excluding 150 mmol/l data)

Source of Variation	df	SSq	MSq	F	
Between Subjects	13	32.72	25.20	5.23	p<0.01
Between Challenges	2	47.97	23.99	31.7	p<0.01
Between Treatments	1	14.07	14.07	11.78	p<0.01
Subjects x Challenges	26	19.67	0.76	1.57	NS
Subjects x Treatment	13	15.52	1.19	2.48	p<0.05
Challenges x Treatments	2	8.55	4.27	8.89	p<0.01
Residual	26	12.50	0.48		
TOTAL	83	150.99			

4.4.2**(b)** Oral Pirenzepine Hydrochloride:

Raw Data Table

Cough Frequencies (coughs/min)

	Chloride Concentration (mmol/l)							
	0		31		75		150	
	P	A	P	A	P	A	P	A
1	0	3	0	8	0	0	0	0
2	26	26	11	8	0	0	0	0
3	8	6	0	3	0	0	0	0
4	9	3	15	4	0	0	0	0
5	22	14	0	22	0	0	0	0
6	22	15	10	6	0	0	0	0
7	14	4	10	0	4	0	0	0
8	15	13	2	9	0	0	0	0
9	11	0	9	0	0	1	0	0
10	33	13	28	5	0	0	0	0
11	10	16	2	11	0	0	0	0
12	32	0	6	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	7	14	0	0	0	0	0	0

ANOVA Table

Transformed Cough Frequencies (excluding 150 mmol/l data)

Source of Variation	df	SSq	MSq	F	
Between Subjects	13	30.87	2.37	3.22	p<0.01
Between Challenges	2	70.88	35.44	34.20	p<0.01
Between Treatments	1	2.47	2.47	1.44	NS
Subjects x Challenges	26	26.95	1.04	1.41	NS
Subjects x Treatment	13	22.24	1.71	2.32	p<0.05
Challenges x Treatments	2	2.49	1.24	1.69	NS
Residual	26	19.10	0.74		
TOTAL	83	175.05			

4.4.2 **(c)** & 4.4.4 **(b)** Inhaled Oxitropium Bromide, Ipratropium Bromide and a Combination of Ipratropium and Fenoterol Hydrobromide in Healthy and Asthmatic Subjects:

Raw Data Tables

Healthy Subjects

Cough Frequencies (coughs/min)

	Placebo			Oxitropium			Ipratropium			Ip/Fen		
	Chloride Concentration (mmol/l)											
	55	31	0	55	31	0	55	31	0	55	31	0
1	11	8	15	1	2	7	2	1	5	0	2	5
2	6	0	14	2	3	10	2	3	8	0	0	4
3	3	5	9	3	0	6	2	3	4	0	3	3
4	3	0	6	0	2	2	3	0	0	0	0	0
5	3	2	30	5	3	7	12	5	36	3	16	35
6	6	0	12	0	0	2	0	0	0	0	0	0
7	0	7	13	0	4	12	15	0	16	2	0	9
8	6	6	21	0	7	13	0	16	15	0	0	2
9	10	4	9	2	0	0	2	2	6	0	2	0
10	2	8	23	0	0	5	0	0	1	1	2	6
11	2	5	14	0	0	0	0	0	0	0	0	4
12	6	4	14	10	0	8	0	0	10	4	6	12
13	0	2	5	0	4	7	0	0	4	0	2	2
14	2	1	8	0	3	10	0	3	8	0	0	8
15	4	7	14	4	12	24	1	8	19	5	4	10
16	3	2	8	0	0	7	0	0	0	2	0	0

4.4.2 **(c)** & 4.4.4 **(b)** Raw Data Tables continued

Asthmatic Subjects

Cough Frequencies (coughs/min)

	Placebo			Oxitropium			Ipratropium			Ip/Fen		
	Chloride Concentration (mmol/l)											
	55	31	0	55	31	0	55	31	0	55	31	0
1	0	0	12	0	0	0	0	5	8	0	0	0
2	0	0	0	1	1	0	2	0	0	2	0	2
3	0	0	16	0	1	15	0	0	17	0	0	1
4	0	0	1	0	0	0	0	0	0	0	0	1
5	9	8	7	0	0	10	0	3	9	1	0	2
6	0	1	8	3	0	0	3	0	0	1	0	0
7	0	0	7	0	0	4	3	0	3	0	0	0
8	1	0	5	1	0	6	3	0	5	1	0	0
9	6	0	8	7	4	9	1	4	6	4	6	14
10	0	0	5	0	0	0	2	0	0	0	0	0

4.4.2 **(c)** & 4.4.4 **(b)** continued

ANOVA Tables

Healthy Subjects Only (transformed cough frequencies)

Source of Variation	df	SSq	MSq	F	
Between Subjects	15	59.93	4.00		
Between Periods	3	1.03	0.34	<1	
Between Treatments	3	20.88	6.96	8.52	p<0.01
Residual	42	34.31	0.82		
Between Challenges	2	58.39	29.19	57.92	p<0.01
Linear	1	47.74	47.74	94.71	p<0.01
Quadratic	1	10.65	10.65	21.12	p<0.01
Challenges x Treatments	6	4.50	0.58	1.16	
Residual	120	60.48	0.50		
TOTAL	191	238.52			

4.4.2 **(c)** & 4.4.4 **(b)** ANOVA Tables continued

Healthy and Asthmatic Groups (transformed data)

Source of Variation	df	SSq	MSq	F	
Between Groups (G)	1	20.29	20.29	6.05	p<0.05
Residual	24	80.56	3.36		
Between Treatments (T)	3	20.89	6.96	10.86	p<0.01
G x T	3	3.26	1.09	1.70	
Residual	72	46.18	0.64		
Between Challenges (C)	2	71.29	35.64	45.69	p<0.01
Linear	1	53.95	53.95	69.16	p<0.01
Quadratic	1	17.34	17.34	22.22	p<0.01
G x C	2	3.43	1.71	2.20	
Residual	48	37.44	0.78		
T x C	6	6.74	1.12	3.00	p<0.01
T x C linear	3	3.55	1.18	3.16	p<0.05
T x C quadratic	3	3.19	1.07	2.85	p<0.05
G x T x C	6	0.58	0.10	<1	
Residual	144	53.88	0.37		
Total	311	344.55			

4.4.1 **(c)** & 4.4.4 **(a)** Inhaled Salbutamol and Procaterol Hydrochloride in Healthy and Asthmatic Subjects:

Raw Data Tables

Healthy Subjects

Cough Frequencies (coughs/min)

Subject	Placebo	Procaterol		Salbutamol
		(10µg)	(20µg)	(200µg)
1	6	1	0	0
2	26	2	2	6
3	7	4	1	4
4	16	2	0	0
5	9	2	1	3
6	13	0	0	0
7	12	1	0	4
8	14	0	3	0
9	10	1	0	0
10	8	6	7	4
11	27	3	1	1
12	6	0	2	0
13	10	4	3	3
14	14	4	1	3
15	5	0	3	2
16	26	12	0	9
17	8	6	0	0
18	9	10	9	1
19	11	10	3	0
20	0	0	0	5

4.4.1 **(c)** & 4.4.4 **(a)** Raw Data Tables continued

Healthy Subjects

FEV_1 (l)

SUB J	PLACEBO			PROCATEROL 20mcg			PROCATEROL 10mcg			SALBUTAMOL		
	BASE LINE	POST TREAT	POST CHAL	BASE LINE	POST TREA	POST CHALL	BASE LINE	POST TREAT	POST CHAL	BASE LINE	POST TREAT	POST CHAL
1	2.25	2.25	2.20	2.20	2.30	2.25	2.30	2.35	2.30	2.15	2.35	2.35
2	2.60	2.60	2.60	2.50	2.60	2.60	2.55	2.60	2.55	2.60	2.60	2.60
3	2.95	2.90	2.80	2.95	2.90	2.90	2.90	2.90	2.80	3.00	2.90	2.90
4	2.80	2.80	2.80	2.80	2.85	2.85	2.85	2.80	2.80	2.75	2.80	2.85
5	2.35	2.40	2.40	2.30	2.45	2.45	2.35	2.45	2.45	2.40	2.50	2.50
6	3.10	3.00	3.15	3.05	3.20	3.20	3.00	3.15	3.20	3.10	3.05	3.15
7	2.55	2.70	2.60	2.50	2.60	2.50	2.70	2.50	2.50	2.55	2.50	2.60
8	3.45	3.40	3.20	3.40	3.50	3.50	3.35	3.50	3.55	3.35	3.55	3.65
9	4.85	4.75	4.65	5.00	5.10	5.20	4.80	4.95	5.00	5.00	5.15	5.30
10	2.75	2.85	2.70	2.85	2.95	2.95	2.80	2.95	2.85	2.65	2.85	2.85
11	3.80	3.75	3.80	3.80	3.70	3.90	3.80	3.90	3.95	3.80	3.95	3.90
12	3.55	3.65	3.75	3.50	3.65	3.75	3.60	3.80	3.90	3.55	3.65	3.70
13	4.35	4.60	4.50	4.35	4.60	4.60	4.60	4.75	4.75	4.40	4.55	4.70
14	3.65	3.60	3.70	3.65	3.65	3.75	3.60	3.70	3.75	3.45	3.50	3.55
15	4.35	4.30	4.45	4.25	4.30	4.40	4.35	4.30	4.30	4.05	4.40	4.30
16	3.80	3.75	3.80	3.75	3.70	3.75	3.70	3.75	3.75	3.75	3.90	3.80
17	1.50	1.30	1.40	1.55	1.55	1.45	1.50	1.50	1.55	1.45	1.40	1.50
18	4.10	4.10	4.10	4.20	4.45	4.45	4.00	4.35	4.40	4.20	4.40	4.45
19	2.85	2.95	2.95	2.75	2.95	2.85	2.65	2.95	2.95	2.80	2.80	2.85
20	3.50	3.45	3.45	3.40	3.50	3.50	3.35	3.40	3.50	3.25	3.40	3.45

4.4.1 **(c)** & 4.4.4 **(a)** Raw Data Tables continued

Healthy Subjects

R_{aw} (kPa/l/s)

SUB	PLACEBO			PROCATEROL 10 µg			PROCATEROL 20 µg			SALBUTAMOL		
	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL
1	0.43	0.38	0.40	0.38	0.37	0.28	0.51	0.42	0.38	0.46	0.42	0.33
2	0.29	0.39	0.35	0.29	0.26	0.29	0.31	0.28	0.25	0.26	0.22	0.24
3	0.42	0.30	0.31	0.26	0.32	0.34	0.31	0.28	0.30	0.52	0.33	0.35
4	0.30	0.25	0.23	0.25	0.25	0.26	0.31	0.29	0.29	0.24	0.26	0.25
5	0.55	0.56	0.63	0.44	0.51	0.39	0.45	0.52	0.45	0.36	0.33	0.39
6	0.34	0.24	0.29	0.25	0.24	0.25	0.26	0.27	0.26	0.34	0.23	0.23
7	0.49	0.43	0.39	0.48	0.42	0.40	0.40	0.32	0.31	0.41	0.32	0.34
8	0.43	0.41	0.44	0.45	0.39	0.35	0.33	0.26	0.36	0.51	0.42	0.31
9	0.33	0.27	0.31	0.28	0.20	0.22	0.20	0.20	0.20	0.31	0.22	0.21
10	0.34	0.40	0.39	0.35	0.38	0.35	0.37	0.35	0.33	0.35	0.34	0.37
11	0.25	0.24	0.26	0.26	0.22	0.19	0.22	0.19	0.21	0.25	0.21	0.20
12	0.32	0.39	0.32	0.34	0.32	0.26	0.29	0.25	0.28	0.34	0.31	0.32
13	0.16	0.17	0.17	0.16	0.14	0.14	0.16	0.14	0.14	0.14	0.11	0.11
14	0.20	0.19	0.23	0.18	0.19	0.19	0.20	0.16	0.18	0.21	0.18	0.16
15	0.30	0.24	0.29	0.23	0.25	0.25	0.28	0.30	0.24	0.28	0.28	0.24
16	0.26	0.22	0.25	0.26	0.21	0.26	0.33	0.31	0.22	0.27	0.25	0.25
17	0.63	0.66	0.48	0.40	0.38	0.39	0.49	0.46	0.37	0.38	0.37	0.37
18	0.15	0.20	0.22	0.15	0.13	0.11	0.14	0.14	0.14	0.17	0.13	0.14
19	0.33	0.24	0.31	0.32	0.29	0.26	0.25	0.25	0.26	0.31	0.25	0.30
20	0.22	0.24	0.26	0.25	0.22	0.25	0.26	0.21	0.25	0.22	0.21	0.25

4.4.1 **(c)** & 4.4.4 **(a)** Raw Data Tables continued

Asthmatic Subjects

		Cough Frequencies (coughs/min)		
SUBJ	PLACEBO	PROCATEROL		SALBUTAMOL
		(10 µg)	(20 µg)	(200 µg)
1	11	4	5	3
2	11	0	4	12
3	8	0	2	0
4	13	5	6	7
5	32	14	4	2
6	19	2	0	5
7	6	0	0	0
8	9	0	0	0
9	22	8	0	0
10	7	3	2	2
11	16	2	0	0
12	15	7	5	5
13	15	8	9	11
14	24	11	11	12
15	16	9	8	0
16	24	16	17	16
17	0	0	0	0
18	11	7	0	7
19	17	9	15	10
20	5	0	2	4

4.4.1 **(c)** & 4.4.4 **(a)** Raw Data Tables continued

Asthmatic Subjects

FEV_1 (l)

SUB	PLACEBO			PROCATEROL 20µg			PROCATEROL 10 µg			SALBUTAMOL		
	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL
1	4.05	3.90	3.70	4.00	4.05	4.00	4.00	4.00	4.00	4.10	4.00	4.00
2	2.75	2.70	2.40	2.40	3.00	3.00	2.40	2.95	2.75	2.75	3.10	3.05
3	3.00	2.80	2.85	2.95	3.15	3.25	2.90	3.15	3.30	2.95	3.25	3.30
4	1.70	1.95	1.80	2.10	2.15	2.35	2.20	2.10	2.15	2.00	2.30	2.30
5	3.25	3.40	3.20	3.40	3.50	3.60	3.45	3.45	3.40	3.35	3.50	3.40
6	3.50	3.40	3.50	3.40	3.50	3.45	3.45	3.50	3.60	3.70	3.90	3.80
7	2.45	2.55	2.15	2.70	3.15	3.15	2.65	3.00	2.90	2.50	2.95	2.95
8	3.35	3.25	3.05	3.30	3.50	3.70	3.50	3.55	3.60	3.50	3.65	3.60
9	3.25	3.05	2.55	3.20	3.05	3.10	3.30	3.05	3.15	3.10	3.15	3.15
10	3.40	3.30	3.50	3.40	3.50	3.50	3.30	3.45	3.65	3.50	3.55	3.55
11	4.80	4.70	4.40	4.90	4.95	4.95	4.60	4.80	4.90	4.70	4.85	5.00
12	4.10	4.10	3.80	4.25	4.25	4.25	4.00	4.15	4.10	3.80	4.10	4.10
13	4.75	4.80	4.55	4.65	5.30	5.30	4.90	5.00	5.00	4.80	5.15	5.00
14	3.85	3.90	4.00	4.00	4.00	4.00	4.10	3.95	4.10	4.00	4.15	4.10
15	1.80	1.55	0.75	1.80	2.70	2.65	1.65	2.40	2.50	1.90	2.40	2.55
16	2.95	2.95	2.90	3.10	3.10	3.10	2.95	3.10	3.05	3.05	3.20	3.20
17	2.50	2.55	2.30	2.25	2.55	2.75	2.30	2.70	2.80	2.15	2.50	2.60
18	3.20	3.20	3.00	3.25	3.35	3.35	3.50	3.80	3.70	3.25	3.75	3.60
19	5.45	5.40	5.45	5.50	5.65	5.60	5.45	5.60	5.65	5.50	5.60	5.55
20	4.30	4.50	4.50	4.50	5.00	5.00	4.30	4.95	5.00	4.40	4.90	5.05

4.4.1 **(c)** & 4.4.4 **(a)** Raw Data Tables continued

Asthmatic Subjects

R_{aw} (kPa/l/s)

SUB	PLACEBO			PROCATEROL 10 µg			PROCATEROL 20 µg			SALBUTAMOL		
	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL	BASE LINE	POST TREA	POST CHAL
1	0.33	0.30	0.32	0.25	0.18	0.20	0.31	0.23	0.25	0.28	0.28	0.25
2	0.36	0.39	0.44	0.44	0.33	0.31	0.33	0.34	0.30	0.36	0.30	0.28
3	0.33	0.38	0.46	0.33	0.23	0.24	0.39	0.25	0.29	0.32	0.21	0.22
4	0.39	0.35	0.46	0.42	0.32	0.34	0.31	0.37	0.35	0.35	0.27	0.34
5	0.32	0.34	0.30	0.36	0.38	0.28	0.24	0.23	0.24	0.31	0.28	0.25
6	0.26	0.23	0.24	0.24	0.16	0.19	0.21	0.16	0.15	0.21	0.16	0.16
7	0.48	0.33	0.50	0.33	0.29	0.26	0.28	0.26	0.24	0.31	0.24	0.27
8	0.39	0.41	0.48	0.52	0.30	0.34	0.39	0.30	0.35	0.39	0.34	0.43
9	0.33	0.32	0.39	0.20	0.40	0.35	0.20	0.24	0.33	0.21	0.20	0.35
10	0.36	0.33	0.33	0.40	0.31	0.38	0.33	0.34	0.29	0.32	0.38	0.32
11	0.24	0.28	0.28	0.33	0.24	0.25	0.27	0.26	0.24	0.31	0.24	0.25
12	0.29	0.28	0.33	0.37	0.36	0.36	0.23	0.23	0.30	0.39	0.31	0.26
13	0.21	0.17	0.22	0.18	0.14	0.17	0.33	0.15	0.15	0.18	0.15	0.15
14	0.32	0.26	0.29	0.32	0.25	0.25	0.29	0.21	0.34	0.28	0.23	0.27
15	0.98	0.66	1.13	0.89	0.66	0.73	0.66	0.68	0.89	0.77	0.67	0.89
16	0.40	0.39	0.38	0.33	0.35	0.36	0.32	0.33	0.29	0.36	0.33	0.32
17	0.56	0.48	0.70	0.83	0.40	0.32	0.61	0.29	0.27	0.83	0.33	0.44
18	0.34	0.35	0.46	0.34	0.22	0.22	0.39	0.25	0.25	0.46	0.19	0.26
19	0.15	0.12	0.16	0.12	0.14	0.11	0.12	0.12	0.11	0.17	0.13	0.12
20	0.22	0.19	0.20	0.21	0.14	0.13	0.25	0.14	0.14	0.17	0.14	0.12

4.4.1 **(c)** & 4.4.4 **(a)** Inhaled Salbutamol and Procaterol Hydrochloride in Healthy and Asthmatic Subjects:

ANOVA Tables

All Subjects (Including 13 Bronchitic Subjects: not included in this thesis)

Transformed Cough Frequencies
(Square-root (cough +1))

Source of Variation	df	SSq	MSq	F
group ptno stratum				
Between Groups (G)	2	8.00	4.00	1.59 p=0.214
Residual	50	125.78	2.52	
grp ptno trt stratum				
Between Treatments (T)	3	71.68	23.89	38.46 p<0.001
G x T	6	12.49	2.08	3.35 p=0.004
Residual	150	93.18	0.62	
Total	211	311.13		

4.4.1 **(c)** & 4.4.4 **(a)** ANOVA Tables continued

All Subjects (Including 13 Bronchitic Subjects)

		FEV_1			
Source of Variation	df	SSq	MSq	F	
grp.ptno.stratum					
Between Groups (G)	2	544.7	272.4	31.03	p<0.001
Residual	50	438.8	8.776		
grp.ptno.trt stratum					
Between Treatments (T)	3	2.256	0.7521	28.60	p<0.001
G x T	6	1.113	0.1855	7.06	p<0.001
Residual	150	3.944	0.02629		
grp.ptno.occas stratum					
Occasion (O)	2	1.329	0.6647	29.93	p<0.001
G x O	4	0.4365	0.1091	4.91	p<0.001
Residual	100	2.221	0.02221		
grp.ptno.trt.occas stratum					
T x O	6	1.431	0.2386	26.89	p<0.001
G x T x O	12	0.4381	0.03651	4.11	p<0.001
Residual	300	2.662	0.008873		
Total	635	999.3			

4.4.1 **(c)** & 4.4.4 **(a)** ANOVA Tables continued

All Subjects (Including 13 Bronchitic Subjects)

Transformed R_{aw}
(Log (R_{aw} x 100))

Source of Variation	df	SSq	MSq	F	
grp.ptno.stratum					
Between Groups (G)	2	7.53	3.76	11.83	p<0.001
Residual	50	15.91	0.32		
grp.ptno.trt stratum					
Between Treatments (T)	3	0.66	0.22	28.13	p<0.001
G x T	6	0.03	0.00	0.62	p=0.712
Residual	150	1.18	0.01		
grp.ptno.occas stratum					
Between Occasion (O)	2	0.39	0.20	27.31	p<0.001
G x O	4	0.09	0.02	3.28	p=0.014
Residual	100	0.72	0.01		
grp.ptno.trt.occas stratum					
T x O	6	0.16	0.03	8.67	p<0.001
G x T x O	12	0.06	0.00	1.51	p=0.121
Residual	300	0.92	0.00		
Total	635	27.63			

4.4.3 Alteration in Airway Tone Associated with Inhibition of Cough

Raw Data Tables

Cough Frequency Pre- and Post -Treatments (n = 6)

	Pl In		Fen		Pir		Sal		Ip		Pl T	
	Pre	Pos	Pre	Pos	Pre	Pos	Pre	Pos	Pre	Pos	Pre	Pos
1	7	19	15	5	9	10	10	4	9	5	6	5
2	54	48	30	0	28	26	48	31	31	7	31	28
3	18	6	26	9	6	6	16	9	7	5	12	13
4	25	28	33	2	33	31	37	20	28	6	33	30
5	18	18	19	13	25	16	31	23	21	22	23	18
6	21	12	18	3	18	20	19	11	13	9	23	17

4.4.3 Raw Data Tables continued

FEV_1 (n=6)

Time 1=baseline
2=post 1st challenge
3=post treatment
4=post 2nd challenge

Subj	Time	Pl In	Fen	Pir	Sal	Ip	Pl T
1	1	4.57	4.62	4.48	4.37	4.58	4.85
	2	4.63	4.64	4.53	4.28	4.63	4.82
	3	4.59	4.70	4.54	4.62	4.65	4.65
	4	4.59	4.80	4.44	4.45	4.84	4.60
2	1	5.05	5.07	5.11	5.91	4.98	4.96
	2	5.16	4.98	4.90	5.21	4.87	4.84
	3	5.01	5.63	5.04	5.27	5.70	4.59
	4	5.23	5.52	4.80	5.68	5.81	4.81
3	1	3.32	3.52	3.26	3.38	3.26	3.30
	2	3.35	3.46	3.28	3.41	3.25	3.25
	3	3.34	3.57	3.15	3.49	3.42	3.26
	4	3.30	3.72	3.24	3.60	3.50	3.23
4	1	3.32	3.20	3.28	2.89	3.05	3.17
	2	3.27	3.17	3.15	3.02	3.00	3.24
	3	3.34	3.42	3.07	3.19	3.30	3.16
	4	3.31	3.42	3.13	3.11	3.33	3.25
5	1	4.10	3.75	3.96	4.33	3.71	3.94
	2	4.10	3.77	3.96	4.19	3.75	3.91
	3	4.10	3.81	4.11	4.07	3.95	3.85
	4	4.22	3.91	4.06	4.08	3.85	3.85
6	1	3.33	3.46	3.19	3.33	3.44	3.48
	2	3.33	3.48	3.16	3.46	3.42	3.26
	3	3.38	3.70	3.33	3.62	3.56	3.35
	4	3.39	3.71	3.23	3.57	3.61	3.28

4.4.3 Raw Data Tables continued

sG_{aw} (n=6)

Time 1=baseline
2=post 1st challenge
3=post treatment
4=post 2nd challenge

Subj	Time	Pl In	Fen	Pir	Sal	Ip	Pl T
1	1	0.222	0.237	0.187	0.226	0.224	0.214
	2	0.216	0.180	0.175	0.178	0.193	0.223
	3	0.191	0.250	0.214	0.230	0.211	0.239
	4	0.200	0.240	0.213	0.243	0.287	0.241
2	1	0.217	0.194	0.193	0.216	0.208	0.190
	2	0.194	0.172	0.225	0.206	0.175	0.174
	3	0.216	0.310	0.186	0.273	0.291	0.184
	4	0.202	0.300	0.173	0.253	0.340	0.172
3	1	0.165	0.137	0.187	0.147	0.156	0.171
	2	0.181	0.273	0.184	0.119	0.185	0.164
	3	0.180	0.241	0.141	0.174	0.250	0.169
	4	0.169	0.227	0.149	0.170	0.312	0.172
4	1	0.225	0.231	0.207	0.165	0.217	0.158
	2	0.234	0.204	0.170	0.166	0.171	0.155
	3	0.207	0.323	0.187	0.223	0.298	0.193
	4	0.203	0.309	0.170	0.198	0.300	0.153
5	1	0.210	0.251	0.233	0.246	0.207	0.250
	2	0.254	0.219	0.222	0.264	0.255	0.255
	3	0.228	0.241	0.239	0.215	0.279	0.232
	4	0.241	0.237	0.244	0.244	0.270	0.278
6	1	0.186	0.202	0.139	0.177	0.187	0.180
	2	0.149	0.190	0.165	0.165	0.168	0.171
	3	0.173	0.237	0.166	0.188	0.241	0.175
	4	0.131	0.260	0.161	0.192	0.230	0.157

4.4.3 Alteration in Airway Tone Associated with Inhibition of Cough

ANOVA Tables

FEV_1 (n = 6)

Analysis of conductance and FEV_1 without carryover effects

Log (cond 3 / cond 2)

Source	Df	SSq	MSq	F	
Bet Patients	5	0.0610	0.0122	2.708	NS
Bet Periods	5	0.0439	0.0088	1.950	NS
Bet Treatments	5	0.1183	0.0237	5.25	p<0.01
Oral Effects	1	0.0212	0.0212	4.701	p<0.05
Drug	2	0.0508	0.0254	5.639	p<0.05
Interaction	2	0.0463	0.0231	5.133	p<0.05
Residual	20	0.0901	0.0045		
TOTAL	35	0.3133			

Log (FEV_1 3 / FEV_1 2)

Source	Df	SSq	MSq	F	
Bet Patients	5	0.0014	0.0003	1.026	NS
Bet Periods	5	0.0014	0.0003	1.022	NS
Bet Treatments	5	0.0058	0.0012	4.38	p<0.01
Oral Effects	1	0.0017	0.0017	6.448	p<0.05
Drug	2	0.0035	0.0018	6.623	p<0.01
Interaction	2	0.0005	0.0003	1.088	NS
Residual	20	0.0053	0.0027		
TOTAL	35	0.0139			

4.4.3 ANOVA Tables continued

Analysis of cough without carryover effects

(square root cough 1) - (square root cough 3)

Source	Df	SSq	MSq	F	
Bet Patients	5	9.486	1.897	1.873	NS
Bet Periods	5	3.616	0.723	0.714	NS
Bet Treatments	5	30.673	6.135	6.056	p<0.01
Oral	1	6.938	6.938		
Drug	2	10.074	9.537		
Interaction	2	4.661	2.331		
Residual	20	20.252	1.013		
TOTAL	35	64.027			

4.6.2 **(a)** Specific LTD_4 Antagonist, SK&F 104353

Raw Data Tables

LTD_4

Cough frequencies (cough/5 min)

Subject	Placebo	50µg	400µg
13	13	9	1
14	7	2	0
15	49	2	19
16	11	0	2
17	26	15	4
18	44	41	0

LTD_4

FEV_1 (l)

Sub	Placebo				50µg				400µg			
	PRE	POST	5 MIN	POST CH	PRE	POST	5MIN	POST CH	PRE	POST	5 MIN	POST CH
13	3.50	3.35	3.40	2.70	3.60	3.60	3.60	2.60	3.50	3.55	3.50	3.40
14	3.70	3.70	3.70	3.15	3.70	3.50	3.55	3.30	3.70	3.60	3.60	3.55
15	2.85	2.75	2.80	2.10	2.90	2.85	2.80	2.60	2.90	2.80	2.90	2.45
16	3.75	3.85	3.85	3.05	3.65	3.75	3.75	3.15	3.75	3.70	3.80	3.45
17	4.05	4.00	4.10	3.20	4.10	4.00	4.00	3.15	4.10	4.10	4.10	3.90
18	4.10	4.00	4.15	3.40	4.10	4.10	4.10	3.40	4.00	3.90	3.90	3.85

4.6.2 **(a)** Raw Data Tables continued

LTD_4

R_{aw} (kPa/l/s)

Su	Placebo					50µg					400µg				
	PRE	POST	5 MIN	POST CH	5MIN POST	PRE	POST	5MIN	POST CH	5 MIN POST	PRE	POST	5 MIN	POST CH	5 MIN POST
13	0.30	0.16	0.30	0.32	0.63	0.27	0.24	0.23	0.32	0.99	0.30	0.19	0.15	0.12	0.19
14	0.26	0.18	0.23	0.32	0.41	0.17	0.15	0.11	0.15	0.19	0.12	0.06	0.28	0.25	0.21
15	0.34	0.11	0.14	0.14	0.46	0.29	0.12	0.18	0.18	0.33	0.31	0.30	0.26	0.29	0.82
16	0.28	0.34	0.22	0.32	0.42	0.33	0.24	0.21	0.25	0.49	0.12	0.14	0.14	0.18	0.19
17	0.20	0.18	0.17	0.20	1.02	0.12	0.13	0.16	0.21	0.45	0.22	0.19	0.21	0.25	0.29
18	0.24	0.20	0.18	0.26	0.49	0.27	0.20	0.19	0.37	0.42	0.24	0.18	0.20	0.17	0.22

4.6.2 **(a)** Specific LTD_4 Antagonist, SK&F 104353

ANOVA Tables

LTD_4

Transformed Cough Frequencies (coughs/min)

Source of Variation	df	SSq	MSq	F
Between Subjects	5	22.39	4.48	2.69
Between Periods	2	8.96	4.48	2.69
Between Treatments	2	25.13	12.56	7.54 $p<0.05$
Residual	8	13.33	1.67	
Total	17	69.80		

LTD_4

Transformed FEV_1

Source of Variation	df	SSq	MSq	F
Between Subjects	5	0.206	0.041	
Between Periods	2	0.0004	0.0002	0.58
Between Treatments	2	0.0035	0.00177	4.73 $p<0.05$
Residual	8	0.003	0.00037	
Between Times	3	0.062	0.0206	80.2 $p<0.001$
Residual	15	0.0038	0.00026	
Time x Period	6	0.00148	0.00025	1.12 NS
Time x Treatment	6	0.0119	0.00198	8.97 $p<0.01$
Residual	24	0.0053	0.00022	
TOTAL	71	0.2970		

4.6.2 **(a)** ANOVA Tables continued

LTD_4

Transformed R_{aw}

Source of Variation	df	SSq	MSq	F
Between Subjects	5	0.21	0.042	
Between Periods	2	0.60	0.300	11.6 p<0.01
Between Treatments	2	0.20	0.098	3.79 NS
Residual	8	0.20	0.026	
Between Times	4	1.40	0.350	18.9 p<0.001
Residual	20	0.37	0.019	
Time x Period	8	0.11	0.014	<1
Time x Treatment	8	0.17	0.022	1.28 NS
Residual	32	0.55	0.017	
TOTAL	89			

4.6.2 **(b)** Non-Specific Inhibition with Salbutamol

Raw Data Tables

FEV_1 (l)

	Placebo				Salbutamol			
Time	1	2	3	4	1	2	3	4
	3.80	3.75	3.45	3.60	3.65	3.80	3.75	3.65
	4.10	4.20	3.50	3.70	4.25	4.45	4.25	4.25
	2.80	2.80	2.30	2.35	2.85	2.80	2.75	2.65
	3.65	3.65	2.90	3.20	3.65	3.65	3.60	3.80
	2.70	2.70	2.35	2.50	2.75	2.85	2.75	2.70

sG_{aw} & Cough Frequency (coughs/5min)

Placebo					Salbutamol				
1	2	3	4	Cough	1	2	3	4	Cough
2.54	2.29	1.84	2.10	0	2.11	2.70	2.29	2.29	0
1.40	1.40	0.38	0.78	25	1.41	3.31	2.20	2.20	0
2.51	3.28	0.45	0.92	11	2.38	4.57	2.67	2.67	0
1.83	1.63	0.36	0.65	31	1.67	2.67	2.13	2.55	0
3.43	3.40	1.07	1.07	10	3.93	4.99	2.37	3.25	3

Times 1, 2, 3 & 4 correspond to baseline, pre and 5 & 15 min post challenge

ANOVA Table

Transformed Cough Frequencies

Source of Variation	df	SSq	MSq	F
Between Subjects	4	6.66	1.66	
Between Treatments	1	15.72	15.72	8.46 $p<0.05$
Residual	4	7.43	1.86	
TOTAL	9	29.81		

SE = 0.61

95% CL = 1.69

APPENDIX 3 - Antitussive Studies

Raw Data And Anova Tables

5.3.1 Opiates

Raw Data Tables

UNDW-Induced Cough

Cough Frequencies (coughs/min)

Subject	Placebo	Noscapine (50mg)	Noscapine (150mg)	Codeine (60mg)
1	27	32	21	24
2	11	2	7	11
3	11	6	2	4
4	10	18	10	15
5	38	39	33	37
6	27	18	10	15
7	0	8	6	0
8	14	23	15	29
9	33	38	23	36
10	23	15	19	31
11	34	25	19	25
12	35	30	39	23
13	10	6	12	2
14	4	11	9	9
15	7	0	4	6
16	10	0	0	13
17	8	18	13	18
18	12	4	23	0
19	30	27	29	35
20	15	19	11	21
21	25	23	13	23
22	0	8	3	22
23	5	10	9	25
24	23	8	21	2

5.3.1 Raw Data Tables continued

Citric Acid-Induced Cough

Cough Frequencies (coughs/min)

Subject	Placebo	Noscapine (50mg)	Noscapine (150mg)	Codeine (60mg)
1	32	24	13	16
2	18	21	11	7
3	5	8	3	2
4	0	14	10	3
5	29	35	41	28
6	17	8	18	10
7	5	21	9	0
8	14	23	15	24
9	34	33	39	27
10	0	0	4	20
11	11	20	18	9
12	10	5	18	5
13	9	6	12	2
14	8	4	6	5
15	9	4	6	5
16	11	0	2	9
17	5	21	15	18
18	17	0	13	0
19	34	26	32	30
20	13	11	14	8
21	18	19	10	14
22	0	9	12	8
23	9	10	14	25
24	12	6	20	13

5.3.1 Opiates

ANOVA Table

UNDW- and Citric Acid-Induced Cough

Transformed cough frequencies

Source of Variation	df	SSq	MSq	F	
Between Subjects (S)	23	222.20	9.66	26.0	p<0.01
Between Treatments (T)	3	0.53	0.18	<1	p>0.05
Between Challenges (C)	1	6.93	6.93	4.62	p<0.05
S x T	69	96.88	1.40	3.78	p<0.01
S x C	23	34.53	1.50	4.04	p<0.01
T x C	3	3.68	1.23	3.31	p<0.05
S x T x C	69	25.61	0.37		
TOTAL	191	390.36			

5.3.2 Nedocromil Sodium

Raw Data Tables

UNDW-Induced Cough

Cough Frequencies (coughs/min + divided into 3 consecutive periods)

Placebo	Fenoterol	Nedocromil
21 (11,4,6)	12 (8,3,1)	10 (6,3,1)
46 (20,13,13)	11 (8,3,0)	27 (19,5,3)
26 (18,2,6)	0 (0,0,0)	26 (17,5,4)
15 (7,4,4)	0 (0,0,0)	0 (0,0,0)
28 (12,9,7)	30 (14,8,8)	30 (17,6,7)
4 (0,0,4)	0 (0,0,0)	0 (0,0,0)
17 (10,5,2)	10 (4,3,3)	18 (12,2,4)
22 (10,7,5)	1 (1,0,0)	16 (12,0,4)
22 (13,6,3)	0 (0,0,0)	0 (0,0,0)
0 (0,0,0)	0 (0,0,0)	0 (0,0,0)
44 (18,18,8)	16 (8,5,3)	30 (15,10,5)
27 (14,9,4)	4 (4,0,0)	8 (8,0,0)
7 (1,6,0)	7 (0,0,7)	0 (0,0,0)
9 (7,2,0)	5 (2,3,0)	9 (9,0,0)
20 (11,8,1)	3 (2,0,1)	6 (2,2,2)
12 (3,3,6)	2 (0,2,0)	13 (3,8,2)
16 (4,8,4)	0 (0,0,0)	1 (0,1,0)
10 (6,3,1)	6 (4,1,1)	4 (4,0,0)

5.3.2 Raw Data Tables continued

Citric Acid-Induced Cough

Cough Frequencies (coughs/min + divided into 3 consecutive periods)

Placebo	Fenoterol	Nedocromil
9 (5,4,0)	6 (6,0,0)	14 (10,3,1)
4 (3,1,0)	0 (0,0,0)	0 (0,0,0)
11 (10,0,1)	10 (10,0,0)	19 (9,9,1)
22 (9,6,7)	4 (4,0,0)	16 (6,4,6)
18 (8,4,6)	35 (13,16,6)	32 (16,13,3)
6 (4,2,0)	0 (0,0,0)	0 (0,0,0)
9 (7,0,2)	14 (7,5,2)	27 (14,8,5)
20 (14,4,2)	1 (1,0,0)	5 (5,0,0)
5 (5,0,0)	9 (9,0,0)	5 (5,0,0)
3 (3,0,0)	0 (0,0,0)	3 (3,0,0)
8 (8,0,0)	7 (5,1,1)	6 (4,2,0)
6 (6,0,0)	3 (3,0,0)	6 (6,0,0)
6 (0,6,0)	0 (0,0,0)	0 (0,0,0)
6 (6,0,0)	5 (5,0,0)	14 (11,3,0)
24 (17,7,0)	18 (10,8,0)	24 (12,12,0)
5 (3,0,2)	6 (2,4,0)	8 (0,4,4)
9 (4,3,2)	9 (3,3,3)	10 (4,4,2)
5 (5,0,0)	7 (7,0,0)	9 (7,2,0)

5.3.2 Raw Data Tables continued

Capsaicin-Induced Cough

Cough Frequencies (coughs/min + divided into 3 consecutive periods)

Placebo	Fenoterol	Nedocromil
17 (10,5,2)	6 (4,2,0)	1 (1,0,0)
9 (9,0,0)	11 (9,2,0)	7 (7,0,0)
10 (5,3,2)	8 (5,3,0)	17 (10,7,0)
21 (10,8,3)	19 (11,4,4)	13 (7,4,2)
25 (12,9,4)	22 (8,6,8)	12 (10,2,0)
25 (14,7,4)	2 (2,0,0)	2 (2,0,0)
19 (9,6,4)	27 (12,9,6)	26 (11,8,7)
30 (13,8,9)	22 (12,7,3)	11 (6,3,2)
14 (12,1,1)	29 (18,11,0)	20 (9,7,4)
14 (12,1,1)	33 (20,5,8)	0 (0,0,0)
29 (12,8,9)	0 (0,0,0)	7 (7,0,0)
23 (8,7,8)	10 (3,3,4)	10 (7,3,0)
13 (0,3,10)	30 (10,14,6)	28 (3,20,5)
8 (6,0,2)	12 (6,4,2)	15 (13,2,0)
22 (14,8,0)	25 (5,12,8)	16 (8,7,1)
6 (0,4,2)	12 (2,6,4)	1 (0,1,0)
10 (2,5,3)	12 (8,4,0)	9 (5,1,3)
8 (6,0,2)	21 (0,16,5)	3 (0,03)

5.3.2 Nedocromil Sodium

ANOVA Table

Transformed Cough Frequencies

Source of Variation	df	SSq	MSq	F
Between Subjects (S)	17	70.96	4.17	4.33 P<0.01
Between Periods (P)	2	0.64	0.32	<1 NS
Between Treatments (T)	2	16.68	8.34	8.66 p<0.01
Residual	32	30.83	0.96	
Between Challenges (C)	2	15.50	7.75	14.1 p<0.01
C x T	34	66.01	1.94	3.53 p<0.01
C x P	4	2.06	0.52	<1 NS
C xT	4	20.11	5.03	9.13 p<0.01
Residual	64	35.24	0.55	
Between Times (20 sec)	2	72.49	36.25	134.6 p<0.01
Times x S	34	38.99	1.15	4.26 p<0.01
Times x P	4	0.39	0.10	<1 NS
Times x T	4	1.60	0.40	1.49 NS
Residual	64	17.23	0.27	
Times x C	4	2.72	0.68	2.73 p<0.05
Times x C x S	68	24.52	0.36	1.45 p<0.05
Times x C x P	8	4.87	0.61	2.45 p<0.05
Times x C x T	8	3.00	0.38	1.51 NS
Residual	128	31.86	0.25	
TOTAL	485	455.71		

5.3.3 Diuretics

Raw Data Tables

Cough Frequencies (coughs/30 sec)

Placebo	Amiloride	Frusemide
12	7	0
8	18	13
30	16	17
23	9	12
1	0	0
0	0	0
0	0	0
14	2	0

FEV_1 (l)

	Placebo			Amiloride			Frusemide		
Time	1	2	3	1	2	3	1	2	3
	2.20	2.10	1.65	2.60	2.40	2.20	2.25	2.20	2.25
	3.80	3.80	3.00	3.50	3.50	3.20	3.90	3.70	3.90
	2.35	2.35	1.80	2.10	2.00	2.00	2.40	2.40	2.40
	2.90	2.80	1.85	2.90	2.75	2.10	2.90	2.90	2.00
	4.85	4.40	3.90	4.95	4.40	3.85	4.60	4.80	4.60
	3.10	3.00	2.55	2.90	2.90	2.50	2.70	2.60	2.60
	2.60	2.60	1.95	2.75	2.65	2.45	2.70	2.70	2.50

Times 1, 2 & 3 represent baseline, post treatment and post UNDW challenge respectively

5.3.3 Raw Data Tables continued

% Fall in FEV_1 at Baseline PD_{20}

Placebo	Amiloride	Frusemide
20	20	0
25	16	0
21	9	0
25	11	7
36	28	31
18	14	4
23	5	0
24	14	5

ANOVA Table

Transformed Cough Frequencies

Source of Variation	df	SSq	MSq	F
Between Subjects	7	43.21	6.17	10.69 p<0.001
Between Treatments	2	3.91	1.95	3.39 p = 0.06
Residual	14	8.08	0.58	
TOTAL	23	55.20		

SE = 0.27; 95%CL = 0.578

Placebo - Frusemide t = 2.55 p < 0.05

Placebo - Amiloride t = 1.68 NS

ANOVA Table

% Fall in FEV_1 at Baseline PD_{20}

Source of Variation	df	SSq	MSq	F
Between Subjects	7	1047.3	149.6	7.39 p<0.001
Between Treatments	2	1314.6	657.3	32.47 p<0.001
Residual	14	283.4	20.2	
TOTAL	23	2645.3		

APPENDIX 4 - Afferent Lung C-Fibre Stimulants And Cough

Raw Data Tables

6.3.2 Adaptation and Cross-Adaptation of Cough

Cough Frequencies (Total + Divided into 3 equal and consecutive periods)

Subject	Baseline	5 min	3 hrs
	UNDW	UNDW	UNDW
1	37 (20,8,9)	9 (6,0,3)	36 (21,6,9)
2	20 (7,9,4)	3 (3,0,0)	18 (5,8,5)
3	23 (15,5,3)	6 (6,0,0)	24 (11,9,4)
4	35 (17,11,7)	12 (5,5,2)	23 (9,8,6)
5	22 (11,9,2)	14 (9,2,3)	28 (12,9,7)
	PGE_2	PGE_2	PGE_2
1	12 (1,6,5)	0 (0,0,0)	8 (0,3,5)
2	16 (4,7,5)	2 (2,0,0)	5 (0,3,2)
3	25 (13,9,3)	6 (0,3,3)	24 (10,8,6)
4	20 (9,7,4)	4 (2,0,2)	12 (8,4,0)
5	12 (5,0,7)	5 (0,5,0)	8 (4,4,0)
	CAPSAICIN	CAPSAICIN	CAPSAICIN
1	22 (5,10,7)	24 (8,7,9)	22 (7,9,6)
2	15 (2,6,7)	15 (5,7,3)	14 (8,6,0)
3	23 (7,8,8)	23 (11,6,6)	28 (13,6,9)
4	13 (3,5,5)	13 (4,4,5)	13 (4,5,4)
5	10 (0,5,5)	9 (0,5,4)	15 (4,5,6)
	UNDW	PGE_2	PGE_2
1	44 (24,12,8)	33 (13,10,10)	17 (8,7,2)
2	23 (5,11,7)	12 (5,5,2)	9 (2,4,3)
3	22 (10,9,3)	27 (16,7,4)	23 (13,9,1)
4	30 (17,8,5)	16 (7,4,5)	15 (8,5,2)
5	10 (10,0,0)	21 (10,6,5)	27 (11,9,7)
	UNDW	CAPSAICIN	CAPSAICIN
1	31 (16,12,3)	9 (1,4,4)	18 (11,3,4)
2	16 (8,6,2)	3 (0,3,0)	22 (8,6,8)
3	10 (8,2,0)	28 (9,13,6)	28 (9,9,10)
4	28 (14,12,2)	8 (4,2,2)	15 (6,4,5)
5	25 (13,8,4)	8 (0,4,4)	12 (2,6,4)

6.3.2 Raw Data Tables continued

	PGE_2	UNDW	UNDW
1	21 (9,7,5)	0 (0,0,0)	33 (18,8,7)
2	17 (9,3,5)	8 (2,2,4)	22 (4,11,7)
3	22 (12,7,3)	9 (6,3,0)	15 (11,2,2)
4	16 (9,5,2)	10 (7,3,0)	24 (13,4,7)
5	24 (13,6,5)	8 (3,3,2)	23 (13,4,6)
	PGE_2	CAPSAICIN	CAPSAICIN
1	12 (4,5,3)	5 (0,3,2)	17 (8,7,2)
2	11 (6,5,0)	20 (6,9,5)	23 (13,5,5)
3	26 (13,10,3)	6 (4,2,0)	17 (8,6,3)
4	20 (10,6,4)	9 (2,2,5)	14 (0,8,6)
5	16 (7,4,5)	24 (8,8,8)	22 (7,8,7)
	CAPSAICIN	UNDW	UNDW
1	25 (8,14,3)	29 (8,12,9)	39 (24,10,5)
2	12 (3,3,6)	21 (5,6,10)	22 (9,7.6)
3	25 (7,6,12)	10 (8,2,0)	11 (8,3,0)
4	10 (2,6,2)	20 (9,9,2)	28 (15,7,6)
5	16 (3,6,7)	25 (16,3,6)	29 (19,7,3)
	CAPSAICIN	PGE_2	PGE_2
1	16 (2,6,8)	34 (19,10,5)	29 (10,12,7)
2	10 (0,3,7)	12 (2,5,5)	14 (2,7,5)
3	21 (8,7,6)	28 (15,11,2)	22 (11,8,3)
4	10 (1,5,4)	12 (5,5,2)	12 (4,4,4)
5	15 (3,6,6)	22 (10,4,8)	24 (12,6,6)

6.3.2 Adaptation and Cross-Adaptation of Cough

ANOVA Tables

Transformed Total Cough Frequencies

(Root (x + 1))

Source of Variation	df	SSq	MSq	F
Subjects	4	8.75	2.107	
Sequences	8	23.23	2.90	
Subjects x Sequences	32	29.90	0.93	
Periods	2	21.29	10.64	
Subjects x Periods	8	3.33	0.42	
Treatments				
Direct effects	2	4.41	2.20	3.07 NS
Carryover effects	2	10.59	5.30	7.38 p<0.01
Residual	76	54.52	0.72	
TOTAL	134	156.00		

SE = 0.379

3 x Consecutive periods of baseline challenge

Challenge	2	5.08	2.54	2.07 NS
Periods	2	7.97	3.99	7.76 p<0.05
Subjects	4	5.04	1.26	5.4 p<0.001
Challenge x Periods	4	18.58	2.65	12.96 p<0.01
Challenge x Subjects	8	9.8	1.23	5.26 p<0.001
Periods x Subjects	8	4.11	0.51	2.21 p<0.05
Challenge x Period x Subjects	16	5.74	0.36	1.54 NS
Residual	90	20.93	0.23	
TOTAL	134	77.24		

SE = 0.125

6.3.2. ANOVA Tables continued

PGE_2 & Capsaicin only

Transformed R_{aw}

Log (During/Pre Challenge)

Source of Variation		df	SSq	MSq	F	
Subjects	4	0.10	0.025			
Challenge	1	0.06	0.061	4.32	NS	
Time of Challenge	2	0.04	0.018	6.10	$p<0.05$	
Subject x Challenge	4	0.06	0.014			
Subject x Time	8	0.02	0.003			
Challenge x Time	2	0.00	0.002	<1	NS	
Subject x Challenge x Time	8	0.11	0.014			
Residual	60	0.34	0.006			
TOTAL	89	0.73				

SE = 0.034

6.3.3 The Antitussive Efficacy of Nedocromil Sodium

Raw Data Tables

Cough Frequencies (Total + Divided into 3 consecutive periods)

ULTRASONIC CHALLENGE

(+ cough during 5 min post challenge)

	PGE_2		Capsaicin	
	Placebo	Nedocromil	Placebo	Nedocromil
1	15 (15,0,0) 9	15 (13,2,0) 11	16 (7,3,6) 0	31 (21,7,3) 4
2	2 (2,0,0) 10	6 (3,0,3) 13	15 (8,5,2) 0	13 (5,4,4) 1
3	15 (11,2,2) 20	16 (5,10,1) 15	7 (6,1,0) 0	8 (8,0,0) 2
4	25 (16,8,1) 5	19 (14,2,3) 3	18 (9,6,3) 0	11 (9,2,0) 0
5	18 (13,3,2) 23	15 (15,0,0) 24	7 (2,2,3) 1	13 (4,2,7) 5
6	8 (8,0,0) 4	11 (11,0,0) 17	27 (16,4,7) 1	26 (21,5,0) 12
7	19 (12,7,0) 12	11 (11,0,0) 4	23 (9,9,5) 4	32 (9,11,12) 6
8	7 (7,0,0) 4	7 (7,0,0) 5	18 (9,5,4) 0	32 (16,10,6) 6

JET CHALLENGE

1	10 (4,2,4)	8 (3,3,2)	12 (5,3,4)	5 (0,3,2)
2	12 (12,0,0)	13 (9,4,0)	8 (3,2,3)	7 (0,1,6)
3	8 (5,0,3)	6 (3,3,0)	12 (6,5,1)	5 (0,3,2)
4	8 (2,2,4)	10 (5,1,4)	16 (6,5,5)	14 (5,4,5)
5	24 (15,9,0)	18 (7,9,2)	20 (4,8,8)	24 (7,10,7)
6	16 (11,5,0)	13 (9,1,3)	19 (6,7,6)	8 (0,5,3)
7	10 (4,6,0)	11 (5,6,0)	19 (3,5,11)	10 (1,4,5)
8	9 (7,2,0)	6 (4,2,0)	16 (4,7,5)	14 (4,4,6)

6.3.3 Raw Data Tables continued

Jet Challenge

R_{aw}

PGE_2

	Placebo				Nedocromil			
	1	2	3	4	1	2	3	4
1	0.43	0.41	0.55	0.42	0.40	0.44	0.48	0.39
2	0.27	0.30	0.37	0.28	0.28	0.27	0.39	0.28
3	0.21	0.19	0.30	0.19	0.20	0.17	0.23	0.22
4	0.39	0.29	0.29	0.31	0.31	0.24	0.34	0.28
5	0.25	0.29	0.31	0.24	0.25	0.25	0.32	0.27
6	0.40	0.30	0.50	0.27	0.36	0.38	0.43	0.34
7	0.39	0.34	0.34	0.40	0.36	0.35	0.40	0.37
8	0.20	0.20	0.26	0.24	0.26	0.25	0.27	0.24

Capsaicin

	Placebo				Nedocromil			
	1	2	3	4	1	2	3	4
1	0.40	0.37	0.47	0.43	0.42	0.41	0.42	0.49
2	0.29	0.33	0.40	0.27	0.29	0.30	0.45	0.29
3	0.19	0.20	0.25	0.18	0.18	0.21	0.25	0.19
4	0.26	0.25	0.24	0.25	0.31	0.29	0.40	0.28
5	0.29	0.33	0.25	0.28	0.27	0.23	0.36	0.23
6	0.32	0.39	0.55	0.33	0.42	0.41	0.46	0.37
7	0.41	0.34	0.32	0.36	0.47	0.30	0.44	0.35
8	0.28	0.17	0.37	0.25	0.24	0.24	0.33	0.23

Times 1, 2, 3 & 4 correspond to Baseline, Pre-challenge, During challenge and Post-challenge respectively.

6.3.3 The Antitussive Efficacy of Nedocromil Sodium
ANOVA Tables

Ultrasonic Challenge
Transformed Cough Frequencies (1 min challenge + 5 min post challenge)

Source of Variation	df	SSq	MSq	F
Between Subjects	7	6.97	0.996	<1 NS
Between Periods	3	2.34	0.78	<1 NS
Between Treatments				
Placebo vs Nedocromil	1	1.95	1.95	1.59 NS
PGE_2 vs Capsaicin	1	3.56	3.56	2.91 NS
Interaction	1	1.85	1.85	1.51 NS
Residual	18	22.07	1.27	
Between Time of Challenge	1	33.48	33.48	119.7 p<0.01
Time x Subjects	7	10.05	1.44	5.13 p<0.01
Time x Periods	3	0.91	0.30	1.09 NS
Time x Treatment	3	21.50	7.17	25.6 p<0.01
Time x Placebo/Nedocromil	1	0.46	0.46	1.65 NS
Time x PGE_2/Capsaicin	1	20.88	20.88	74.6 p<0.01
Time x Interaction	1	0.156	0.156	<1 NS
Residual	18	5.04	0.28	
TOTAL	63	109.72		

Jet Challenge
Transformed Cough Frequencies (1 min challenge + 5 min post challenge)

Between Subjects	7	7.29	1.04	7.72
Between Periods	3	1.17	0.39	2.89
Between Treatments	3	2.62	0.88	6.48 p<0.01
Placebo vs Nedocromil	1	1.01	1.01	7.47 p<0.01
PGE_2 vs Capsaicin	1	0.94	0.94	6.94 p<0.01
Interaction	1	0.68	0.68	
Residual	18	2.43	0.135	
Between times of challenge	2	2.83	1.42	4.04 p<0.05
Times x Subjects	14	4.45	0.32	<1 NS
Times x Periods	6	1.10	0.18	<1 NS
Times x Treatments	6	12.08	2.01	5.75 p<0.01
Times x Placebo/Nedocromil	2	1.13	0.57	1.62 NS
Times x PGE_2/Capsaicin	2	10.85	5.43	15.5 p<0.01
Times x interaction	2	0.09	0.04	<1 NS
Residual	36	12.6	0.35	
TOTAL	95	4.66		

6.3.3 ANOVA Tables continued

Log Transformed R_{aw}

Source of Variation	df	SSq	MSq	F
Between Subjects	7	1.24	0.178	70.3 p<0.01
Between Periods	3	0.007	0.002	<1 NS
Between Treatments	3	0.0085	0.0028	1.12 NS
Placebo vs Nedocromil	1	0.0031	0.0031	1.22 NS
PGE_2 vs Capsaicin	1	0.0014	0.0014	<1 NS
Interaction	1	0.004	0.004	1.59 NS
Residual	18	0.0455	0.0025	
Between Times of Challenge	3	0.189	0.063	21.4 p<0.01
Times x Subjects	21	0.092	0.004	1.48 NS
Times x Periods	9	0.010	0.001	<1 NS
Times x Treatments	9	0.007	0.001	<1 NS
Times x Placebo/Nedocromil	3	0.002	0.001	<1 NS
Times x PGE_2/Capsaicin	3	0.001	0.0003	<1 NS
Times x Interaction	3	0.004	0.001	<1 NS
Residual	54	0.159	0.0029	
TOTAL	127	1.761		

SE = 0.0218

APPENDIX 5 - Cough Associated With Viral Infection
Raw Data And Anova Tables

7.4 Effect of Bronchodilator Treatment on Cough Associated with URTI

Placebo Group

Baseline - Day 1				Day 10			
FEV_1	FVC	PEF	UNDW	FEV_1	FVC	PEF	UNDW
2.90	3.90	300	34	3.10	3.80	390	37
5.30	6.30	490	22	5.30	6.20	570	2
3.00	3.50	390	22	3.00	3.50	420	7
3.35	3.75	500	6	3.30	3.50	430	11
4.30	5.60	610	0	4.20	5.80	655	0
3.70	4.20	490	4	3.70	4.20	490	2
3.70	4.20	490	8	3.80	4.20	510	9
2.60	3.20	420	61	2.60	3.20	450	38
4.40	5.00	550	29	4.50	5.10	550	17
3.30	4.40	410	40	3.40	4.40	460	20
5.50	6.70	680	37	5.80	7.00	610	8
3.40	3.95	490	28	3.40	3.90	510	8
3.90	4.70	510	27	3.90	4.60	500	21
3.65	4.25	410	41	3.90	4.55	450	12
4.00	4.15	510	19	3.90	4.15	490	26
4.80	4.95	545	19	5.00	5.05	580	9
3.70	4.60	640	8	3.80	4.60	690	14
3.55	4.2	490	12	3.35	4.15	450	3
3.70	4.40	415	4	3.80	4.35	440	6
2.95	3.10	420	9	3.00	3.30	500	10
3.70	3.95	510	46	3.75	3.90	465	6
3.85	4.50	495	12	4.00	4.50	530	0
3.65	4.40	420	10	3.75	4.35	470	31
2.75	3.30	380	20	2.75	3.40	450	13
3.75	4.80	500	24	3.85	5.00	540	5
3.35	4.40	370	37	3.50	4.30	425	17
3.95	4.35	470	14	3.60	4.20	510	20
4.50	5.90	600	46	4.80	5.80	590	32

7.4 Raw Data Tables continued

Oxitropium Group

Baseline - Day 1				Day 10			
FEV_1	FVC	PEF	UNDW	FEV_1	FVC	PEF	UNDW
3.65	3.90	480	38	3.50	3.70	460	32
2.50	2.80	385	26	2.80	3.10	400	24
3.90	4.30	550	26	4.20	4.50	570	5
3.60	4.45	390	41	3.65	4.40	440	34
4.50	4.90	480	31	4.40	4.90	500	27
3.20	3.70	420	33	3.60	3.90	430	42
3.35	3.75	490	0	3.35	3.85	510	0
3.55	4.00	380	20	3.85	4.10	430	0
3.85	4.20	550	17	3.70	4.00	500	2
6.20	7.30	550	21	6.35	7.50	560	11
3.15	4.00	400	21	3.25	4.00	440	2
5.10	6.10	600	21	5.15	6.20	720	28
4.20	5.70	580	31	3.90	5.40	560	25
3.65	3.90	380	31	3.70	4.15	480	29
3.20	4.90	440	25	2.45	4.20	430	5
3.05	3.40	360	18	3.05	3.20	410	10
5.05	5.70	590	3	5.35	5.80	610	2
4.00	4.40	530	16	3.90	4.55	540	30
3.00	3.40	410	22	3.40	3.75	420	24
3.30	3.50	480	58	3.35	3.60	470	24
3.60	4.20	430	9	3.45	4.10	440	14
3.50	4.00	400	40	3.60	4.30	490	0
1.50	1.80	305	16	1.50	1.85	340	7
3.75	4.35	485	2	3.50	4.10	470	0
4.65	5.90	560	4	4.60	5.90	570	5
3.25	3.45	340	35	3.30	3.40	410	15

7.4 ANOVA Tables

UNDW-Induced Cough

Transformed Cough Frequencies

Placebo Group

Source of Variation	df	SSq	MSq	F
Between Subjects	30	104.44	3.48	3.308 p<0.001
Between Visits (Day1/10)	1	14.85	14.85	14.12 p<0.001
Residual	30	31.57	1.05	
TOTAL	61	150.86		

SE = 0.18

95%CL = 0.375

Oxitropium Group

Source of Variation	df	SSq	MSq	F
Between Subjects	27	120.44	4.46	3.51 p<0.001
Between Visits	1	18.21	18.21	14.32 p<0.001
Residual	27	34.32	1.27	
TOTAL	55	172.96		

SE = 0.21

95%CL = 0.437

7.4 ANOVA Tables continued

Diary PEFR (l/min)

Time Effect

Source of Variation	df	SSq	MSq	F
Model	56	1006741	17978	53.9 p<0.0001
Error	102	34048	333.8	
Total	158	1040788		
Between Treatment	1	2488.9	2488.9	7.46 p<0.01
Between Subjects	51	998185	19572	58.63 p<0.001
Between Times	2	5963.2	2981.6	8.93 p<0.001
Treatment x Time	2	104.0	52.02	0.16 NS

Day x Time Effects

Source of Variation	df	SSq	MSq	F
Model	56	3899.5	69.6	8.34 p<0.001
Error	102	851.6	8.35	
Total	158	4751.3		
Between Treatment	1	8.98	8.98	1.08 NS
Between Subjects	51	3865	75.8	9.08 p<0.0001
Day x Time	2	23.6	11.8	1.41 NS
Treat xDayxTime	2	1.92	0.96	0.12 NS

7.4 ANOVA Tables continued

ANOVA Lung Function, Day 10 - Day1

Source of Variation	df	SSq	MSq	F
FEV_1				
Treatment	1	0.01	0.014	0.35 NS
Error	52	2.07	0.040	
Total	53	2.08		
FVC				
Treatment	1	0.00	0.000	0.01 NS
Error	52	1.80	0.035	
Total	53	1.80		
PEFR				
Treatment	1	70.10	70.096	0.04 NS
Error	52	87487.8	1682.5	
Total	53	87557.9		

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