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INVESTIGATION OF THE SENSITIVITY OF THE COUGH REFLEX IN HUMANS

Thesis submitted by

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DEDICATED TO MY PARENTS

Indian Proverb

Lust, fire, cough, these three are not concealed.

Sir Thomas Browne 1605 -1682 (English physician)

The ancient Inhabitants of this Island
were less troubled with Coughs when
they went naked, and slept in Caves
and Woods, than Men now in Cham-
bers and Feather-beds

"A letter to a friend"

ABSTRACT

Cough is the most common symptom of patients with respiratory disease. It maybe associated with the physical stimulation of the cough reflex by either a structural abnormality (eg. carcinoma of the bronchus) or by the presence of material including increased secretions within the airways (eg. mucous in smokers hypersecretion or the acute phase of infections). In these instances the cough can be explained on the basis that there is evidence of the stimulus to the reflex. Persistent cough may also be troublesome symptom in the absence of such obvious stimuli. This occurs in patients with cough following some respiratory tract infections where cough persists for many weeks after evidence of active infection or increased secretions have settled. Another group where persistent dry cough occurs, is in the proportion of patients taking angiotensin converting enzyme inhibitors, who develop cough as a side effect of the therapy.

The purpose of this project was to develop a method for quantitating the sensitivity of the cough reflex in volunteers and subsequently to investigate the sensitivity reflex in patients with cough. The cough challenge developed, involved the use of inhaled capsaicin, the active ingredient of red peppers. This challenge does not appear to be subject to tachyphylaxis, enabling repeated challenges to be performed with reproducible results.

Studies performed in controls, patients with productive cough and patients with non-productive cough showed that those in the latter group had an abnormal increase in the sensitivity of the cough reflex compared to the two other groups. Those patients with a productive cough had a normal sensitivity of the cough reflex.

Potential mechanisms involved in the abnormal sensitivity of the cough reflex

were studied. The possibility that various mediators may increase the sensitivity of the reflex was investigated, and this study showed that prostaglandin E_2 but not bradykinin enhanced the cough response to inhaled capsaicin. Further, sulindac, a cyclooxygenase inhibitor, reduced the sensitivity of the cough reflex in patients with ACE-I cough but not those with an idiopathic cough. The effect of altering airway tone was also investigated with various anti-asthma therapies and airway spasmogens. However, cough was not altered either by decreasing or by increasing airway tone.

The effect of putative and traditional antitussives was also assessed. The sensitivity of the cough reflex was reduced by systemic opiates and inhaled local anaesthetics, but not inhaled opiates and nedocromil sodium.

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DECLARATION

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All studies were approved by the local Ethical Committee and written informed consent was obtained.

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CHAPTER 1

INTRODUCTION

SECTION 1

General Introduction

Cough is "a sudden and violent expiratory effort that explosively expels alveolar gas out through the glottis at velocities of 75 - 100 mph, usually to clear airways of foreign matter. It is a culmination of a co-ordinated sequence of events mediated by the medulla (via the vagus) and initiated by chemical and mechanical irritation of the respiratory passages" (Saunders 1984).

The commonest causes of cough are the virally induced acute upper respiratory tract infections when the cough is usually non-productive and self-limiting. In other patients, cough can be associated with increased mucus production or a chronic respiratory disease such as asthma. However, in a proportion of patients there is no obvious cause at presentation and such a cough can persist for many years. Many respiratory diseases are associated with cough which can be due to anatomical abnormalities within the airways or respiratory tract infection. When a pulmonary cause can not be identified then the possibility that cough may arise from conditions outside the respiratory tract, such as within the cardiovascular system, nasal sinuses, ears and gastrointestinal tract need to be considered.

The incidence of cough in the population is modified by the frequency of cigarette smoking and other environmental factors. Surveys have variously reported the prevalence of cough within the range of 5 - 40% (Wynder *et al.* 1964; Cullen *et al.* 1968; Corroa *et al.* 1979; Gulsvik 1982; Sears *et al.* 1986; Woolcock *et al.* 1987).

Treatment of cough can either be specifically directed at the underlying cause or be symptomatic. Symptomatic treatment is non-specifically directed at the symptom using such drugs as opiates and local anaesthetics. In the UK, sales of "over the counter" antitussives have been estimated to be in excess of £38 million and cough and cold remedies comprise by far the largest category of "over the counter" sales (Drug & Therapeutics Bullettin, 1985). Upto 50% of a General Practitioners' visits during winter are prompted by a complaint of cough (Korpas & Tomori 1979). However, most commonly available antitussives are only marginally beneficial to patients, since most of the therapy is symptomatic and the precise mechanism of the cough is often unclear.

There is a need then to further investigate the mechanism of cough in order to develop more effective anti-tussive therapy.

SECTION 2

Diseases: which are associated with cough.

When Cabot (1917) wrote his textbook on clinical symptoms in the early part of this century, persistent cough was most frequently reported to be due to tuberculosis. However, due to improved nutrition and living conditions and development of effective therapy, tuberculosis is now rare in the UK.

Today cough can be caused by numerous diseases such as bronchial cancer, bronchitis, emphysema, asthma and whooping cough in children, and is either productive or non-productive. Diseases causing cough can be classified into various categories depending on their etiology.

Diseases such as pharyngitis, laryngitis, and post-nasal drip from chronically infected sinuses, undoubtedly lead to irritation of the larynx and so result in coughing. For example, Irwin and co-workers found that of patients presenting with chronic cough 29 % could be attributed to post-nasal drip, as seen in patients with allergic rhinitis, chronic sinusitis and obstruction of the nasopharynx by enlarged adenoids (Irwin *et al.* 1981). Cough associated with oesophageal reflux has also been reported in several studies (Irwin *et al.* 1989 & 1990), and cough is found to be the most frequent symptom of the common cold (Irwin *et al* 1977).

Cough may also be associated with infections of the lower airways such as with chronic bronchitis, bronchiectasis, cystic fibrosis, asthma, tumours and granuloma. Chronic cough is a major symptom in bronchiectasis and bronchitis, it is usually productive. In fibrosing conditions such as fibrosing alveolitis the disease

leads to a distortion of the normal architecture of the airways, causing a persistent cough. Bronchial carcinoma often presents initially with cough (Irwin *et al.* 1977). Those patients with bronchial carcinoma who never complain of cough may have more peripheral tumours arising from smaller bronchi where receptors responsible for cough are few in number or even absent.

Asthma may also be responsible for severe persistent cough and is often associated with a wheeze. In fact this symptom may be the only manifestation of the disease (Corrao *et al* 1979, McFadden 1975). It is important to identify the aetiology of such a cough since specific therapy directed at asthma would relieve this cough.

Angiotensin converting enzyme inhibitors (ACE-I) are now used extensively in the treatment of both hypertension and heart failure. In general, these drugs are well tolerated, but during the past 5 years cough has been increasingly recognised as a common side effect with an incidence of between 1-30 % (Stumpe & Kolloch 1984; Sesoko *et al.* 1985; Israel-Biet *et al.* 1986; Webb *et al.* 1986; Semple & Herd 1986; Coulter & Edwards 1987). The cough is dry, irritating and non-productive, and is reportedly commoner in women (Berkin & Ball 1988) and generally develops some weeks after starting therapy, so that the patient may not associate the cough with their therapy. The development of this side effect is likely to increase with increasing use of ACE-I; both as a first line therapy of hypertension and in heart failure. By 1988 the committee on Safety of Medicines had received 365 reports of cough related to ACE-I (Berkin & Ball 1988) and now there are likely to be more. Further in a large post- marketing surveillance study of 13,713 patients, 2,990 presented with cough, 15 % of the reports occurring in the first month of treatment (Inman 1988).

Cardiovascular diseases such as aortic aneurysm may cause cough where it is

severe and non-productive. A persistent non-productive cough is also seen in left ventricular failure (Cabanes *et al.* 1989).

Extra-thoracic causes of cough include stimulation of the external auditory meatus (Wolf *et al.* 1973), environmental irritants which may stimulate receptors in the larynx, trachea or bronchi and smoking, by far the most commonly implicated aetiological factor for chronic persistent cough (Wynder *et al* 1964). Cough may also be psychological.

Due to the many causes and in some cases unknown aetiology of cough, investigation of cough has proven to be difficult. Studies have tended to investigate the efficacy of antitussives, rather than understanding the mechanism involved in chronic cough or whether cough is dissimilar in different disease states, and furthermore if different types of cough ie. productive and unproductive differ in response to tussive stimulants.

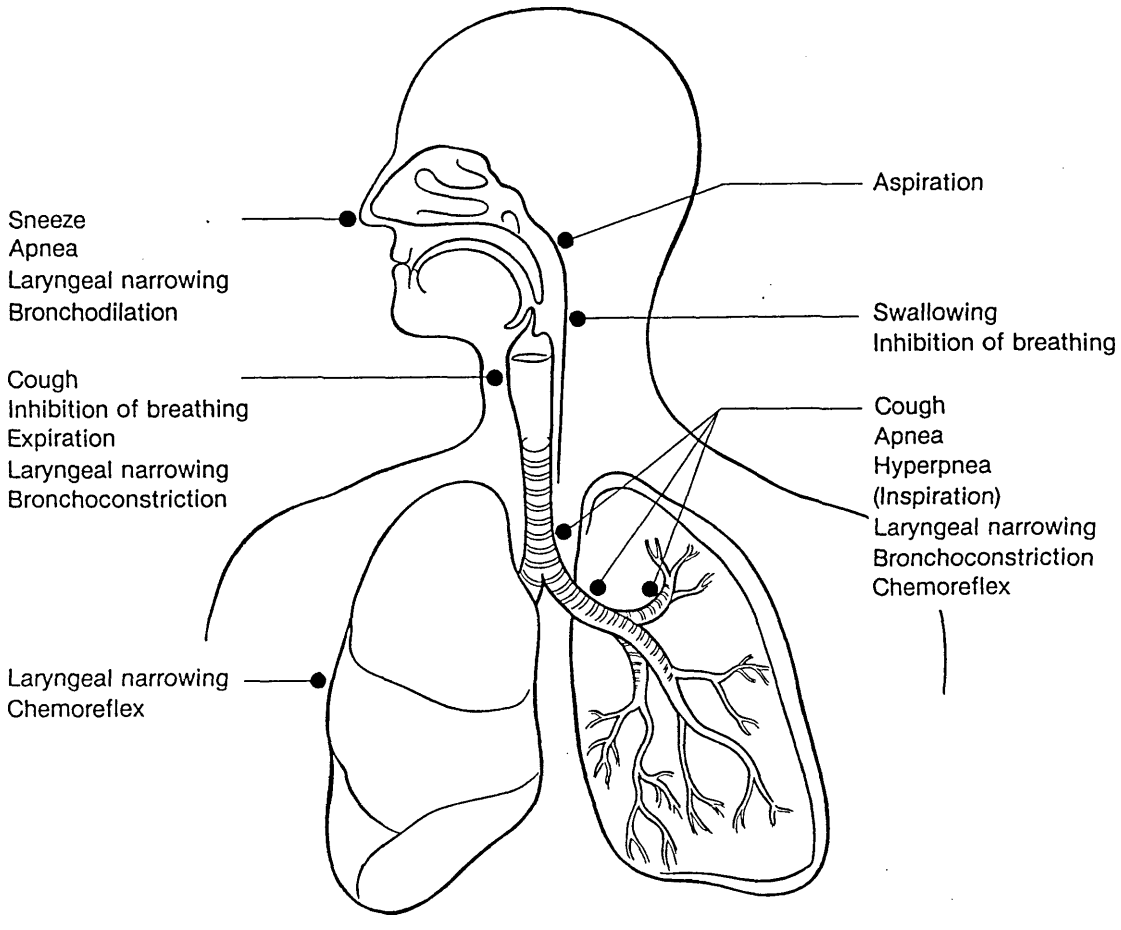
SECTION 3

Physiology of cough

Cough is a reflex transmitted via the vagus nerve (Klassen *et al.* 1951) and results from the stimulation of sensory nerves in the airways. A single cough consists of a rapid inspiration followed by a forced expiration against a closed glottis with continued expiratory effort to expel air and any foreign body which may have stimulated the cough. Sensory nerve endings which can be stimulated are found in the mucous membrane in various regions of the respiratory tract. The localisation of these regions has been deduced from experiments of controlled irritation and the most sensitive areas for production of cough are the larynx, trachea and the large bronchi (Larsell & Gurget 1924). These particular areas seem to be especially sensitive to tussigenic stimuli (Widdicombe 1954) as is seen in conscious dogs who cough and become very disturbed by the passage of cigarette smoke through their larynx, which caused a marked increase in activity of laryngeal irritant receptors (Lee 1987). In view of its sensitivity the larynx seems to be the most plausible trigger site for cough in man, as indicated by inhibition with topical local anaesthetics restricted to the larynx and airway bifurcation area (Steinhaus & Gaskin 1963, Poulton & James 1979), and because of its location, would receive a greater exposure to tussigenic stimuli. The main afferent pathways originating from the larynx, and responsible for cough, are the superior laryngeal nerves (Tomori & Widdicombe 1969). Figure 1.1 shows the trigger sites for respiratory reflexes including cough in man.

Figure 1.1

Trigger sites for respiratory reflexes in man.



Role of sensory nerves in cough.

As with any reflex, cough can be considered to have afferent (peripheral sensory receptors), integrative (brainstem and higher central nervous system centres) and efferent (motor neurons to respiratory muscles) components. The afferent pathway of the cough reflex is carried in the vagus nerves as indicated in experimental animals (Richardson & Peatfield 1981) and in man (Klassen 1951) where cough from one side of the bronchial tree is abolished by ipsilateral vagotomy. Central co-ordination of the cough reflex appears to be from the 'cough centre.' Its localisation in man is unknown, however, in the cat the centre has been localised within the medulla oblongata (Chou & Wang 1975). This centre is distinct from that controlling tidal breathing (Widdicombe 1977). Evidence of this is found from the work of May & Widdicombe (1954) who found that pholcodeine prevented coughing but ventilation was not affected, while phenobarbitone caused apnoea but had no effect on the cough threshold.

Although the structure of the cough receptors is unclear, examination, by electron microscopy, has shown the fine nerve endings of the airways to interdigitate between the epithelial cells of the mucosa (Jeffery & Reid 1973, Hung *et al.* 1973). It is assumed that the fine unmyelinated nerve processes observed extending close to the airway lumen between epithelial cells act as cough receptors, largely because such fibres are: 1) numerous at tussigenic sites (Fillenz & Widdicombe 1972) and 2) sensitive to both mechanical and chemical stimuli known to produce cough (Coleridge & Coleridge 1984). Some of these unmyelinated terminal processes possess fibres with myelinated axons with central projections via the vagus and the superior laryngeal nerves and these are referred to as rapidly adapting irritant receptors. Such

nerves are also sensitive to solutions of low chloride ion (Cl^-) content, which are tussigenic when inhaled as aerosols by human volunteers (Eschenbacher *et al.* 1984; Fuller & Collier 1984). Studies, recording the activity in mechanically stimulated afferent fibres of the larynx (Andrew 1956; Boushey *et al.* 1974), showed two types of discharge patterns, suggesting the presence of two receptors: one with slowly adapting activity and the other with rapidly adapting responses. Studies have also suggested a role for airway C-fibres in cough (Karlsson *et al.* 1988a, Korpas & Tomori 1979).

1. Slowly adapting receptors

Slowly adapting receptors are myelinated nerve terminals located mainly to the smooth muscle of conducting airways (Fillenz & Widdicombe 1972) and as their name implies adapt very slowly to changes in the length of this muscle. They have the ultrastructural appearance of mechanoreceptors and are stimulated by changes in tension across the airway wall. They increase their activity during inspiration and are responsible for the termination of inspiration and prolongation of expiration on lung inflation (Karlsson *et al.* 1988b). These receptors are also responsible for the Hering-Breuer inflation reflex (Hanacek *et al.* 1984)

It is unlikely that the slowly adapting receptors are directly involved in the cough reflex since these do not respond to tussive stimuli. However, these myelinated fibres were implicated in coughing by Bucher (1958) who suggested that during the inspiratory effort, which is an integral part of cough, the stretch receptors are more intensively stimulated hence increasing the inhibitory influence on central inspiratory activity and thus strengthening the subsequent expiration. He also suggested that the

intensity of the cough reflex could be reduced by anaesthetizing lung stretch receptors. Davies *et al* (1978) demonstrated that slowly adapting receptors are selectively blocked by exposure to high concentration of sulphur dioxide (SO_2) as indicated by the abolition of the Hering-Breuer reflex in rabbits. Further, apnoea and tracheal probing failed to cause cough. This was confirmed by Hanacek and co-workers (1984) who demonstrated that the inhibitory action of SO_2 was specific to slowly adapting receptors, the rapidly adapting receptors being only transiently affected by SO_2.

The lower activity of slowly adapting receptors activity in newborns (Fisher & Sant'Ambrogio 1982) may also explain the weaker response to tussigenic stimuli in the early stages of development (Korpas & Tomari 1979). It seems likely that the cough suppressing action of pulmonary stretch receptor block is associated with central suppression of afferent information necessary for the initiation or co-ordination of complex reflex activity of the cough.

2. Rapidly adapting receptors.

These are also myelinated nerve fibres which differ from slowly adapting receptors in that they adapt more quickly and fire irregularly without stimulation. The receptors discharge sporadically during normal respiration but there may be bursts of activity during inspiration (Fillenz & Widdicombe 1972). A study, where a partial block of vagal conduction, limited to the myelinated fibres was produced, has shown inhibition of the cough reflex (Kaufman *et al.* 1980), thus implicating a role for nerve endings with myelinated fibres. These fibres are stimulated by mechanical and chemical stimuli and are therefore known as "irritant receptors". Intrapulmonary

irritant receptors are stimulated by gases such as ammonia, SO_2, ozone and also by several inflammatory mediators such as histamine, serotonin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Sampson & Vidruk 1975). Stimulation of rapidly adapting stretch receptors with chemicals can also be indirect. Any substance which alters the bronchial smooth muscle calibre (bronchoconstrictor or bronchodilator agents) will indirectly stimulate the rapidly adapting stretch receptors and therefore, can induce cough.

Anatomically these receptors are believed situated below the epithelium and between the epithelial cells and their sensory information is conducted in the vagus nerves by myelinated nerves (Agostoni *et al* 1957). The receptors are found around the whole circumference of conducting airways and their density is greatest at bifurcations (Sant'Ambrogio *et al.* 1984). They have been further described in the cat (Das *et al.* 1978) and in humans (Laitenen 1985).

Evidence for the role of rapidly adapting receptors in cough comes from the fact that many tussigenic stimuli can stimulate or sensitize them to other stimuli. In the trachea and proximal bronchi when stimulated, they produce cough and those further down in the lung bring about augmented breaths and hyperpnoea and possibly cough (Fillenz & Widdicombe 1972). Furthermore in view of their distribution rapidly adapting receptors seem to most likely to be the cause of coughing (Mortola *et al.* 1975).

However, there is some evidence to suggest that these receptors may not have a large role in the genesis of cough. Correlation between the presence of intraepithelial nerves and the presence of cough is poor, as demonstrated by the absence of intraepithelial nerves in the ferret who coughs, and the rat which has intraepithelial nerves in the trachea but does not cough when the lung is stimulated (Jeffery & Reid 1973).

3. C-fibres.

The majority of fibres originating from the larynx and lower airways are non-myelinated, the ratio of non-myelinated to myelinated fibres being 11:1 in the cat (Jammes *et al.* 1982). Lung C-fibre endings are relatively insensitive to mechanical stimulation and, unlike the myelinated nerve endings, are found throughout the lung (Karlsson *et al.* 1988b). C-fibre endings are generally silent and have been studied by inducing activity with exogenous substances such as capsaicin and phenyl biguanide. Boushey and co-workers (1974) reported that phenyl biguanide topically applied to the laryngeal mucosa of the cat stimulated laryngeal C-fibre receptors. They also showed that SO_2 was a more potent stimulant of C-fibres than of myelinated fibres in the superiorlaryngeal nerve, and that cats coughed when they inhaled SO_2. When given to conscious dogs through a tracheostomy, SO_2 caused coughing and stimulated the C-fibres in both bronchi and extra-pulmonary airways, but in contrast had little effect on irritant receptors when inhaled by dogs (Roberts *et al.* 1981), suggesting a role for C-fibres in the mediation of cough. Capsaicin, a selective C-fibre stimulant, evokes cough in man and guinea pigs (Collier & Fuller 1984; Forsberg & Karlsson 1986). Bradykinin and prostaglandins also stimulate C-fibres, as seen in dogs (Karlsson *et al.* 1988b) and evoke cough in man (Costello *et al.* 1985; Fuller *et al.* 1987a). Coughing, however, is abolished by a selective cold block of vagal conduction which preserves C-fibre function (Widdicombe 1954).

The data for the role of both C-fibres and rapidly adapting fibres in the mediation of cough is therefore not clear cut. It is most likely that under appropriate circumstances, stimulation of one or the other, or of both C-fibres and rapidly adapting fibres can cause cough.

Mechanics of cough

Afferent impulses pass from the respiratory passages through the vagus nerve to the medulla. There, an automatic sequence of events is integrated by the 'cough centre', leading to the several phases of the efferent limb of the cough reflex (Leith 1985). These are designed to create maximum airflow in the upper airway and are achieved due to the high linear gas velocities (distance travelled by gas molecules per unit time) that are a function of the cross sectional area of the airways:

Velocity = flow/cross sectional area.

For a given flow the linear velocity is high in airways that are dynamically compressed by high intra-thoracic pressure. These high linear velocities are important for the removal of secretions and they also vibrate the lung and airway tissues causing the characteristic coughing sound.

While the sensory limb of cough is still largely unknown, in man the effect of coordinated activation of the efferent pathway is well established (Leith *et al.* 1986). There are four important phases to cough and are as follows:

1. Inspiration

During this phase, inhalation of a widely variable volume of air occurs with the glottis open (averaging 2.5 litres) to allow greater expiratory flows and pressures. The variability in the amount of air inhaled ranges from 50% of the tidal volume to 50% of the vital capacity. The high pressures produced at high lung volume are important in increasing the cough effectiveness, because at high lung volumes the length-tension relationship of the expiratory muscles is at a peak. Hence these muscles are capable of producing greater expiratory pressures and flows.

2. Compression

This starts with the simultaneous closure of the glottis and the onset of expiratory activity in muscles of the rib cage and abdomen. The glottis remains closed for approximately 0.2 seconds and the intrinsic pressure may be as high as 300 cm H_2O (McCool & Leith 1987). The rate and the amount of change in lung volume during this time is large. The force velocity relationship for skeletal muscle contraction dictates that the greater the velocity of contraction the smaller the force developed. Expiratory flow rate and the rate of compression of intra-thoracic gas dictate the expiratory muscle shortening velocity. Hence when the glottis is closed there is no expiratory flow and the expiratory muscle shortening is small. However, when the glottis opens, shortening velocity is reflected by expiratory flow and rate of volume change due to gas compression. Hence the velocity of expiratory muscle shortening is much greater when the glottis is open. Therefore the pressure developed decreases as expiratory flow increases and intra-thoracic pressure is not as high as when the glottis is closed.

3. Expression

This begins with the opening of the glottis; there is an explosive release of trapped intra-thoracic air. As the glottis opens pressure in the central intra-thoracic airways drops to that of atmosphere, whilst that in the alveoli and pleural spaces remains positive. The high intra-pleural and intra-alveolar pressures produce high expiratory flow rates, such that the pleural pressure exceeds that within the central airways and so the airways collapse. The decrease in the airway volume may be small but as it occurs so quickly the instantaneous flows may be very high eg. a change in volume of 50 ml in 10 msec would result in a mean flow of 5 l/s. This phase may

last a few tenths of a second during which time a litre or less of air is expired.

4. Relaxation

This phase is associated with relaxation of expiratory muscles, with a fall in pleural and alveolar pressures, and with transient bronchodilatation. The glottis may or may not be closed. The compressed central airways expand.

SECTION 4

Assessment of cough

Various methods over the years have been employed to assess and quantify cough such as clinical evaluation, sound recording, diary cards and questionnaires and provocative challenges.

Clinical

As with any other symptom, a full clinical assessment of cough can be performed. This includes reporting of a history eg. duration and character of cough, recent respiratory tract infections, smoking history and any other relevant occupational and environmental exposures. This is followed by a physical examination and appropriate investigations which may include chest and sinus x-rays, barium studies pH monitoring and pulmonary function testing. These will give information towards a clinical diagnosis but add no further information relating to the quantification of the cough.

Sound recording

This method gives an objective assessment of the frequency and number of coughs. For an individual, the most practical use has been to record night time cough with a sound operated recorder in the patient's bedroom (Archer & Simpson 1985). Another technique has been employed by the Food and Drug Administration for assessing tussive agents, which requires patients with chronic productive cough to

gather together in a room. The cough sounds are recorded over a period of time. This technique is limited, however, by marked inter-individual differences in cough frequency.

Davenport (1938) used kymographic recordings of chest movements to determine frequency of coughing, but found it an unsatisfactory method for monitoring cough. Gravenstein and co-workers (1954), introduced a sound recorder to a patients room, connected to a microphone, from which the number of coughs were counted and the antitussive assessment of heroin and codeine was made. A direct writing system was used to record all sounds in the patients room and the sound-induced electrical impulses were registered kymographically. The technique was found to be tedious as one of the investigators had to sit outside the room and personally note which sounds were coughs.

A study of the spectral analysis of cough sounds (Debreczeni *et al.* 1986) was made for patients and normals. From the study they were able to demonstrate that the duration of cough was longer in the patients. However, these results may not be useful as the sounds were recorded with a mouthpiece and nose clip on the patient, and the microphone attached to the cheek, which may have altered the cough sound.

When studying cough it seems that any attachments of apparatus to the subject such as the microphone as above or as in other studies electrodes, for recording abdominal electromyograms (Cox *et al.* 1984) are undesirable, hence studies recording cough should be designed to be simple and unobtrusive to the subject. In using the above methods it also may be impossible to distinguish cough from other noise. The sound recordings may also have to be made over long observation periods, severely limiting its usefulness.

Diary cards and Questionnaires

Diary cards represent a standard method for recording any symptom and are applicable to cough. Some studies have shown a poor correlation with night time cough and reported symptoms. Archer & Simpson (1985) measured cough at home with a microphone attached above the subjects head and also on a diary card. Objective cough counts and half hour periods containing cough recordings on a tape recorder did not correlate with diary card scores awarded to eight children on seven nights each. This study implies that night cough diary scores may mislead in the assessment of symptom severity. Another group (Fyans *et al.* 1989) using diary cards to assess symptoms in a 12 week study with nedocromil sodium in asthmatics, found that although lung function had significantly improved whilst taking the drug, none of the parameters recorded on the diary card, including cough, reached statistical significance, suggesting that symptoms evaluated using a dairy card does not provide reliable information. Alternatively, it can be concluded that the observations from these two studies do not reflect the disease state. Furthermore diary cards are less useful in providing information which can increase our understanding of the mechanisms and pathology of chronic cough. Despite these problems, recording on diary cards remains the most practical method in studying large groups of patients and in long term assessment of symptoms such as cough (Tinkelman *et al.* 1985). They are practical, as it is an uncomplicated procedure to use with patients outside laboratories and allows the patient to monitor their symptoms over any length of time.

A number of "questioning techniques" have been used in an attempt to quantify cough, such as the Medical Research Council questionnaire for bronchitis which has been well validated (Medical Research Council Committee on Research into Chronic

Bronchitis 1976). Another well validated approach has been to ask a series of questions in random order which have then been used to give a numerical score for the patient's cough severity (Gulsvik & Refvem 1988).

Gravenstein and co-workers (1954) used a questionnaire in their study to obtain information on frequency of cough, distress caused by cough, sputum production and side effects of the therapy given. The questionnaire was setup assuming that the patient would establish a baseline for their "usual cough", that is the cough they had experienced for a long time. However, this suffers the disadvantage that the patient may not have used their 'average cough' as their baseline, but compared any given days cough with that of the previous day.

An epidemiological study on persistent cough (Wynder *et al.* 1964) found that reactions to the questions varied and were highly subjective. It was also found that the frequency of a history of persistent cough amongst various smoking categories showed an inconsistent rate of increase with age, whereas the study described that the older non-smokers reported more frequently a history of persistent cough than did the younger ones.

In a standardised respiratory symptom questionnaire administered to 200 males (Dales *et al.* 1987), the value of the information obtained in predicting airway reactivity was analyzed. They found that it had a specificity ranging from 5% - 67%, this was due to a weak association between airway reactivity and information on the cough, eg. wheeze or an episodic cough with sputum.

Coughs vary greatly in their individual severity, duration and strength. These qualities are difficult to measure, and as a consequence answers to questions on diary cards and questionnaires can be highly subjective. Unblinded interviewers can further

bias the results of a study. Interviewers may also differ in the methods they use, how they obtain their information and interpret and report the answers they receive to questionnaires. This was clearly demonstrated by Cochrane and co-workers (1951).

However, it appears that questionnaires can be useful for epidemiological studies but in the assessment of the efficacy of antitussive agents the results may be too subjective to be valid.

Provocative challenges

In order to assess the sensitivity of the cough reflex in patients and to test and compare objectively the relative effects of antitussive agents, it was thought necessary to develop methods to experimentally stimulate cough. An ideal challenge should possess the following properties : 1) a volunteer should show consistent responses to the same dose of stimulating agent, within a study day, 2) reproducible response during the course of the study, and 3) the method for eliciting the cough should be non-toxic and relatively simple to perform.

The development of methods for experimentally stimulating cough has spanned over 40 years, and a variety of stimuli have been employed to induce cough, and to assess the effect of antitussive agents on this cough. The agents used to experimentally induce cough are discussed below.

Early Challenges

Hoglund and Michaelson (1950) published the first report on experimental cough provoked in man, by inhalation of a jet of ammonia vapour to induce coughing in human volunteers, in an attempt to measure the antitussive effect of various drugs.

Gravenstein and co-workers (1954) used a modified version of that used by Hoglund & Michaelson (1950). Although similar methods were used in these studies, they showed conflicting data in the evaluation of opiates. It seems that the design of experiments of the these studies was not satisfactory. They did not use a placebo or double-blind conditions. Furthermore the ammonia mixture used to stimulate cough was readily lost from the rubber container as shown by Gravenstein *et al* (1954), in contrast to that reported by Hoglund & Michaelson (1950) who found levels to remain constant.

A method using acetylcholine was developed by Tiffeneau (1957) to elicit cough. The threshold of excitability, frequency, intensity, onset and duration of acetylcholine cough was measured. This method was further modified by two groups to evaluate antitussives (Gregoire *et al* 1958; Prime 1961) . Gregoire *et al* (1958) used acetylcholine at various concentrations to obtain a threshold concentration for the production of cough. This dose was then given, after the intravenous application of a local anaesthetic to ten subjects. Prime *et al* (1961) had subjects continually breathing a 1 % acetylcholine solution until coughing began; the number of coughs were counted for the following 20 minutes. Results obtained from these studies could not be repeated in a later study by Sevelius and co-workers (1965) using Tiffeneau's method (Tiffeneau 1957), suggesting that the use of acetylcholine in assessing cough was not satisfactory.

Citric acid

Bickerman & Barach (1954) noticed that when patients with bronchial asthma were given nebulized bronchodilator drugs, irritation occurred in the upper respiratory

tract. This lead to the development of a citric acid aerosol challenge which subsequently was used as an effective method for inducing cough. Gravenstein and co-workers (1954) used this aerosol model as a means to investigate the effect of antitussives such as codeine and heroin on citric acid induced cough. They found great variability in the subjects' response to the citric acid, this was undoubtedly due to the fact that such aerosols did not provide fixed concentrations, as noted by Bickerman and co-workers (1956). Consequently the method was further developed and refined whereby subjects inhaled substances from various nebulizers which delivered particles of different sizes ranging between 1.8 - 4.2 μm. The dose of citric acid delivered was measured accurately by the use of a reservoir placed between the ventilometer and the mask. A total of 33 potential inducers of cough were tested including tartaric, citric, hydrochloric, sulphuric and benzoic acids, ethyl alcohol, diethyl ether and concentrated ammonium hydroxide. Aerosols composed of citric, tartaric or benzoic acid produced the most consistent results. This method was further refined to not only record the number of coughs but also peak airflows and volumes of air expelled, as induced by a standard dose of citric acid aerosol (Bickerman *et al.* 1956). However, in the screening of patients Bickerman *et al.* (1956) discarded 80% of the subjects, retaining only those who gave a constant response to citric acid. Despite this, he was able to demonstrate a 50 % reduction in the citric acid cough in response to the use of antitussives. The usefulness of this cough challenge is thus severely restricted by the need for careful patient selection.

This method has been extensively used for inducing cough over the years. Archibald and co-workers (1959) and Shane and Krzyski (1957) used citric acid solutions at concentrations of 10 % and 15 %. A further modification of this model

was made where the latent period, that is, the period from the beginning of inhalation of citric acid to the onset of cough was measured, any lengthening of this period being indicative of cough suppression (Calesnick & Christensen 1967). These groups demonstrated the antitussive effectiveness of codeine.

Empey and co-workers (1979) further simplified and improved the technique of Bickerman *et al* (1956) by avoiding the use of complex equipment and increasing the sensitivity by using smaller increments in concentration of citric acid, to establish the cough threshold. It seems likely that the screening procedure in this study gives rise to problems with having a select population of responders to citric acid. Subjects who did not cough after inhalation of one breath of 10 % citric acid were excluded. However, 16 of the 18 subjects chosen for the study failed to cough at even 20 % citric acid on baseline challenges.

Packman & London (1980) used the original method of Bickerman *et al* (1956) to evaluate the antitussive effects of aromatics delivered by inunction. A disadvantage of this study was the unnecessary use of complex recording equipment of the cough response. A further disadvantage of this method was the use of "trained" subjects, who had worked with the method through many months, and hence may have shown a reasonable degree of adaptation to the procedure.

Two studies (Rees & Clark 1983; Belcher & Rees 1986) used citric acid solutions between 1 % and 50 % to assess several antitussives and ß-adrenoceptor stimulants. Considerable variation in the pre-treatment cough thresholds between individuals (2 - 50 %) and to a lesser extent within subjects was found. Also steadily increasing doses of citric acid were used to induce cough, which may have had the potential problem of producing cumulative effects of sensitivity to citric acid as shown

by a previous study (Calesnick & Christensen 1967).

With the use of citric acid to stimulate cough over the years, a study to investigate the diurnal variation and adaptation of the cough response to citric acid was performed (Pounsford & Saunders 1985). Six of the eight subjects studied were found to have a higher threshold in the afternoons and the mean thresholds tended to decrease as the experiments proceeded. In ten subjects, two series of citric acid inhalations were given, separated by an hour. The total number of coughs was significantly lower during the second run.

The technique pioneered by Bickerman *et al* 1956) still remains the most popular cough inducing model in the evaluation of the efficacy of antitussives. However, from the literature reviewed it is clear that there is a wide variation between subjects in their response to citric acid and the effects of antitussive agents appears to produce different results in different studies. In those studies where the subjects were breathing citric acid continuously, it seems probable that any irritation or coughing provoked during inhalation would lead to an altered pattern of deposition of the citric acid in the airways. The studies cited also fail to mention whether they were performed at the same time of day, when assessing antitussives. Diurnal variation has been shown to be an important factor in the expression of an antitussive effect (Pounsford & Saunders 1985).

Low chloride solutions

Several studies have shown that the inhalation of ultrasonically nebulized aqueous solutions which contain a low concentration of permeant anion cause bronchoconstriction (Allegra & Bianco 1980; Schoeffel *et al* 1981) and Boushey *et*

al (1974) showed that *in vivo* stimulation of vagal afferent receptors in the laryngeal epithelium appeared sensitive to falls in Cl^- concentrations. Sheppard *et al.* (1983) have also investigated whether or not cough was induced under these conditions. They found that the inhalation of distilled water caused significantly more coughing than did saline. Eschenbacher *et al.* (1984) suggested that the alterations of osmolarity, as well as the low permeant anion concentrations, of inhaled aerosols, also induce cough. However, Lowry *et al* (1988) when investigating the effects of pH and osmolarity on aerosol-induced cough, could not find any change in the cough elicited by D-glucose aerosols over a range of osmolarities. Fuller & Collier (1984) also studied the cough response to inhalation of distilled water and as in Sheppard and co-workers (1983) study, found that the cough response to inhaled distilled water was poorly reproducible and in asthmatics marked bronchoconstriction as well as cough was produced. This may affect interpretation of results when using such methods to assess the efficacy of antitussives.

Godden and co-workers (1986) developed a method of inducing cough by inhalation of fluids of various osmolarities, pH and ionic content. Coughs were identified by their flow pattern displayed on a two channel recorder and cough frequency, flow rate and volume were also analyzed. From their data they concluded that cough frequency progressively increased with the reduction in chloride ion concentration. This group applied their method to investigate the antitussive effects of bronchodilators on low chloride ion induced cough (Lowry *et al* 1987). However, their results conflict with data from other groups where these bronchodilators showed no effect on chemically induced cough (Pounsford *et al* 1985; Belcher & Rees 1986), suggesting that use of different agents to induce cough produce different results.

Miscellaneous challenges

Intravenous para-aldehyde was used by Gravenstein & co-workers (1954) to induce cough in assessing opiates as antitussives. Repetitive injections of paraldehyde produced a mild degree of phlebitis and it also produced intense and painful vasospasms. There was also an after burst of coughing after the initial cough and great variation was seen in the response within the same subject at different times of the day and between subject to subject. Hence the differences between values before and after opiates were too small to be significant.

Another agent lobeline, which is thought to have a direct effect on the "cough centre" was administered intravenously in a study and was found to cause cough followed by tachypnoea (Eddy *et al* 1969a). However, in view of its possible toxic reactions it has not been used widely to assess cough.

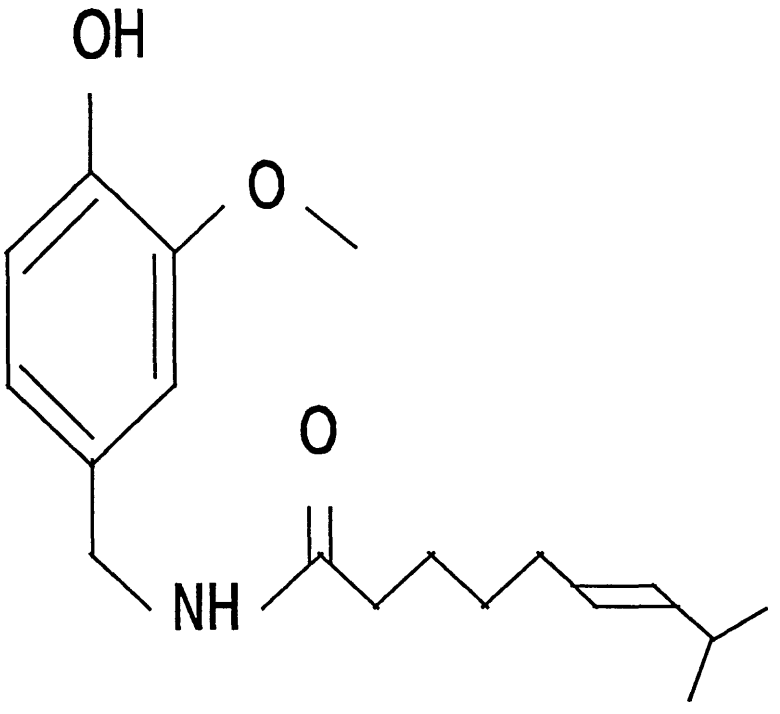
Hillis (1952) used a peppermint-water (1:40) solution to induce cough, by spraying it ten times directly into the larynx, via an endo-tracheal tube, of one patient over the period of one year. This study has little value since the results from the study of one subject, may obviously not reflect results from a larger more representative population. The administration technique is unpleasant and further tachyphylaxsis may have occurred in the single subject over the course of the study.

Capsaicin

Collier & Fuller (1984) first demonstrated in humans that inhaled capsaicin (Figure 1.2) was a powerful cough stimulant, which was well tolerated. Capsaicin is a selective rather than specific stimulant of pulmonary C-fibre endings. In the study of Collier & Fuller (1984), capsaicin produced a dose-dependent cough response and number of coughs produced by a fixed dose of capsaicin in any subject was highly reproducible. There was also little evidence of either acute desensitisation or of desensitisation occurring over longer periods as the same cough response occurred when subjects were challenged at intervals of several days. Thus capsaicin-induced cough appears to have an advantage over previously studied challenges, and hence was considered to be suitable for further development, as described in the following chapter.

Figure 1.2.

Chemical structure of capsaicin (8-methyl-N-vanilyl-6 nonemide).



SECTION 5

Treatment of cough

It is perhaps surprising that such a common symptom as cough, is without effective therapy (Banner 1986). Where there is an underlying cause for cough, treatment is relatively simple, since adequate therapy for the underlying condition may well relieve the patient of cough. This is typically seen in patients where the cough is associated with asthma. The real therapeutic challenge is the relief of cough whose aetiology is unknown (so called idiopathic cough) or whose origin is not directly treatable eg. viral infections. Previous studies with antitussives have given conflicting results (Hoglund & Michaelsson 1950; Bickerman *et al.* 1956; Tiffeneau 1957; Eddy *et al.* 1969a & b; Rees & Clarke 1983).

Due to the many causes and sometimes unknown aetiology of cough, treatment can be confusing and often haphazard, being directed at relieving the symptom rather than treating the underlying disease. One of the aims of this study was first to investigate further the mechanism of non-productive cough and then to study the effects of potential antitussive agents on the cough reflex in normal volunteers and in patients with cough.

Treatments currently available

Several agents are available for the treatment of cough and are discussed below:

(a) Demulcents

These comprise the majority of "over the counter" medications. These

preparations are purchased in multi-million pound quantities each year and there is some evidence that apart from tasting pleasant that they do actually suppress cough (Packman & London 1980).

The mechanism by which they do this is unknown because very little research has been done to investigate their mode of action. There are three possible modes of action:

1. The sugar content of the cough mixture encourages saliva production and swallowing,
2. The sugar solution may coat sensory nerve endings in the epipharynx and cause their stimulation, this stimulation may suppress cough by a "gating" process.
3. The sugar solution may act as a protective barrier to sensory receptors which can either produce cough or heighten the cough reflex.

(b) Opiates

The opiates are the oldest and the most studied of all cough suppressants, and are the standard against which all other cough suppressants are measured. Until the 1950's codeine was the only commonly available preparation. However, in the last 30 years numerous studies have screened for new antitussive agents such as dextromethorphan and noscapine (Kase 1980), but the popularity of codeine continues and its annual consumption remains high. There is no doubt that systemic opiates can suppress cough (Eddy *et al.* 1969b) but the dose needed is far higher than that found in the majority of proprietary cough mixtures. The British National Formulary lists seven opiate analogues as antitussives and the most commonly used contain codeine,

pholcodeine and dextromethorphan. The opiates exert their pharmacological action via the μ-subtype of opiate receptors, however, the site of these receptors is unclear. The three most likely sites based on the review of Fuller & Jackson (1990) are as follows:

1. On the sensory nerve endings responsible for initiating cough: an opioid pentapeptide which suppresses cough in animals, has an action on μ receptors located on sensory receptors in the lung (Adcock *et al.* 1988),
2. Within the central nervous system: this may be either directly on the cough centre in the medulla or by an action on the brain stem respiratory centres, and
3. μ receptor stimulation may reduce mucus production or increase mucociliary clearance and so reduce the need for cough.

(c) Local anaesthetics

Although potentially hazardous, the obvious effectiveness of inhaled local anaesthetics as antitussive agents has led to their use in intractable cough (Karlsson 1983). Local anaesthetics prevent sensory nerve traffic in both myelinated and non-myelinated nerves and are the most consistently effective antitussive agents (Karlsson 1983). Since these drugs could remove all the protective reflexes of the lung, they must be used with extreme care. The British National Formulary lists a number of local anaesthetic lozenges and sprays for the symptom of the common cold.

SECTION 6

Aims of the thesis

The aim of this thesis was to develop a method of quantitating the sensitivity of the cough reflex in patients with a cough. The method developed was a modification of the cough challenge used by Collier & Fuller (1984).

The hypothesis tested was that patients with non-productive cough would have an abnormal increase in the sensitivity of their cough reflex. Thus it would be possible to treat the symptom by use of drugs which would reduce the sensitivity of the reflex to normal, thereby not interfering with the normal defence role of the reflex. A second aim was to evaluate the effect of potential antitussives on the sensitivity of the cough reflex in normal volunteers and patients with cough.

CHAPTER 2

METHODS

Supplementary information for section 1

Development of Methods: The method was based on the study of Collier & Fuller (1984) in which the concentration of capsaicin used ranged from 2 - 65 x 10^{-6} M. A single inhalation at a starting concentration of 1.75 x 10^{-6} M (dose of 0.4 nmol) was administered to 3 subjects followed by doubling concentrations to a final concentration of 5 x 10^{-4} M (dose of 100 nmol). Higher concentrations were not tolerated and in most subjects produced no further increase in the number of coughs recorded. A concentration of 5 x 10^{-4} M was chosen as the end point.

Reproducibility: Once the concentration range and dosing interval were established the reproducibility of the method was assessed in normal subjects. Same day: nine people were challenged twice at an interval of one hour and 6 people were challenged three times at one hour intervals. The mean change in log D_2 between challenges was 0.03 ± 0.14 (± CI) and the log D_5 was 0.00 ± 0.09. Between days: thirteen people had three capsaicin challenges at least one day apart. The mean change from day to day in log D_2 was 0.1 ± 0.1 and the log D_5 was 0.08 ± 0.12. Therefore all 95% CI were < 0.15, hence 95% of the time a repeat challenge will be less than one doubling dilution ie. 0.3 in log units (0.15 x 2). This degree of reproducibility compares closely to that seen in the bronchial challenge described by Yan *et al* (1983).

Tachyphylaxis: There was no tachyphylaxis seen in the Collier & Fuller study (1984). There was also no tachyphylaxis seen either in the study above where subjects were challenged 1 hour apart or in the placebo-controlled studies of chapter 5. The initial finding that the dose-response curve to capsaicin was not altered by dosing either at random or with ascending concentrations further supports the lack of any significant tachyphylaxis. Therefore, although I did not formally exclude tachyphylaxis by the repetitive inhalation of high doses of capsaicin, it is unlikely that it would affect the interpretation of the dose-response to capsaicin in the studies reported in this thesis.

Dosing schedule: After demonstrating an increase in the sensitivity of the cough reflex in patients with chronic cough, it was considered unacceptable to challenge them using random dosing in case they experienced severe coughing spasms following high concentrations of capsaicin. In contrast, normal subjects were blinded to the challenging dose of capsaicin by administering them in a random fashion.

Age: Although a normal elderly population was not formally studied, the non-coughing control groups in chapter 3 (table 3.1) contained substantial numbers of elderly patients and these groups had essentially normal responses to capsaicin independent of their age.

SECTION 1.

Cough challenge

Cough was induced using capsaicin (Sigma, Poole, UK) which was dissolved in absolute alcohol to make a stock solution of 10^{-2} M. This was further diluted in saline ([0.9 % NaCl] Sterac: Ivex Pharmaceuticals Ltd, Larne, N. Ireland) to provide solutions of capsaicin between 1.75 x 10^{-6} M and 5 x 10^{-4} M. The solutions were stored at 4°C and renewed every three to four days.

A standard challenge was used in most studies but some variations were made for the drug intervention studies. The standard challenge consisted of the subjects inhaling single breaths of saline and capsaicin solution given via a breath activated nebuliser controlled by a dosimeter (PK Morgan Ltd). The solutions were given in a random order in the normal volunteer studies, but not in the patient studies when the solutions were given in ascending order and inhalations of saline were interspersed at random during the challenge for both normal volunteer and patient studies as a blinding procedure. Each challenge was given at one minute intervals. The number of coughs within the minute following the inhalation, were recorded using a microphone attached to a mingograph recorder (Siemans Elema AB, Solma, Sweden) running at 25mm/sec. The challenge was stopped when the subject coughed 5 or more times. Coughs were identified as a discrete complex of deflections on the paper; each complex was counted as a cough.

Statistical analysis

The results are expressed as the log of the capsaicin dose or concentration causing two or more coughs (D_2 or C_2) and the log of the capsaicin dose or concentration causing five or more coughs (D_5 or C_5). These parameters were chosen from the data obtained in the preliminary studies: D_2 was chosen as the lower parameter and not the threshold, D_1, to increase the likelihood that the cough recorded a real event related to capsaicin dosing and not a coincidental chance occurrence which may be the case if single coughs are recorded; the D_5 was the dose where most subjects reached the plateau of the dose response curve.

These parameters are presented as means or medians with 99 % confidence intervals (CI) or standard error (SE). A typical cough dose response curve is shown in Figure 2.1 and it also shows how the D_2 and the D_5 are obtained from the curve.

The data for the control population was normally distributed. However, for the patient groups this was not the case. In all studies data was therefore analyzed by non-parametric analysis of variance and the Mann-Whitney U test in Section 3 of Chapter 3. The difference in the number of coughs for each subject before and after drug treatment was analyzed by the paired Wilcoxon test.

All the data in the treatment section remained blind until the studies were completed.

Figure 2.1.

Capsaicin cough-dose response curve of one subject demonstrating the calculation of the dose of capsaicin causing two or more coughs (D_2) and five or more coughs (D_5).

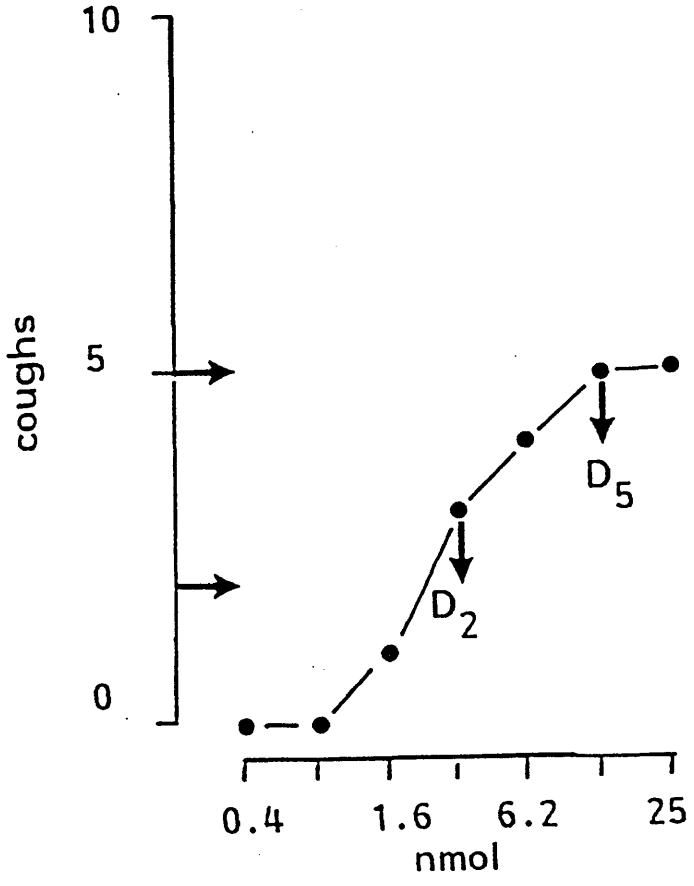
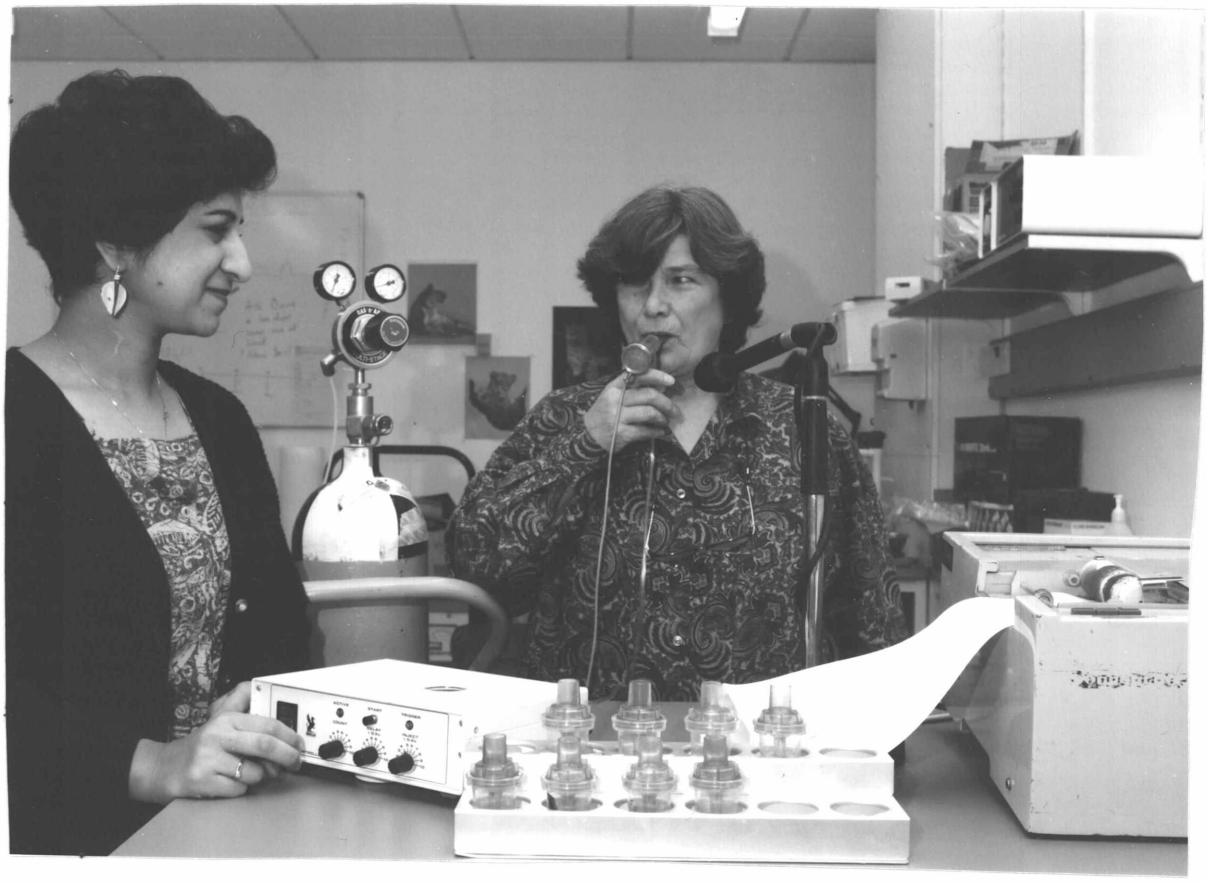


Figure 2.2.

Photograph of a patient inhaling a dose of capsaicin from a nebuliser attached to a breath activated dosimeter. The number of coughs were recorded on a mingograph.



SECTION 2.

Bronchial Challenge

Histamine sensitivity was measured by modification of the method described by Yan *et al.* (1983). Spirometry was measured using a Vitalograph spirometer (Vitalograph UK).

Histamine acid phosphate (Indis Ltd, UK) was diluted in 0.9 % saline to make a 32 mg/ml stock solution from which concentrations of 8 mg/ml and 2 mg/ml were made. The solutions were stored at 4°C and renewed once a week. 0.9 % saline was used as the control solution. The solutions were administered using a hand held Devilbiss No. 40 nebuliser. Following baseline measurements of FEV_1 (forced expiratory volume in one second) a saline inhalation was given. The patient sitting with their head slightly extended was asked to take large breaths through their open mouth, with the open end of the nebuliser placed just inside their front teeth and directed towards the back of the throat. The bulb of the nebuliser was then given a firm squeeze as the subject inhaled towards total lung capacity. Two measurements of FEV_1 were made one minute after the inhalation of saline. The mean FEV_1 after challenge with 0.9 % saline was taken as the reference level from which the required 20 % fall was estimated. Increasing doses of histamine were then administered and FEV_1 measured 60 seconds after each dose. Histamine was administered until a 20 % fall in the FEV_1 was reached or after a cumulative dose of 4.94 µmol was reached (Table 2.1). A bronchodilator aerosol was given to help recovery when required. The percent change in FEV_1 was plotted against the dose of histamine on a logarithmic scale and the dose in µmol which caused a 20 % fall (PD_{20} FEV_1) was recorded.

Table 2.1.

Cumulative doses of histamine administered during a bronchial challenge using a Devilbiss No. 40 nebuliser.

No. of puffs of histamine	Cumulative dose of histamine (μmol)
Baseline	0
Normal saline x 2 puffs	0
2 mg/ml x 2 puffs	0.04
2 mg/ml x 4 puffs	0.12
8 mg/ml x 2 puffs	0.28
8 mg/ml x 4 puffs	0.60
32 mg/ml x 2 puffs	1.24
32 mg/ml x 4 puffs	2.44
32 mg/ml x 8 puffs	4.94

PD_{20} (as defined by Yan *et al* (1983)

>0.08 μmol - severe asthma

0.08 - 0.6 μmol - moderate asthma

0.6 - 1.5 μmol - mild asthma

>1.5 μmol - normal

SECTION 3.

Respiratory resistance

Total respiratory resistance was measured using the forced oscillation technique developed by Landser and co-workers (1976). This technique was designed to measure the resistance of the total respiratory system using sound waves. The sound waves, which are sine-wave oscillations, are generated by a loudspeaker connected to the tube through which the subject breathes, thus oscillating the respiratory system. This results in sine-wave pressure and flow oscillations which are measured at the mouth. Total respiratory resistance, R_{osc}, is calculated from the ratio of mouth pressure and flow (R_{osc} = pressure/flow). The measurement is made with the subjects cheeks and floor of the mouth supported by the palms of the hands, breathing quietly on a mouth piece that is attached via a screen pneumotachograph to the oscillation apparatus. A bias flow prevents changes in oxygen and carbon dioxide. Pseudo-random noise is applied at the mouth by a loudspeaker at 2-Hz intervals over the range 2 to 26 Hz for a 16 second period. Mouth pressure and flow across the pnuemotachograph are recorded by two identical differential pressure transducers (PM5; Validyne, Northbridge, California) and the pressure and flow are separated by Fourier analysis. From these signals, $R_{osc,}$ at different frequencies, is computed and this is the mean values for inspiration and expiration over the 16 second period of tidal breathing. The apparatus is checked and calibrated to ensure that the response of both transducers and attached tubing is matched at all frequencies upto 26 Hz using a reference linear resistance. The reliability of the desired values is indicated by a coherence function for each frequency; only results with coherence function > 0.95 were accepted.

CHAPTER 3

SENSITIVITY OF THE COUGH REFLEX IN PATIENTS WITH CHRONIC COUGH

SECTION 1

Introduction

Chronic cough may be either a productive or non-productive of sputum, and many patients attend Chest Clinics from both groups.

Having designed a reproducible, tolerant and safe method to quantify the sensitivity of the cough reflex in man, I investigated the hypothesis of whether those patients with a non-productive cough, had an abnormal increase in the sensitivity of their cough reflex, before studying the effects of various antitussives on the capsaicin-induced cough. With the presence of two populations of chronic coughers, ie productive and non-productive, I also questioned whether there was a difference in the sensitivity of the cough reflex between patients with productive and non-productive cough and whether this was related to different disease states. As a control group I have studied a population of normal volunteers and patients without cough.

SECTION 2

The sensitivity of the cough reflex in normal volunteers and patients without cough

In order to assess the sensitivity of the cough reflex in patients with cough, it was first necessary to establish the sensitivity of the cough reflex in both normal volunteers and patients without cough. The data on the sensitivity of the cough reflex in these groups, is presented below.

Methods

Subjects

One hundred and fifty-eight normal volunteers from The Royal Postgraduate Medical School and patients without cough from various clinics at Hammersmith Hospital were studied (Table 3.1). The groups of subjects studied consisted of normal volunteers and clinic patients without cough. These included stable asthmatics with no current cough, patients taking ACE-I therapy who had not developed cough and patients on hypertensive therapy other than ACE-I. All subjects had no recent history of a respiratory tract infection.

Cough challenge and Bronchial challenge

As described in chapter 2.

Protocol

All subjects had a capsaicin cough dose response measured. In addition asthmatic subjects underwent a bronchial histamine challenge.

Table 3.1

Characteristics of normal volunteers and patients without cough.

GROUP	No.	Sex	Mean age (range)	Smoking history			
				NS	EX	S	NK
Normals	90	37F	29.2 (19-56)	57	6	15	13
ACE-I no cough	35	19F	56.6 (25-73)	26	2	5	0
Hypertension	15	10F	55.1 (43-68)	10	1	3	1
Asthma no cough	18	12F	33.6 (5-76)	10	0	2	6

NS non smoking

EX ex-smoker

S smoker

NK not known

Results

The cough challenge was well tolerated with no adverse reactions nor any bronchoconstriction in the patients with asthma. The results for the C_2 and C_5 are shown in Table 3.2. There was no difference between the patients sub-groups, and no difference was found between the normal volunteers and the control patient group. Bronchial challenges with histamine were found to be positive in all asthmatics tested.

Within the normal volunteers and control patient group no difference in the sensitivity to the capsaicin challenge between the sexes was seen (expressed as the C_2 and C_5). The log mean (99 % CI) C_2 and C_5 for the females was 1.05 (0.17) and 1.80 (0.22) respectively, and for the males was 1.02 (0.14) and 1.81 (0.14) respectively. Within the study sample as a whole there was no difference in the C_2 and C_5 values between non-smokers, smokers and ex-smokers. The log mean (99% CI) C_2 for the non-smokers, smokers, ex-smokers and unknowns was 0.962 (0.08), 1.00 (0.16), 0.99 (0.20) and 1.01 (0.21) respectively. The log mean (99 % CI) C_5 for the non-smokers, smokers, ex-smokers and unknowns was 1.69 (0.10), 1.90 (0.18), 2.06 (0.20) and 1.84 (0.27) respectively.

Table 3.2.

The mean C_2 and C_5 with 99 % confidence intervals (CI) in normal volunteers and patients without cough.

Group (without- cough)	log C_2(μM)(99% CI)	log C_5(μM)(99% CI)	Histamine	
			+ve	-
Normals	1.04 (0.02)	1.81 (0.14)	ND	ND
ACE-I	0.94 (0.18)	1.80 (0.19)	ND	ND
Hypertension	0.91 (0.27)	1.77 (0.36)	ND	ND
Asthma	0.87 (0.22)	1.49 (0.37)	18	0

ND not done

Discussion

The data shows that there is little variation of the sensitivity of the cough reflex in the normal population and in those patients without cough. Therapy for the treatment of either hypertension or asthma appear to have no effect on the sensitivity of the cough reflex, to inhaled capsaicin. The data also demonstrates that the sex of a subject and smoking, also have no effect on the sensitivity of the cough reflex.

From this study I can conclude that the method developed to investigate the cough reflex is safe and well tolerant, and can be used to investigate the sensitivity of the cough reflex in patients with cough.

This group of normals and patients with no cough were used as the normals described in the following patient studies, unless stated otherwise.

SECTION 3

Cough sensitivity in patients with ACE-I cough

During the first three months of my research five patients with ACE-I cough were referred for investigation of their chronic cough. Some had been taking ACE-I for many years and their cough remained unaltered by therapy.

This study led to the first demonstration of an increased cough reflex in this group of patients.

Methods

Subjects

Study 1. Five patients (mean age 60 ± 12 years, 3 females) attending the chest clinic were studied. These patients had developed cough between one week and six months after starting treatment with either enalapril or captopril for hypertension or heart failure. Two patients had stable asthma, which was being treated with inhaled steroids and $ß_2$ agonists.

Study 2. From a survey in the Hammersmith Hospital Hypertension Clinic thirty-five patients (described in Section 2) taking ACE-I therapy, who had no cough and forty-three patients with ACE-I cough (mean age 56 ± 24 years, 30 females) were also studied.

Cough challenge

As described in chapter 2.

Protocol 1

The five patients described had a capsaicin cough response measured whilst taking their anti-hypertensive medication and one week after stopping the ACE-I.

Protocol 2

The patients described on ACE-I with and without cough, all had a capsaicin cough response measured, whilst on their anti-hypertensive therapy.

Results

Study 1. In these patients the D_2 (99% CI) and D_5 (99% CI) were lower, than normal 0.53 (0.38-0.74) nmol and 1.59 (0.75-3.36) nmol respectively, and the maximum number of coughs was also higher (median (range) 18 (15-20)) during treatment. The cough in these patients subsided within one week of stopping treatment. These values returned towards normal when treatment was stopped, the D_2 and D_5 being 1.59 (0.75-3.36) nmol and 3.61 (1.32-9.88) nmol respectively, and the maximum number of coughs decreasing to 8 (5-11) (Figure 3.1).

Study 2. In the group of patients who were taking ACE-I but had no cough and those with cough whilst on ACE-I the C_2 (99 % CI) was 0.94 (0.18) and 0.53 (0.18) respectively, and the C_5 was 1.80 (0.19) and 1.13 (0.25) respectively.

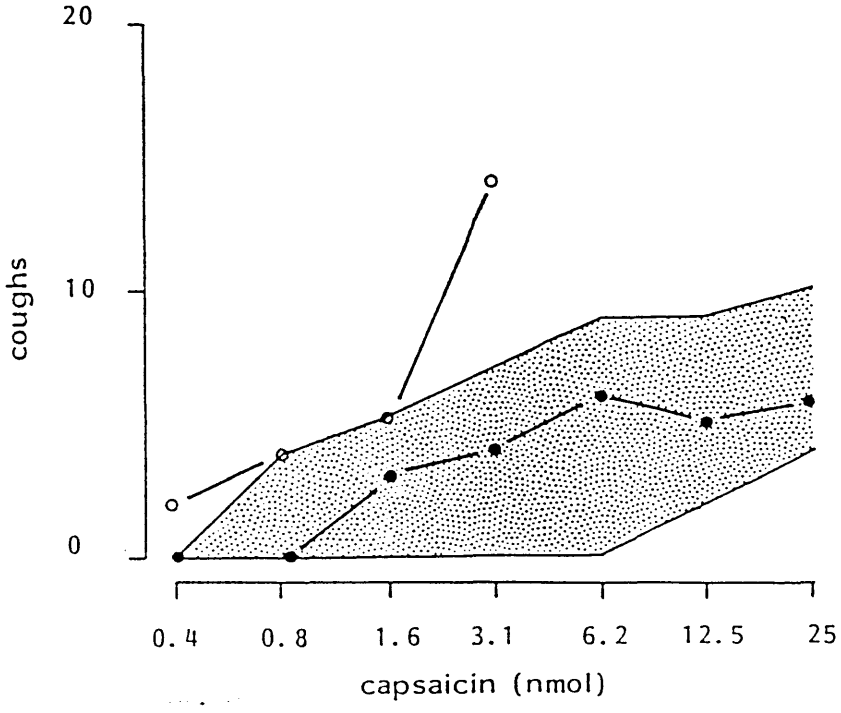
These results, for the latter two groups, were significantly different from each other ($P < 0.0001$).

On the basis of previous reports that the ACE-I cough is commoner in women, the data was analyzed to find any differences in the sensitivity of the cough reflex

amongst females and males. The C_2 (99 % CI) was 0.48 (0.17) and 0.63 (0.38) for females and males respectively. The C_5 (99 % CI) was 1.3 (0.29) and 1.12 (0.42) for females and males respectively, in the group of patients with ACE-I cough. These data demonstrate that there was no difference between female and male patients.

Figure 3.1.

Ninety-six per cent observation intervals (⁝) of number of coughs after inhalation of capsaicin in 50 normal subjects and median number of coughs in five patients with cough related to angiotensin converting enzyme inhibitor during (O) and one week after stopping (●) therapy.



Discussion

The first of these studies was the original demonstration that cough during ACE-I therapy was associated with an increase in the sensitivity of the cough reflex. Cough reactivity, measured as the response to inhaled capsaicin, was increased while on therapy and returned towards normal after stopping the ACE-I, coinciding with the resolution of the symptoms.

These observations in patients with ACE-I cough, after taking appropriate controls into account, suggests that there is an association between the presence of cough and an abnormal increase in the sensitivity of the cough reflex. However, the study was retrospective and to investigate further, a possible causal role, studies were undertaken of the acute and chronic effects of ACE-I on capsaicin induced cough.

Change in the cough reflex during therapy with angiotensin converting enzyme inhibitors

Following the initial observation in five patients it was not certain whether the reflex was abnormal in all patients taking ACE-I or only those with cough. The survey of patients on ACE-I, with and without cough did imply that the abnormality was not universal, but only occurred in patients with cough. However, this was a retrospective study and hence could not prove this.

Therefore in a prospective study the effect of two ACE-I drugs, ramipril and enalapril was investigated in hypertensive patients who had not been treated with an ACE-I previously, to assess this question. Both enalapril and ramipril have a long duration of action and are potent inhibitors of plasma ACE. The effect of these two ACE-I on blood pressure, plasma ACE activity and cough was examined in a randomized double blind, cross-over study comparing the effect of daily enalapril 10 mg, ramipril 10 mg and placebo for seven days.

Methods

Patients

Twenty patients, 11 females, were studied, with a mean age of 54 years, range 37-69 years. All had hypertension and required additional therapy in whom the introduction of an ACE-I was being considered. Patient characteristics are summarized in Table 3.3. Other anti-hypertensive treatment was continued: 11 were taking a ß blocker, 14 were taking a diuretic, one a calcium channel blocker, while 3 received only the ACE-I. Eleven of the patients were current smokers, 2 were asthmatic and used inhaled ß adrenoreceptor agonists regularly, two subjects were diabetic,one of whom was insulin dependent. One patient used prednisolone (5 mg) throughout the study as maintenance therapy for psoriatic arthritis while another took terfenadine (60 mg) twice daily for hay fever.

Protocol

Patients attended the clinical laboratory on 6 occasions at the same time of day. Following baseline measurements of blood pressure, blood was taken for plasma urea and creatinine and serum ACE activity extractions, and a capsaicin cough challenge was performed, each subject then received either enalapril (10 mg), ramipril (10 mg), or placebo, once daily for one week, in random order, with a washout period of at least one week between each treatment period. The patients were instructed to take the capsules containing the medication at night. All measurements were made in the morning. Plasma urea and creatinine and serum ACE activity were measured before the study and at the end of each active limb of the study, while blood pressure measurements and cough challenges were carried out before the study and at the start

and end of each limb. Patients were asked on each visit if they had developed a cough or other new symptoms.

Blood Pressure Measurements

At each visit blood pressure was measured, with the patient sitting, after a 15 minute rest, using a Dynomap automatic pressure recorder. Two measurements were made 10 min apart and the mean of the two used in the analysis.

Cough Challenge

As described in chapter 2.

Serum ACE activity and urea and creatinine

Blood was drawn at the start of the study and at the end of each limb of the study for measurement of urea and electrolytes. Blood was also taken and immediately centrifuged and serum stored at -20°C for subsequent analysis of ACE activity. The ACE activity was measured by a sensitive fluorometric technique (Friedland & Silverstein 1976). Serum ACE activity was measured to ensure compliance of the patients and to assess any association beteween blood pressure and serum ACE activity.

Table 3.3. Characteristics of 20 patients who needed to take angiotensin converting enzyme inhibitors to control their hypertension.

Age	Sex	Concurrent disease	Concurrent treatment	Current smoker
56	F	Hayfever	ß blocker and terfenadine	No
54	M	Non-insulin dependent diabetes mellitus	ß blocker	No
68	F		ß blocker & diuretic	No
69	M		diuretic & calcium channel blocker	Yes
62	F		ß blocker & diuretic	Yes
42	F		ß blocker & diuretic	Yes
56	M		ß blocker & diuretic	No
63	F	Insulin dependent diabetes mellitus	diuretic	No
64	F		diuretic	Yes
60	F		diuretic	Yes
60	F		ß blocker & diuretic	Yes
65	M	Asthma		Yes
42	F	Arthritis	diuretic & prednisolone	Yes
38	M	Asthma		Yes
56	M		ß blocker & diuretic	Yes
37	M		diuretic	Yes
51	M		ß blocker & diuretic	No
54	M		ß blocker & diuretic	No
55	M		ß Blocker	No
56	F			No

M - male

F - female

Results

Blood Pressure

No significant difference was found between baseline blood pressure on any of the pre-study days: blood pressure, mean (± standard error), was 159 (± 3)/97 (± 2) mm Hg on pre-study visit one and 154 (± 3)/93 (± 2) mm Hg on the second pre-study day and 154 (± 3)/95 ± 2 mm Hg on the third pre-study visit. There was no significant change in blood pressure after placebo therapy (152 (± 4)/92 (± 3) mm Hg): blood pressure was 143 (± 3)/88 (± 2) mm Hg after enalapril therapy and 147 (± 4)/86 (± 3) mm Hg after ramipril. Only the systolic blood pressure after enalapril was significantly different from that after placebo ($P < 0.05$). (Figure 3.2)

Plasma urea and creatinine and ACE activity

Mean (± standard error) plasma urea was 6.1 (± 0.5) mM and creatinine 98.3 (± 3.7) μM before the start of the study and did not change significantly during the study. Serum ACE activity was 37 ± 4 nmol. $ml^{-1.}$ min^{-1} before the study and was unchanged by placebo, (37 ± 3 nmol. $ml^{-1.}$ min^{-1}) but fell significantly to 22 ± 2 nmol. $ml^{-1.}$ min^{-1} during enalapril therapy and to 7 ± 1 nmol. $ml^{-1.}$ min^{-1} during ramipril therapy. The ACE activity after ramipril was significantly lower than that after enalapril (Figure 3.3).

Figure 3.2.

Change in blood pressure after placebo (P), enalapril (E) and ramipril (R) therapy.

(* = P < 0.05)

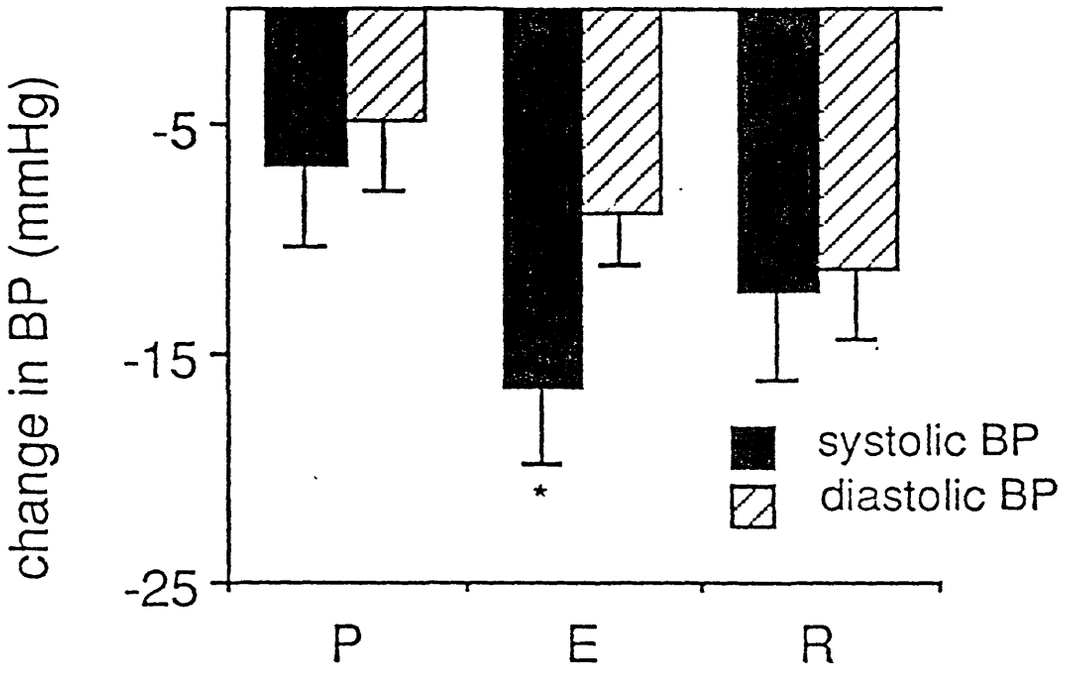
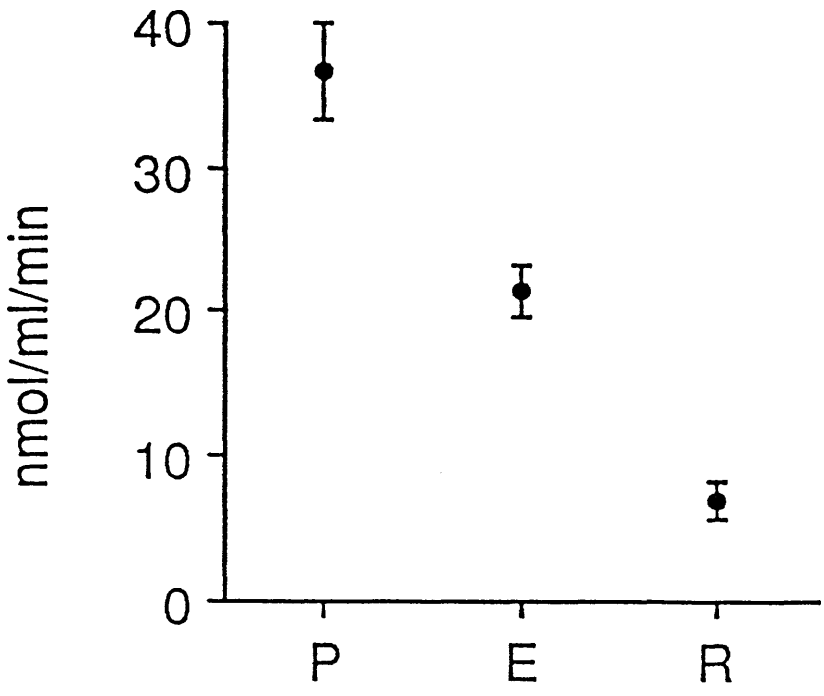


Figure 3.3.

Serum ACE activity (nmol/ml/min) after placebo (P) enalapril (E) and ramipril (R) therapy. Both active treatments caused significant falls in serum ACE activity from baseline values, that after ramipril being significantly lower than that after enalapril.



Cough Challenge Test

On the first pre-study day mean D_2 (99 % CI) was 2.3 (1.25-3.39) nmol of capsaicin and did not change significantly on the second or third pre-study days (2.2 (1.7-2.9) nmol and 2.4 (1.8-3.2) nmol respectively) or after placebo 2.4 (1.5-4.0) nmol. During enalapril therapy D_2 was 1.3 (1.12-2.82) nmol and during ramipril therapy D_2 was 2.2 (1.7-3.0) nmol (Figure 3.4 a). On the first pre-study day mean D_5 (99 % CI) was 20.2 (13.9-29.6) nmol of capsaicin and unchanged at 20.3 (14.5-28.3) nmol and 17.6 (12.0-25.8) nmol on the second and third pre-study days respectively. On placebo, D_5 was 18.9 (13.9-25.8) nmol. On enalapril D_5 was 14.4 (8.4 - 24.5) nmol and on ramipril was 15.3 (10.8 -21.18) nmol (Figure 3.4 b). None of these changes were significant. The probability of type two error (ß) missing a change of one dilution of capsaicin for D_2 was low being < 0.04 and for $D_5 < 0.02$. This was calculated from the between group variance of the data from the pre-study days.

No patient complained of cough during placebo therapy but three of the twenty patients reported cough during both enalapril and ramipril therapy. Although all three patients had a normal sensitivity to capsaicin on placebo they each showed an increase in the sensitivity of the cough reflex while on ACE-I (Figure 3.4 a & b). While on enalapril, two of these patients coughed excessively at the second lowest dose of capsaicin administered, thus making D_2 and D_5 the same. On enalapril D_2 and D_5 for each of the three patients fell below the 95 % confidence intervals for the group as a whole (D_2 0.8, 0.8, 0.4 nmol respectively; D_5 1.4, 1.4, 6.2 respectively). On ramipril the D_2 for each of the three patients fell below the 95 % confidence intervals of the group as a whole (D_2 0.8, 1.6, 1.6 nmol respectively, and the D_5 of patient 5 was below the 95 % confidence intervals at 6.2 nmol. Interestingly, the other two patients

described the cough on ramipril (which they received after enalapril) as being less severe than that experienced on enalapril.

There was no correlation between the plasma change in ACE activity or the change in blood pressure in response to ACE-I therapy and alteration in the sensitivity of the cough reflex.

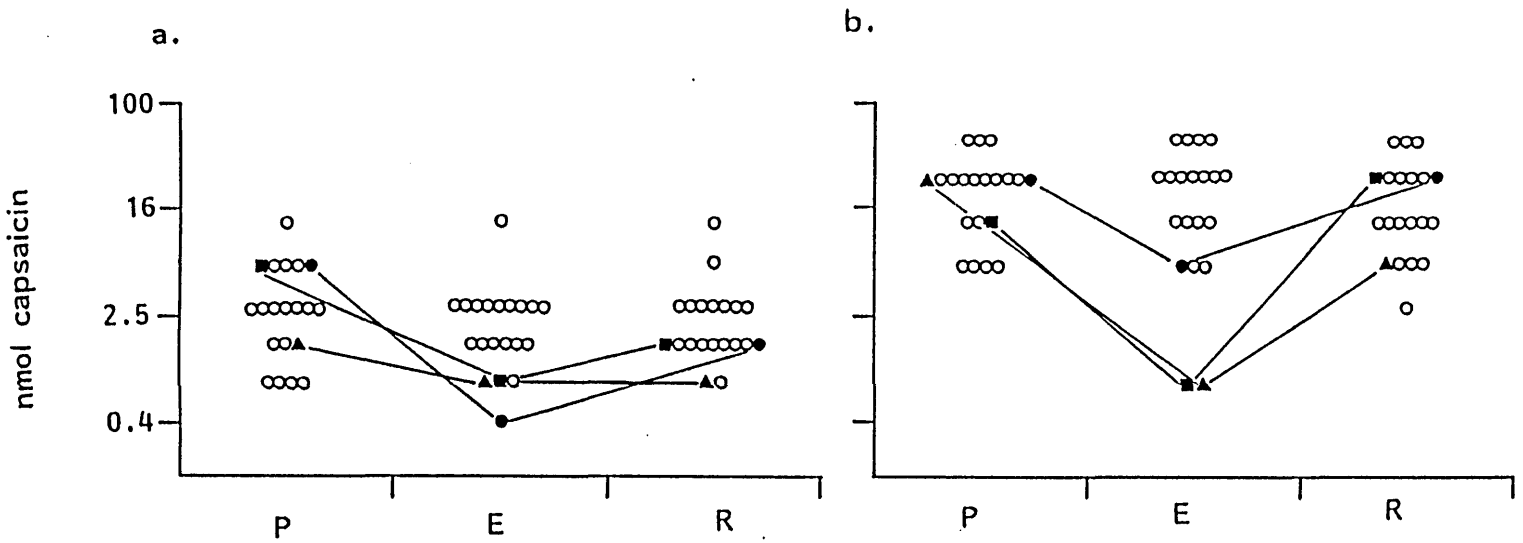


Figure 3.4 a & b.

a) D_2 and b) D_5 in the twenty subjects after placebo (P), enalapril (E) and ramipril (R). The values for the three patients who developed cough are shown by solid symbols.

Discussion

This study demonstrated that there was no generalized change in the cough reflex sensitivity in patients given an ACE-I. However, there was an increase in the sensitivity to inhaled capsaicin in three of the patients given ACE-I and these were patients also complained of cough during treatment with both enalapril and ramipril. However, Morice and co-workers were able to show an increase in the sensitivity of the cough reflex in normal volunteers after ACE-I therapy (Morice *et al* 1987). The patients who coughed on the ACE-I had a normal cough response to capsaicin on placebo, therefore the cough is not due to any abnormal sensitivity of the reflex before treatment. The incidence of cough in this study would appear to be at the higher end of the range previously reported retrospective analysis of symptoms (Coulter & Edwards 1987, Stumpe *et al.* 1984, Hood *et al.* 1987). There was no correlation between cough reflex sensitivity and serum ACE activity or blood pressure.

Mechanisms of the abnormal cough reflex associated with ACE-I cough

The mechanism of increased cough during ACE-I therapy is unknown, possible factors involved in this cough are discussed below.

1. Asthma

In the reports of patients who developed cough during ACE-I therapy there appears to have been a preponderance of asthmatic patients (Semple & Herd 1986). One study identified a group of patients who coughed on ACE-I and had their bronchial reactivity measured whilst on and off ACE-I therapy, and found the bronchial reactivity to increase while on ACE-I therapy. However, the present study included two asthmatic subjects neither of whom had a change in their asthma symptoms or developed cough while on ACE-I therapy.

2. Sex

In the present study, each of the patients who complained of cough were female. Previously, women have accounted for two thirds of reported cases of cough during ACE-I therapy (Berkin & Ball 1988), however, from the data shown in Section 2 there appears to be no significant difference in the cough sensitivity between females and males.

3. Mediators

Angiotensin converting enzymes will hydrolyse peptides other than angiotensin I, such as bradykinin. Subcutaneous injection of bradykinin causes a wheal to form and this response can be potentiated by enalapril (Fuller *et al.* 1987b). However, the interactions of bradykinin with the mediators of cough may be more complex, and a role for prostaglandins has been suggested by the effect of a cyclo-oxygenase inhibitor, sulindac in reducing the sensitivity of the reflex (Nicholls & Gilchrist 1987).

It is not clear why only a three of the twenty patients were affected by this side effect and the present study does not point to clear differences in the ability of various ACE-I to generate cough. The development of cough and the change in the cough reflex sensitivity seen in the three patients occurred with both enalapril and ramipril. Pharmacokinetic factors and access to tissue sites may be important in the development of cough but, as demonstrated here, the serum ACE activity is not related to blood pressure or to the occurrence of cough.

The cough of ACE-I therapy will be of increasing importance as these drugs are even more widely prescribed. Further studies to elucidate the mechanism of this side effect will be required and may in addition help to clarify the mechanisms involved in chronic cough.

SECTION 4

Patients with chronic cough

From the observation that an increased sensitivity of the cough response can be shown in patients with chronic cough associated with ACE-I therapy a large population of patients with productive and non-productive cough not on ACE-I were investigated. In this study the effect of various disease states on the cough reflex was also examined.

Methods

Subjects

The study was carried out on 162 individuals (96 females) who were consecutive patients attending the chest clinic with and without a diagnosis of cough (Table 3.4). The respiratory diagnosis was given by the referring consultant pulmonary physician using standard clinical criteria. Patients were diagnosed as having asthma on the basis of positive history, clinical symptoms and a positive histamine test as described by Yan et al. (1983) (described in Chapter 2). Patients were grouped into non-productive and productive cough on the basis of the questionnaire described below.

Questionnaire

The respiratory symptoms questionnaire was modified from that devised by Gulsvik (1982), which was originally used in a population of non smokers in Oslo.

The questionnaire included background information such as age and sex. The subjects were asked if they had a regular cough and if so how often. If the answer was 'yes' then the subjects were also asked whether they produced phlegm and how often. All patients were then asked if they suffered from shortness of breath or chest pain. Detailed information regarding smoking habits was also obtained. This included the number of cigarettes smoked per day, time over which the subject was a regular smoker and for former smokers, for how long a period they had smoked before ceasing. Subjects were also asked about their disease and current treatment, and for any other medical history. From the questionnaire the patients were divided into two groups: those patients who had cough which was never or rarely associated with phlegm production, ie. non-productive cough (group A), and those patients who mostly or always produced phlegm when coughing, ie. productive cough (group B).

Cough challenge & Bronchial challenge

As described in chapter 2.

Protocol

Patients first answered the questionnaire, then had a cough challenge followed, if practicable (ie. a history of wheeze or shortness of breath), by a bronchial challenge. The diagnostic labels (group assignment) were therefore assigned to patients before the challenges were performed.

Table 3.4.

Characteristics of patients in group A and group B.

GROUP	No.	Sex	Mean age (range)	Smoking history			
				NS	EX	S	NK
Group A							
Idiopathic	65	46F	44.5 (5-80)	33	9	4	19
Post-nasal drip	13	8F	48.8 (23-74)	5	1	1	6
Post-viral cough	5	3F	46.0 (14-73)	2	0	2	1
Asthma + cough	23	13F	41.3 (7-71)	15	2	6	0
Oesophageal reflux	14	7F	53.4 (31-79)	14	0	0	0
Group B							
Bronchiectasis	12	6F	46.3 (19-73)	5	1	6	0
Bronchiectasis with no infection	7	4F	45.7 (19-64)	3	4	0	0
COPD	11	5F	65.2 (48-76)	0	6	5	0
ILD	12	4F	51.0 (32-85)	4	2	6	0

NS non-smoking

EX ex-smoker

S smoker

NK not known

COPD chronic obstructive pulmonary disease

ILD interstitial lung disease

Results

Group A consisted of patients with a non-productive cough associated with asthma, post-viral cough, post-nasal drip, oesophageal reflux and those without diagnosis (idiopathic). The patients without diagnosis had no clear history of previous viral infections, asthma, post nasal drip or oesophageal reflux and/or had failed to respond to adequate therapy for the latter two diseases and had a negative histamine test. Group B consisted of patients with productive cough including those with chronic obstructive pulmonary disease (COPD), bronchiectasis and interstitial lung disease (ILD) consisting of 8 patients with sarcoidosis and 4 with fibrosing alveolitis. Bronchial challenges with histamine were found to be positive in all asthmatics tested. Fifty-two of the sixty-five patients in the idiopathic group were tested and all had a negative bronchial challenge.

Results are shown in Table 3.5 and in Figure 3.5. There was no significant difference between the normal volunteers and patients without cough from Section 2 and group B. Within group B, all patients had a similar sensitivity except those with bronchiectasis and current infective exacerbations in whom the sensitivity of the cough reflex was increased compared to those without infection ($P < 0.01$) and the normal control group. The means for group A were significantly different from those of the other groups ($P < 0.0001$). In group A although most patients had an increase in the sensitivity of the cough reflex this was not the case however, with the group who had post-nasal drip who had significantly higher C_2 and C_5 values compared to the remainder of group A ($P < 0.01$).

Table 3.5.

The mean C_2 and C_5 with 99 % confidence intervals (CI) in groups A and B.

Group	log C_2(μM)(99%)	log C_5(μM)(99%)	Histamine		
			+ve	-ve	NK
Group A					
Idiopathic	0.6 (0.12)	1.08 (0.19)	0	52	13
Post-nasal drip	1.3 (0.49)	1.93 (0.3)	2	9	2
Post-viral cough	0.59 (0.35)	1.14 (0.62)	ND	ND	-
Asthma + cough	0.65 (0.25)	1.06 (0.41)	23	0	0
Oesophageal reflux	0.54 (0.07)	0.8 (0.12)	1	6	7
Group B					
Bronchiectasis	1.25 (0.41)	1.94 (0.38)	ND	ND	-
Bronchiectasis with infection	0.5 (0.22)	1.11 (0.58)	ND	ND	-
COPD	0.92 (0.43)	1.44 (0.52)	ND	ND	-
ILD	1.29 (0.53)	1.50 (0.51)	ND	ND	-

ND not done

NK not known

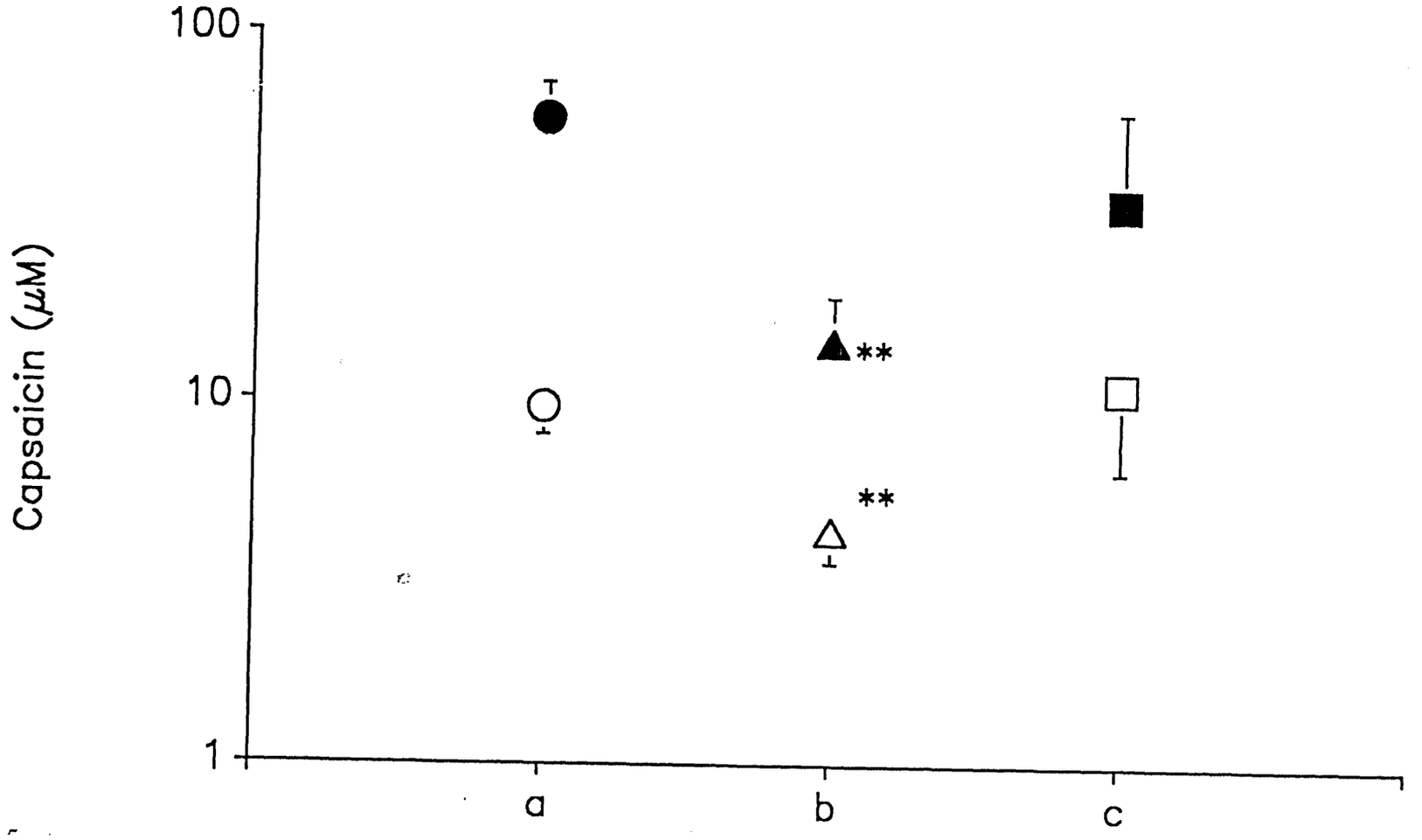


Figure 3.5.

The mean C_2 (open symbols) and C_5 (closed symbols) with 99 % confidence intervals: a) group containing normal volunteers and patients without cough from Section 2; b) group containing non-productive coughers (** $P < 0.0001$, Group A in text); c) group containing productive coughers (Group B in text).

Discussion

Patients with a non-productive cough (group A) have a heightened sensitivity of the cough reflex, when compared to non-coughing subjects (Section 2) and to patients with stable productive cough (group B). There were two unexpected inclusions in the sub-groups; patients with post-nasal drip had a non-productive cough, whereas patients with post-nasal drip are usually known to have a productive cough and those with interstitial lung disease who reported a productive cough rather than a non-productive cough which is usually associated with interstitial lung disease. Those with interstitial lung disease had a similar cough sensitivity to group B as a whole suggesting a normal sensitivity of the cough reflex, however those with post-nasal drip had a markedly different response from group A as a whole. There were other exceptions eg. patients with bronchiectasis with active infection had an increased sensitivity of the reflex. These observations support the hypothesis that cough can be caused by both increased mucus production and/or an increased sensitivity of the cough reflex. These results are therefore consistent with previous reports of increased cough responses (Empey *et al.* 1976) in volunteers with viral infections.

Non-productive cough associated with an increase in capsaicin sensitivity was found in 23 of 41 asthmatics; all of the non-coughing asthmatics had normal capsaicin sensitivities. It is possible that airway inflammation in subacute asthma leads to sensitization of the cough receptors (Simonsson *et al.* 1967) since an increase in the responsiveness of cough receptors has been reported previously in patients with bronchial asthma (Mitsuhashi *et al.* 1985).

Gastroesophageal reflux has been implicated in the pathogenesis of respiratory diseases, in particular asthma (Pack 1990). Four of the fourteen patients had

symptoms of gastroesophageal reflux with a non- productive cough and increased sensitivity of the cough reflex, their cough responded to treatment with a combination of antacids, H_2 antagonists or the proton pump inhibitor omeprazole. It is possible that in the patients who did not respond to therapy the cough reflex was abnormal for other reasons.

Patients with post-nasal drip have secretions which originate in the nasopharynx, drain down to the posterior pharynx and are thought to provoke cough by direct stimulation of the receptors in this area (Irwin *et al.* 1981). There is evidence that their cough can be treated with appropriate therapy (Irwin *et al.* 1990). This would be consistent with our finding of a normal cough reflex in this group. However, these patients unexpectedly report a non-productive cough.

Since several diseases result in chronic non-productive cough, there is probably more than one mechanism responsible for the abnormal cough reflex. The presence of mediators in the airways or bronchial wall may cause hyperalgesia of the sensory nerves. Such an increase in inflammatory mediators may well occur in patients with asthma and bronchiectasis with infection. Sensitisation has been shown to occur following the inhalation of $PGF_{2\alpha}$ (Nichol *et al.* 1988). A further possible mechanism is epithelial damage in patients with non-productive cough which may occur in viral respiratory tract infections (Higenbottam 1984) leading to an increased access of cough stimulants such as cold air and dust.

It has been reported that females may have a difference in the sensitivity of the cough reflex compared to males. In this study, however, the response between sexes was not significantly different in either the non-coughing or the coughing groups. Smoking has also been suggested to have an influence on the cough reflex (Wynder

et al 1964), however, the data in this study did not show a difference in the results of the normal volunteers and control groups when compared to smokers and non-smokers in the various groups studied.

The population of patients studied obviously does not reflect the general population, as there is a higher proportion of coughers than non-coughers in the various groups described in this study. The study group was also a highly selected tertiary referral group. Nevertheless, this does not alter the validity of the study, which was not intended to be epidemiological.

From this study it appears that cough can occur from either excessive bronchial secretions or an increase in the sensitivity of the cough reflex. Clinically it may be possible to use cough reflex testing in the investigation of cough and also to direct therapy to normalise this increase.

CHAPTER 4.

INVESTIGATIONS OF THE MECHANISM OF THE ABNORMAL COUGH REFLEX

SECTION 1

Introduction

There was an abnormal increase in the sensitivity of the cough reflex as described in Chapter 3, in a group of patients with a complaint of non-productive cough due to various aetiologies. An increase in the cough reflex has been reported in patients with respiratory tract viral infections challenged with citric acid (Empey *et al.* 1976) and acetylcholine (Tiffeneau 1957). In patients with viral infections loss or damage to the epithelial barrier results in increased access of cough stimulants to the sensory nerve endings, thereby increasing the sensitivity of the reflex (Higenbottam 1984). Alternatively, the presence of inflammatory mediators such as bradykinin may alter just the sensitivity of the cough reflex (Fuller *et al.* 1987a).

Studies have linked the cough associated with ACE-I therapy in asthmatic patients who are also on ACE-I, with their asthma (Bucknall *et al.* 1988), and our observations have shown an abnormal cough reflex in some patients with asthma. The role of bronchial tone on the cough reflex therefore warrants further investigation.

In this chapter I report a series of investigations of the effect of mediators such as bradykinin, prostaglandin E_2 and histamine on the sensitivity of the cough reflex. I have also investigated the role of prostaglandins in chronic cough in two groups of patients. Furthermore, I report studies in which airway tone has been manipulated by both bronchodilators and bronchoconstrictors, and how they effect the capsaicin-induced cough.

SECTION 2

Effect of inflammatory mediators on the sensitivity of the cough reflex

Inhaled prostaglandins (PG), $PGF_{2\alpha}$, PGE_2 or PGE_1, produce coughing, retrosternal soreness and significant changes in airway tone in normal and asthmatic volunteers (Cuthbert 1969; Costello *et al.* 1985). Thus it is not unreasonable to suggest that prostaglandins may have a role to play in the physiological or pathophysiological development of cough.

Prostaglandin E_2, $PGF_{2\alpha}$ and PGD_2 have all been shown to increase the bronchoconstrictor response (Fuller *et al.* 1986) and prostaglandin E_2 is known to produce hyperalgesia in the skin (Ferreira 1972, Graves *et al.* 1971). Nadel (1979) has suggested that bronchial epithelial damage and inflammation results in sensitisation of epithelial irritant receptors. Prostaglandins may be released as a consequence of epithelial damage and prostaglandin $F_{2\alpha}$ is known to stimulate vagal sensory receptors in the lung (Coleridge & Coleridge 1986). Hence prostaglandins may be the mediators of the bronchial epithelial irritant receptor sensitization postulated by Nadel (1979). Similarly, in respiratory tract infections, which involve a significant inflammatory component, generation of arachidonate metabolites such as prostaglandins, is almost certain to occur and these may possibly initiate cough as seen in the non-productive coughers referred to in Chapter 3, therefore the prostaglandin PGE_2 was investigated in this study.

Blockade of angiotensin converting enzyme leads not only to a fall in angiotensin II formation, but also to a reduction in bradykinin breakdown (Hood *et al.*

1987), and bradykinin can alter the cutaneous response to enalapril (Fuller *et al.* 1987b) and stimulates the production of prostaglandins (Rossini *et al.* 1988). Hence, the role of bradykinin in the cough reflex was also investigated in this study.

In this study designed to evaluate the role of mediators in cough, the tussive agent citric acid and the spasmogen histamine were used as controls.

Methods

Subjects

Six normal volunteers, two females, with a mean age of 36 years (range 22-62 years) were used. None of the subjects had a history of recent respiratory infection or cough and none was taking any medication other than an oral contraceptive.

Materials

PGE_2 (Upjohn, Crawley, UK), bradykinin (Sigma) histamine acid phosphate and citric acid (BDH, Poole, UK) were all made up as 7.6 mM solutions in 0.9% NaCl, this being the maximal soluble concentration possible for bradykinin.

Cough Challenge

As described in chapter 2.

Protocol

Subjects attended the laboratory on four separate occasions at the same time of day. A capsaicin dose cough response was measured for each subject. Subjects then inhaled five breaths of either histamine, bradykinin, PGE_2, or citric acid solutions, in a randomized double-blind manner. Five breaths were taken, at one minute intervals from the same nebulizer system as used for the capsaicin challenge, and the number of coughs after each breath of the test mediator was recorded. Immediately following inhalation of the test mediators a cough dose-response to capsaicin was obtained. The cough dose-response to capsaicin was further repeated 30 minutes following inhalation of the test mediators.

Results

Baseline Responses to Inhaled Mediators

The first dose of 0.15 μmol for each test mediator caused some coughing. PGE_2 and bradykinin caused coughs in all six subjects, and histamine and citric acid in only three of the subjects. On the first and fifth inhalations, respectively, the sum total of coughs for subjects for each subsequent dose of mediator was: bradykinin, 4 and 3; citric acid, 5 and 1; prostaglandin E_2, 36 and 14; histamine 15 and 0.

Effect of Mediators on Capsaicin Response to Cough

The median cough dose-response before and after inhalation of the 0.76 μmol dose of the test mediators is shown in Figure 4.1 and the geometric mean data for the D_2 and D_5 is shown in Table 4.1. Neither citric acid, bradykinin nor histamine altered the cough response. However, PGE_2, while not altering the D_2, did significantly increase the D_5 ($P < 0.05$ when compared to all the other mediators). Thirty minutes after the inhalation of PGE_2, the capsaicin cough response had returned to baseline; the geometric mean D_2 being 3.5 (1.5 to 8.1) nmol, and D_5 was 11.1 (5.7 to 21.6) nmol.

Figure 4.1

The median number of coughs after increasing doses of capsaicin before (●), 5 minutes (○) and 30 minutes (□) after inhaling 5 breaths of either citric acid (C), bradykinin (BK), prostaglandin E_2 (PGE_2) or histamine (H). (n=6). S = saline control.

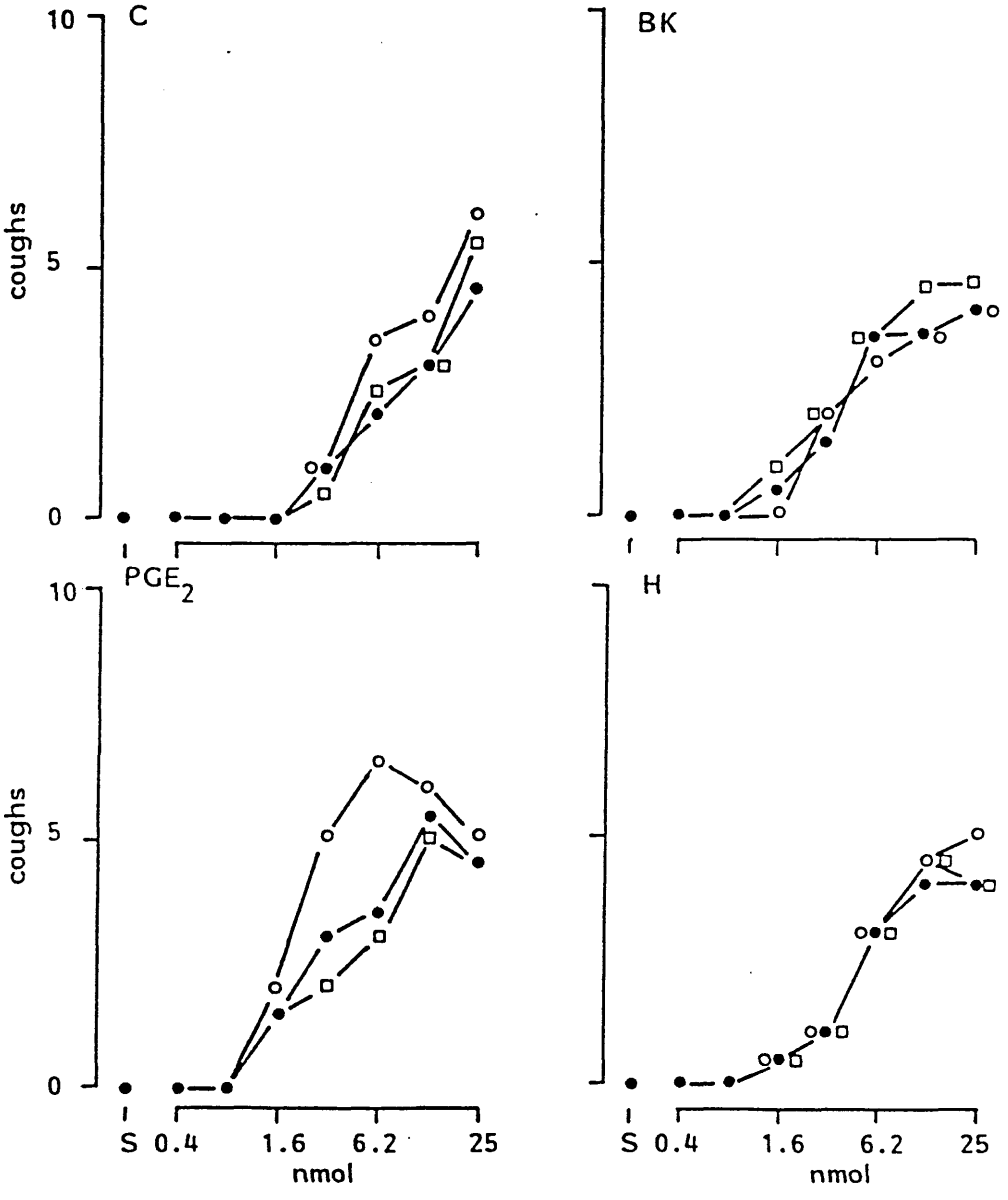


Table 4.1.

Effect of citric acid (C), bradykinin (BK), histamine (H) and prostaglandin E_2 (PGE_2) on capsaicin-induced cough.

	D_2		D_5	
	Pre	Post	Pre	Post
C	4.4 (2.2-8.8)	2.8 (1.7-4.4)	15.7 (8.8-28.3)	15.7 (9.8-12.5)
BK	2.8 (2.1-3.7)	3.5 (2.8-4.3)	17.7 (13.9-22.4)	25.0 (25.0-25.0)
H	3.9 (3.1-5.0)	3.9 (3.1-5.0)	19.8(17.2-23.0)	15.7 (12.5-19.3)
PGE_2	2.8 (2.1-3.7)	1.7 (1.0-3.1)	12.5 (9.2-17.6)	4.4 (2.4-7.9)

Results are geometric means (95 % confidence intervals)

Discussion

This study showed that capsaicin-induced cough is significantly enhanced after the inhalation of PGE_2 at a dose that causes immediate coughing. Inhalation of the same dose of bradykinin, histamine or citric acid, all of which also caused immediate coughing failed to enhance the capsaicin response. All tussive agents showed marked tachyphylaxis, as subsequent doses of these agents caused fewer coughs.

Inhaled PGE_2 increased the cough response to a high dose of capsaicin without affecting the sensitivity. This suggests that the threshold of sensory nerves to stimulation by capsaicin was unchanged, but that the number of impulses following stimulation was increased. It is unlikely that PGE_2 alters epithelial function, since damage to this area would enhance access of capsaicin to the sensory nerve endings and thus alter the threshold as well as the reactivity. In animal studies, prostaglandins can stimulate unmyelinated sensory nerves in the airways (Coleridge *et al.* 1983). In human skin, intradermal application of PGE_2 causes local flare with little axon reflex flare (Crunkhorn & Willis 1971). However, at the site of local flare, there is persistent hyperalgesia (Ferreira *et al.* 1978), suggesting that the unmyelinated nerve receptors have changed their responsiveness due to the presence of the prostaglandin, whereas the lack of axon reflex implies that the prostaglandin does not itself stimulate these fibres.

PGE_2 may be generated in the airway wall during airway inflammation or under the influence of drugs such as angiotensin-converting enzyme inhibitors, which are known to potentiate prostaglandin formation (Galler *et al.* 1982). Increases in the levels of bradykinin in the airway wall have also been suggested as the cause of enhanced cough during treatment with these drugs (Hood *et al.* 1987). A further study

in support of this suggestion shows that the wheal response to intradermal application of bradykinin is enhanced by treatment with Enalapril (Fuller *et al.* 1987b). However, the airway constrictor responses to inhaled bradykinin were not potentiated by treatment with another converting enzyme inhibitor, ramipril, implying that the converting enzyme per se is not important for the metabolism of bradykinin in the airway (Dixon *et al.* 1987a). Indeed, the major peptidase in the airway appears to be enkephalinase, which is not inhibited by these drugs (Sekizawa *et al.* 1987). Even if bradykinin levels are increased in the airway, the results of this study suggest that it would not affect cough responsiveness despite reports that bradykinin may potentiate the response to sensory nerves in the skin (Regoli & Barabe 1980).

The implication of this study is that the presence of some inflammatory mediators in the airway may increase the sensitivity of the cough reflex, leading to cough in subjects exposed to normal environmental stimulants such as cold air. Thus, drugs that inhibit prostaglandin formation or action should be examined as potential therapies for persistent cough.

SECTION 3

The effect of sulindac on the abnormal cough reflex associated with dry cough.

ACE-I have been demonstrated to potentiate tissue production of prostaglandins (Galler et al. 1982). A role for prostaglandins in the dry cough associated with ACE-I was suggested by a case report of the disappearance of such a cough during treatment with a non-steroidal anti-inflammatory agent, sulindac (Nicholls and Gilchrist, 1987). It has also been demonstrated that prostaglandin synthesis is inhibited in hypertensive patients by pretreatment with indomethacin (Swartz & Williams 1982). Mullane & Moncada (1980) found that ACE-I potentiated the release of a prostacyclin-like substance, and the effect was completely reversed by indomethacin.

This phenomenon has been further investigated here in the cough produced during or as a result of ACE-I therapy, and in idiopathic, dry, unproductive cough. Where in the following study the effect of the cyclooxygenase inhibitor, sulindac, on cough symptoms and cough sensitivity was examined.

Methods

Subjects

Group A consisted of six patients (3 female), with an age range 45-66 (mean 58) years who complained of cough while on ACE-I therapy. All had been prescribed the ACE-I, four were taking captopril and two enalapril, as part of their treatment for essential hypertension. We also examined six patients (group B), 3 female, with an age range of 36-73 (mean 58) years who had long standing dry non-productive cough with no known cause. All patients had normal chest x-rays, forced vital capacity, and FEV_1. None of the patients were smokers or had symptoms of asthma and the nine patients tested had a normal sensitivity to inhaled histamine. The characteristics of the patients are summarized in Table 4.2 and 4.3. The results of these study groups have been compared to those, normals and patients without cough described in Chapter 3, Section 2.

Table 4.2. Clinical details of patients with angiotensin converting enzyme inhibitor (ACE-I) associated cough.

Age	Sex	ACE-I Daily Dose	Other treatment	Histamine challenge PD_{20}
46	M	Enalapril 10mg	Frusemide 40mg Atenolol 100mg	ND
56	F	Captopril 50mg	Frusemide 20mg Atenolol 50mg Thyroxine 100µg	>2.5 µmol
61	M	Captopril 75mg		ND
57	F	Captopril	Atenolol 100mg Frusemide 20mg	>2.5 µmol
56	F	Captopril 150mg	Frusemide 80mg Nifedipine 40mg Amioderone 400mg	>2.56 µmol
59	M	Enalapril 20mg	Bendofluazide 5mg	ND

ND: Not done

Table 4.3. Clinical details of patients with idiopathic dry, non-productive cough

Age	Sex	Medication	Histamine challenge PD_{20}
58	F	-	1.68 μmol
57	M	-	>2.56 μmol
73	F	-	1.51 μmol
59	F	-	>2.56 μmol
36	M	-	>2.56 μmol
63	M	Digoxin 0.25mg Dipyridamole 300mg	>2.56 μmol

PD_{20}: Dose of histamine causing a 20 % reduction in the FEV_1.

Materials

Sulindac (200 mg/day) and placebo tablets were supplied by Merck Sharpe & Dohme.

Protocol

Each patient was studied on two occasions once before and once one week after therapy, with each treatment period being at least one week apart. Placebo and sulindac were given in a double-blind random manner. The hypertensive patients continued their ACE-I and other therapy throughout the study (Table 4.2 and 4.3). Cough reflex sensitivity, and for the hypertensive patients only, supine blood pressure, were recorded before and at the end of each of the two treatment periods. Symptoms of cough were assessed at the end of each treatment period. Blood pressure was measured using a dynamap automatic pressure recorder.

Cough symptoms and cough challenge

The symptom of cough over the week was assessed by the sum of 7 daily scores of 0 to 20 (10 points allocated to day time cough and 10 to night time cough), with 10 being most troublesome and 0 no cough at all.

Cough reflex sensitivity was measured as described in chapter 2. The highest dose given was either that which caused 5 or more coughs or the highest concentration of capsaicin.

Results

Blood pressure

Mean blood pressure (±SE) of group A before placebo therapy was 169/97 (±10/5) mm Hg, 146/90 (±4/6) mm Hg before sulindac therapy, 153/94 (±3/4) mm Hg after placebo and 159/94 (±5/6) after sulindac. None of these changes was significant.

Cough reflex sensitivity

Group A had a mean D_2 (range) of 0.28 (0.16-0.49) nmol before placebo and was not different from that following placebo, 0.35 (0.22-0.58) nmol, or before sulindac, 0.25 (0.18-0.36) nmol. However, after sulindac, D_2 was significantly increased to 0.63 (0.28-1.39) nmol ($P < 0.05$). The D_5 (range) for group A was 0.89 (0.25-3.16) nmol before placebo, and was not different from that following placebo 0.63 (0.28-1.39) nmol or before sulindac, 1.26 (0.5-3.08) nmol, but following sulindac D_5 was significantly increased, 4.01 (1.0-15.3 nmol, $P < 0.01$) .

Group B had a D_2 of 0.25 (0.18-0.35) nmol before placebo and this was not different from that after placebo, 0.35 (0.12-1.05) nmol, before sulindac, 0.22 (0.17-0.3) nmol, or following sulindac, 0.29 (0.16-0.5) nmol. D_5 for group B was 0.89 (0.22-3.5) nmol before placebo and this was not different from that after placebo 1.4 (0.33-6.03) nmol, before sulindac 1.12 (0.26-4.90) nmol, or following sulindac, 0.71 (0.24-3.31) nmol.

Figures 4.2 and 4.3 show the D_2 and D_5 in both patient groups.

Cough score

Mean cough scores (range) for placebo were 83 (25-104) for group A and 52 (9-62) for group B. There was no significant difference between these scores as determined by the Mann-Whitney U test. The cough score for group A fell significantly to 52 (9-62) during the week on sulindac while that of group B did not change significantly on sulindac therapy, 30 (8-61) (Figure 4.4).

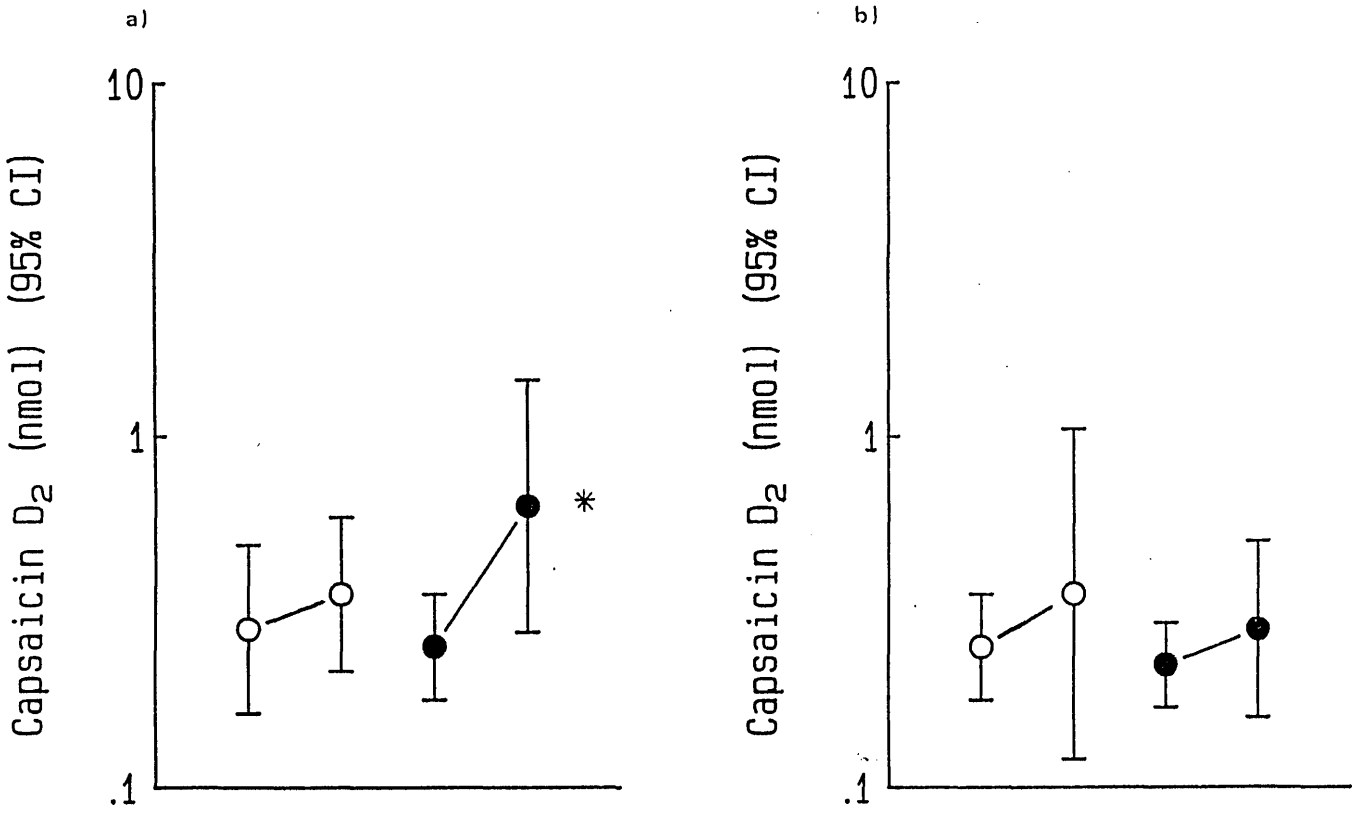


Figure 4.2.

D_2 before and after placebo (O) and before and after sulindac (●) in (a) 6 patients with ACE-I associated cough (* $P < 0.05$); (b) 6 patients with dry idiopathic cough.

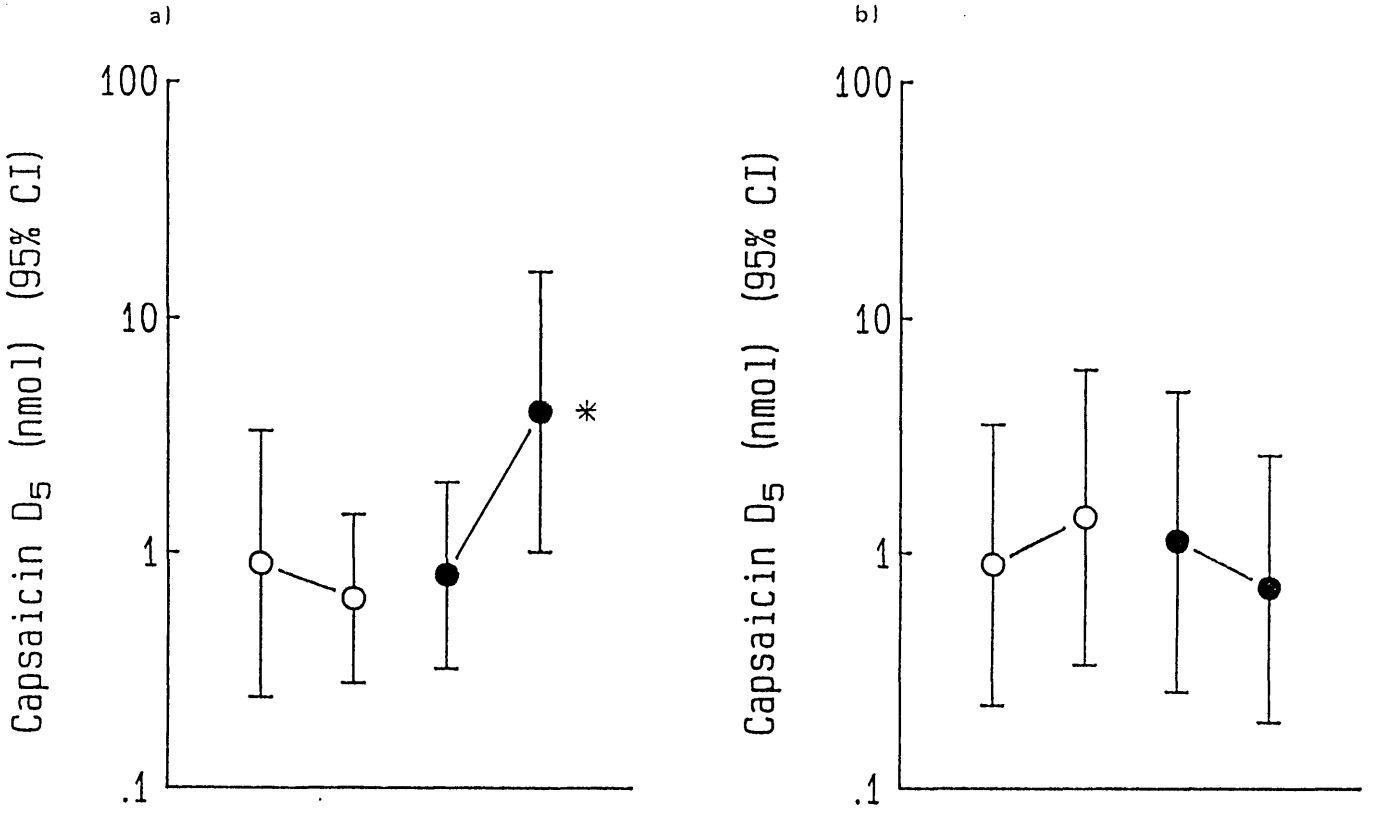
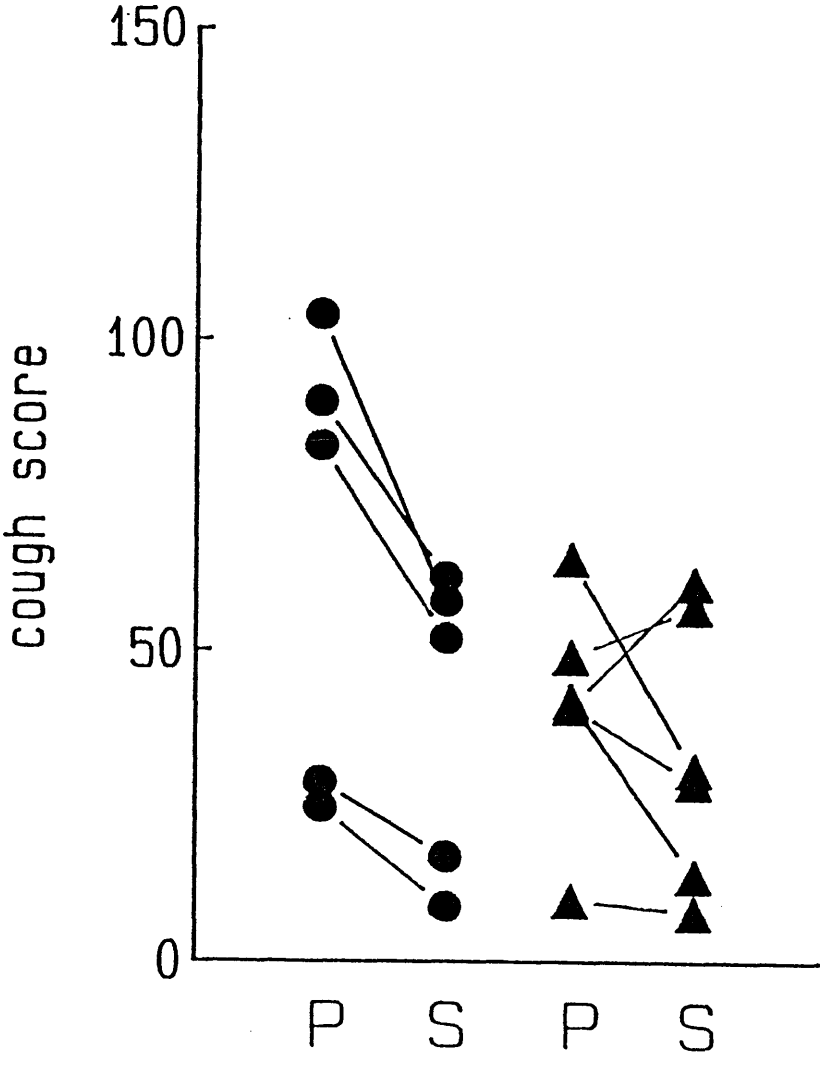


Figure 4.3.

D_5 before and after placebo (○) and before and after sulindac (●) in (a) 6 patients with ACE-I associated cough (*P < 0.01); (b) 6 patients with dry, idiopathic cough.

Figure 4.4.

Total weekly cough scores while on placebo (P) and sulindac (S) of individual patients with ACE-I associated cough (●) and idiopathic dry non-productive cough (▲). One patient in the ACE-I associated cough group failed to return cough symptom scores.



Discussion

This study shows that the non-steroidal anti-inflammatory agent sulindac reduced both the symptom of cough and the associated increase in the sensitivity of the cough reflex in patients with ACE-I cough. In contrast, sulindac does not appear to change either the symptom of cough or the increased cough reflex in patients who have an non-productive cough for other reasons. Sulindac did not cause any significant change in the blood pressure of the patients on ACE-I therapy.

It has been proposed that non-steroidal anti-inflammatory agents act through inhibition of the synthesis of prostaglandins (Vane, 1971). The effect of this drug on reducing the cough reflex sensitivity towards a normal response, thus greatly reducing the cough symptom, supports a role for prostaglandins in ACE-I cough. However, no measurement of prostaglandin production in the lung was made in the present study and a suppressive effect on other inflammatory mediators cannot be excluded.

The dose of sulindac used in this study was within the usual range for therapeutic efficacy (Molina & Poal, 1980) but it is possible that a higher dose may have resulted in further improvement in symptoms and cough reflex response. The lack of response in the other patient group, while not ruling out a role for prostaglandins in some patients, suggests that abnormal prostaglandin metabolism is not a usual cause of all cough.

SECTION 4

Effects of airway tone on the sensitivity of the cough reflex

Cough is a prominent symptom of some asthmatics (Corrao *et al.* 1979). McFadden (1972) described a group of asthmatic children, for whom persistent cough was the sole complaint of their asthma.

The hyper-responsiveness of cough receptors was evaluated using the acetic acid inhalation test in patients with asthma (Mitsuhashi *et al.* 1985), and this study demonstrated that these patients had a sensitive reaction to less than 10 % acetic acid in comparison to healthy adults and children with and without cough who responded to concentrations greater than 20 %. Salem & Ariado (1964) proposed that, rather than the cough stimulus interacting directly with cough receptors, cough receptors are stimulated by local bronchoconstriction.

Other studies have also demonstrated the antitussive actions of some $ß_2$-adrenergic and anticholinergic drugs to artificially induced cough. For example, in two studies, there was a reduced response in asthmatics to inhaled tussive agents such as ultrasonically nebulised water, hypotonic saline or citric acid (Lowry *et al.* 1987, Pounsford *et al.* 1985). Reports have also shown reduction in self-reported daily coughing in patients suffering from acute cough associated with respiratory infections (Tukiainen *et al.* 1986), as well as in chronic coughers presenting at an Allergy Clinic (Ellul-Micallef 1983), after the use of anti-asthma drugs. Vesco *et al.* (1988) have reported a reduction in cough frequency in patients given $ß_2$-agonists prior to fibreoptic bronchoscopy.

Pounsford *et al.* (1985), however, reported no effects on the cough response to inhaled citric acid in normals after the use of salbutamol, a $ß_2$-agonist; although they did report that ipratropium bromide an anticholinergic drug, reduced cough in asthmatics.

The object of this study was to determine whether alteration of airway tone caused by the bronchodilating agents, salbutamol and ipratropium bromide, and the bronchoconstricting agent, methacholine, had any effect upon the sensitivity of the cough reflex.

Methods

Subjects

Twelve subjects (4 females) with a mean age of 21 years (age range 20-22) took part in these double blind, randomised studies.

Materials

The drugs used in this study included salbutamol 2.5 mg (Ventolin: Glaxo, Greenford, Middlesex), ipratropium bromide 0.25 mg (Atrovent: Boehringer Ingelheim Ltd, Bracknell, Berkshire) and methacholine 2-64 mg (BDP, Poole, UK).

Lung Function

The FEV_1 was measured using a dry wedge spirometer (Vitalograph); the best of three values was recorded. Total respiratory resistance (Rrs) was measured using the forced oscillation technique as described in chapter 2.

Cough Challenge

As described in chapter 2.

Methacholine challenge

Subjects inhaled 5 breaths of saline from the breath activated nebuliser. Airway resistance (Rrs) was measured after 30 seconds using the forced oscillation technique. A dose response to methacholine was then performed starting with 5 inhalations of the 2 mg/ml concentration of methacholine and the Rrs measured 30 seconds later. This procedure was repeated until the PD_{40} (the dose of methacholine

that provoked a 40 % increase in the Rrs) was reached. The mean of the post-saline Rrs was used as the baseline from which the 40 % increase in Rrs was calculated.

Protocol 1

Six subjects (1 female) made four visits to the laboratory, each visit being one week apart. On the first visit, baseline measurements were made of FEV_1 which were followed by a methacholine challenge to determine the PD_{40}. After 30 minutes, the baseline sensitivity of each subject's cough reflex was measured by capsaicin challenge. At the subsequent three visits FEV_1 was measured followed by a capsaicin challenge. After 15 minutes, each subject was given one of three treatments (saline, salbutamol or methacholine (PD_{40})) in a, randomised, double blind manner. All drugs were delivered by nebulization via compressed oxygen at a pressure of 8l/min. At 1 minute and 10 minutes after completion of the drug administration, the measurements of FEV_1 and cough sensitivity to capsaicin were repeated.

Protocol II

Six subjects (3 females) attended the laboratory on three occasions, each one week apart. At each visit 3 baseline readings of airway resistance (Rrs). This was followed by a measurement of the sensitivity of the cough reflex to capsaicin. Fifteen minutes later, the subject was given one of the three treatments (saline, salbutamol or ipratropium bromide) in a randomised, double blind manner. Again each drug was administered in a nebulised form with compressed oxygen at a pressure of 8 l/min. On complete administration of the drug, the airways resistance was again measured by the oscillaire immediately after a challenge with the lowest dose of capsaicin that caused 2 coughs.

Results

Protocol I

Figure 4.5 shows that inhaled methacholine produced a statistically significant reduction in FEV_1 in normals ($P < 0.05$); the maximum reduction observed was 9 %. In addition, Figure 4.5 shows that salbutamol caused a significant increase in FEV_1 ($P < 0.05$), reaching a 6 % increase at 10 minutes post-treatment. No significant changes in FEV_1 were observed with saline.

Figure 4.6 shows that there was no statistically significant changes in following any treatment at the lowest concentration of capsaicin causing 2 or more coughs (C_2).

Protocol II

Figure 4.7 shows that baseline Rrs was not reduced significantly by ipratropium bromide but was reduced significantly by salbutamol, however, both treatments reduced the capsaicin induced, increase in Rrs.

Neither with saline, salbutamol nor ipratropium bromide treatment had a significant effect on the lowest concentration of capsaicin required to produce 2 or more coughs (C_2). (Figure 4.8).

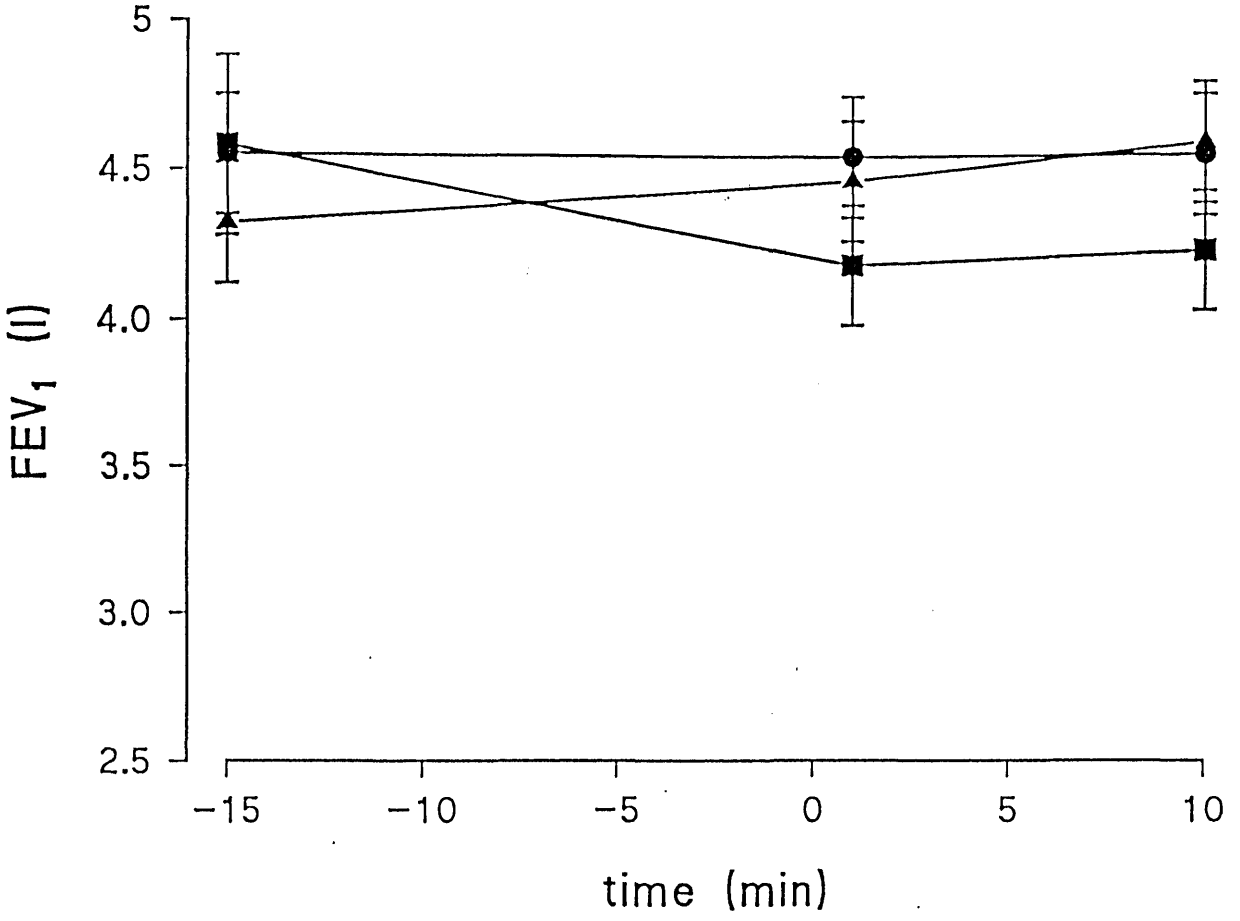


Figure 4.5.

Changes in FEV_1 (l) at various times (mins) after inhalation of saline (●), methacholine (■) and salbutamol (▲), in 6 subjects.

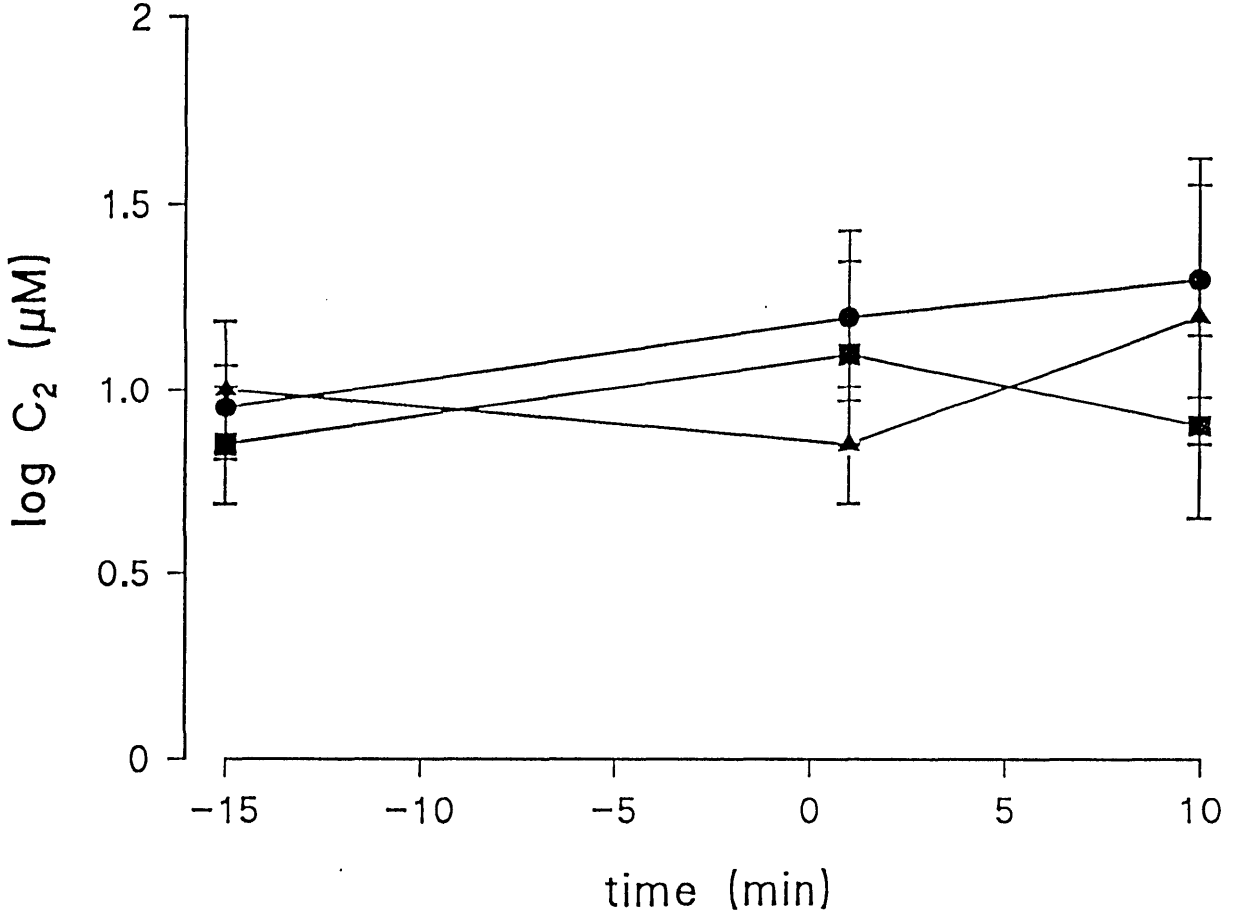


Figure 4.6

Changes in the C_2 at various times (mins) after inhalation of saline (●), methacholine (■) and salbutamol (▲) in 6 subjects.

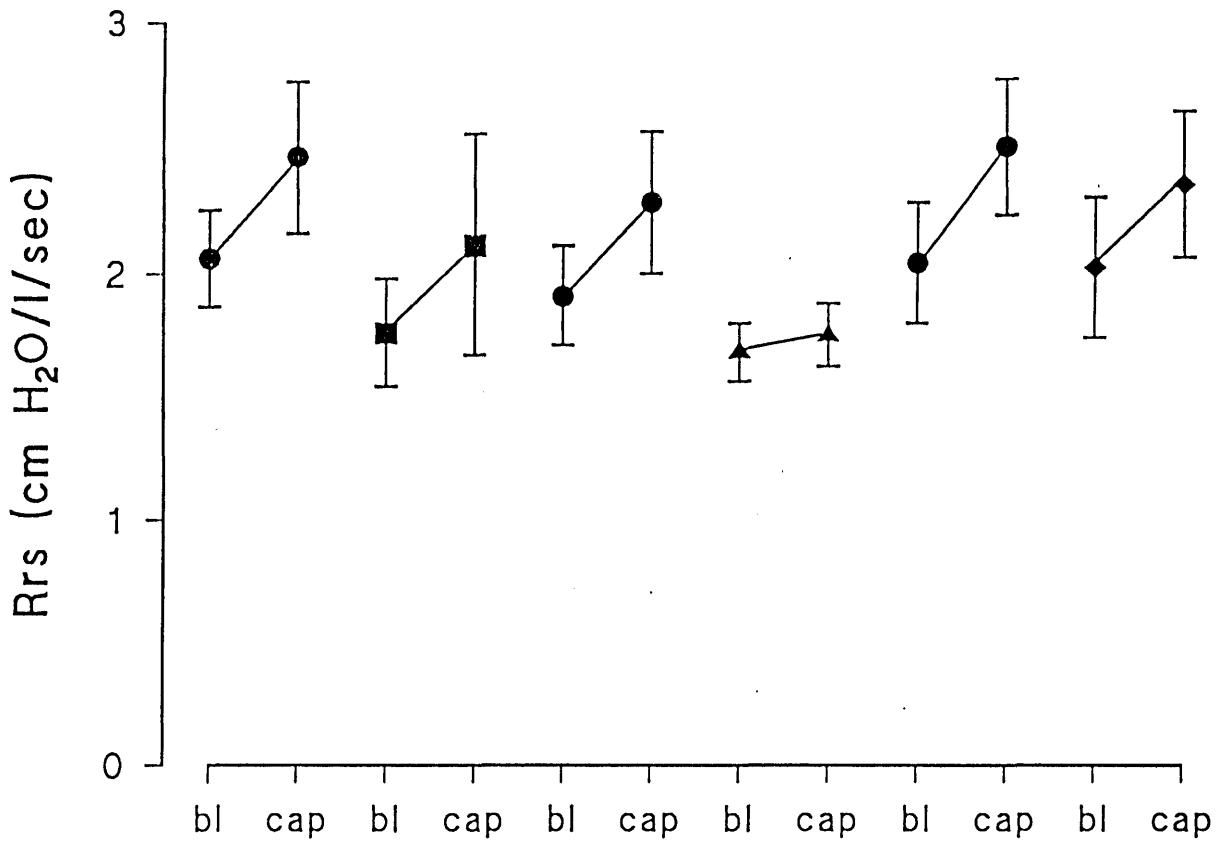


Figure 4.7.

Changes in airways resistance (Rrs) before (●) and after ipratropium bromide (■), salbutamol (▲) and saline (♦), in 6 subjects.

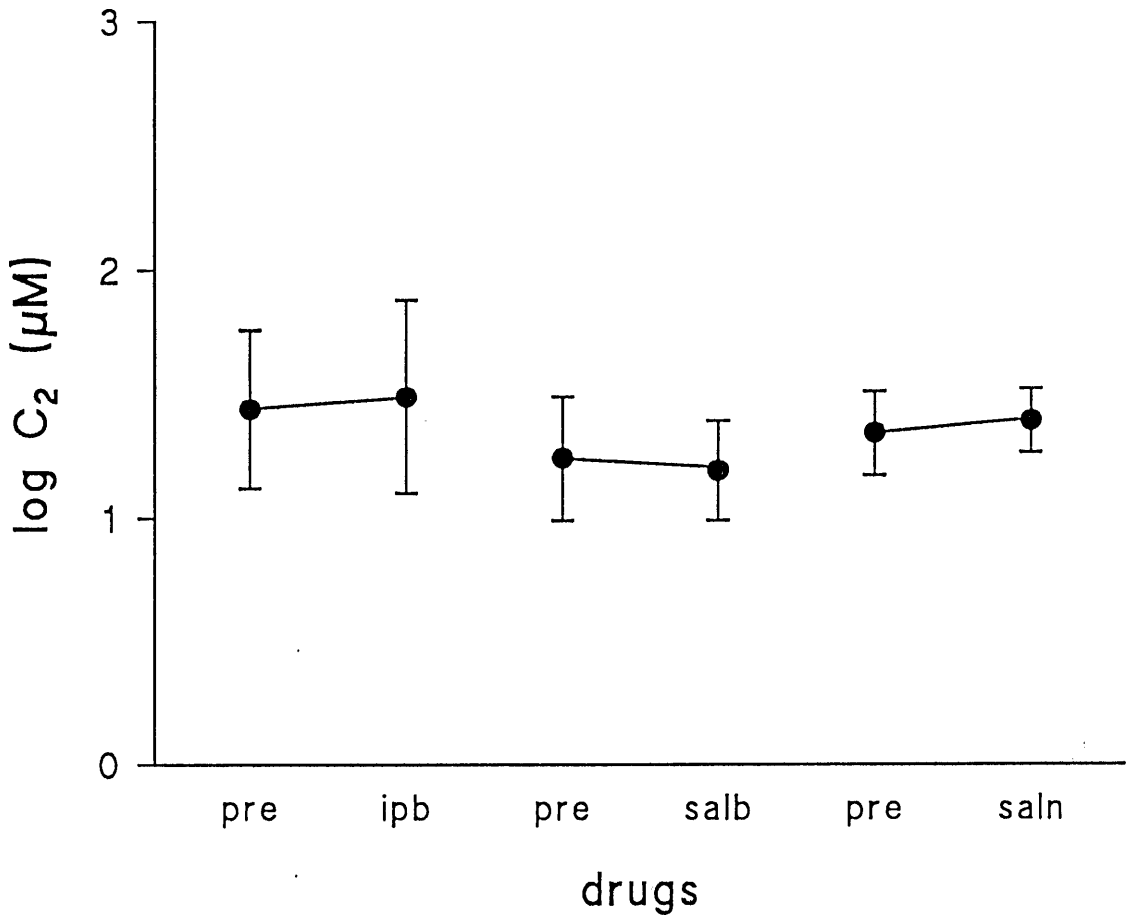


Figure 4.8.

Changes in the C_2 before and after ipratropium bromide (ipb), salbutamol (salb) or saline (saln) in 6 subjects.

Discussion

Significant increases or decreases in airway tone brought about by the drugs in these normal volunteers did not cause a change in sensitivity of the cough reflex. Thus indicating that antitussive action of salbutamol or ipratropium bromide in asthmatics must be produced either by an effect on airway tone or airway secretions, rather than via sensory pathways.

Cough associated with asthma is thought in part to be due to an increase in airway mediators and trigger airway receptors to bring about bronchoconstriction (Empey *et al.* 1976) which produces changes in airflow, that may trigger cough receptors leading to cough.

There is considerable evidence to suggest that cough receptors differ from those responsible for bronchoconstriction. Mild asthmatics, when exposed to different aerosols of varying osmolarity, bronchoconstrict to hypo- or hyper osmolaric solutions. Furthermore, the absence of a small permeant anion (eg, chloride) in an isomolar solution causes cough, but not bronchoconstriction (Eschenbacher *et al.* 1984, Godden *et al.* 1986). In addition, the inhalation of lignocaine blocks cough, but not bronchoconstriction while disodium cromoglycate blocks bronchoconstriction, but not cough, induced by a distilled water aerosol (Sheppard *et al.* 1983; Fuller & Collier 1984), these observations also suggest that the cough and bronchoconstriction reflex are separate.

Bronchodilators may alter the sensitivity of the cough reflex either by reducing the input from airway stretch receptors, or by altering mucocillary clearance (Fuller & Jackson 1990). It has also been suggested that $ß_2$-agonists may exert direct effects

upon the airway epithelium to alter the penetration of the tussive agent to the nerves (Lowry *et al.* 1988).

The findings presented in this study contrast with those of Lowry *et al.* (1987) who found that ipratropium bromide significantly reduces cough induced by ultrasonically nebulized water. The discrepancy between our data might be explained by the fact that the ultrasonically nebulised water used by Lowry *et al* (1987) induces cough by triggering different nerves in the airways from capsaicin.

The data from this study, however do corroborate the findings of Pounsford *et al.* (1985), who reported the cough response to citric acid was diminished by both ipratropium bromide and salbutamol in asthmatics, but these bronchodilators had no effect in the normals studied.

The airway calibre in asthmatic subjects receiving bronchodilators is significantly increased and this in turn has a demonstrable effect on the cough response. In contrast, however, the change in airway calibre due to bronchodilators in normal subjects is much smaller and hence has no effect on the cough response.

CHAPTER 5

ASSESSMENT OF THE EFFECT OF CURRENTLY AVAILABLE AND POTENTIAL ANTITUSSIVE THERAPIES ON THE COUGH REFLEX.

SECTION 1

Introduction

Although there are many formulations of antitussive remedies there is little evidence to support their effectiveness against cough. I have used the capsaicin-challenge for producing cough to evaluate some currently available 'over the counter' antitussives as well as some novel antitussive agents. The reproducibility and simplicity of the capsaicin challenge, makes it an ideal tool for these investigations.

In this chapter the following antitussives and potential antitussives have been studied:

1) Topical opiates - I have studied the effect of inhaled codeine, morphine and the opioid 443C81, all of which could cause inhibition of cough through stimulation of airway opiate receptors.

3) Local anaesthetics - Topically administered local anaesthetics are used in bronchoscopy to block reflexes, including cough. In this respect, lignocaine also been reported to suppress chronic cough in a few patients (Howard *et al.* 1977, Sanders & Kirkpatrick 1984). Hence, I studied three local anaesthetics, lignocaine, dyclonine and phenol and the effect they had on capsaicin induced cough.

2) Nedocromil sodium - In view of its potential neural inhibitory effect, this drug, despite the lack of effect of sodium cromoglycate on capsaicin induced cough (Collier & Fuller 1984), I felt it would be useful to study it in this model due to its greater potency compared with sodium cromoglycate in both normal volunteers and patients with cough.

SECTION 2

Effect of intravenous, oral and inhaled opiates on the capsaicin cough response.

As the opiates are held to be the "gold standard" of and the most used antitussives, I carried out the following studies to investigate the effects of two opiate analgesics, codeine and morphine.

First I have examined the effect of parental morphine and secondly the effect of oral codeine and dextromethorphan, and finally the effect of inhaled codeine, morphine and 443C81.

Dextromethorphan is the most widely used non-opioid cough suppressant, representing the active ingredient in several "over the counter" antitussive formulations. It has been shown to be free from central nervous system effects, yet its antitussive activity is comparable in potency to codeine on a mg for mg basis (Benson *et al.* 1953).

443C81 (Tyr-D.Arg-Gly-Phe(4-NO_2)-Pro NH_2) is a peripherally acting analogue of the opioid peptide met-enkephalin, it has limited ability to penetrate the CNS and which is therefore a potential therapeutic agent with opioid activity confined to the periphery and devoid of central narcotic properties. In anaesthesised cats, intravenous 443C81 has been shown to inhibit capsaicin-induced increases in pulmonary and bronchial C-afferent fibre discharge (Adcock 1987). In guinea pigs, intravenous 443C81 inhibited citric acid aerosol-induced cough (Adcock *et al.* 1988) which was significantly antagonised by the peripheral opiate antagonist N-methyl nalorphine. This suggests that the antitussive action of 443C81 may be peripherally mediated.

Methods

Subjects

Forty-two subjects (22 males and 20 females, with an age range of 19 - 45 years) with no history of recent respiratory disease and none were taking any medication. In two studies, parental morphine and inhaled 443C81 male subjects only were used.

Materials

Codeine phosphate at 50 mg/ml and morphine sulphate at 10 mg/ml (both from McFarlan Smith, Edinburgh, UK) (both were dissolved in 0.9 % saline) were used in the inhalation studies. Codeine phosphate (60 mg) and matched placebo were used in the oral study. Morphine sulphate (1 mg/ml), naloxone (0.4 mg/ml, Dupont Pharmaceuticals, Stevenage, UK), and 0.9% (w/v) saline for injection were used in the intravenous study. Dextromethorphan syrup (1.5 mg/mls) was supplied by Proctor and Gamble (Egham, Surrey) and 443C81 1 mg/ml and 4 mg/ml was supplied by Wellcome (Beckenham, Kent).

Capsaicin Cough Challenge

As described in chapter 2.

Study Protocols

1. Intravenous morphine.

Six male subjects were studied on two occasions. After baseline measurement of the capsaicin cough response, each subject received an intravenous infusion of either morphine (0.15 mg/kg) or 0.9 % saline given in a randomized double-blind

manner over 15 minutes. During the infusion the subjects were supine and heart rate and blood pressure were monitored at 5 minute intervals. After the 15 minute infusion the subjects sat up and a capsaicin cough response was performed. Subjects then returned to the supine position and were given a slow bolus injection of 4 mg of naloxone after which further measurements of the capsaicin cough response were made.

2. Oral opiates

a) Codeine.

Six subjects participated on two separate occasions. After baseline measurement of a capsaicin cough response either 60 mg of codeine or matched placebo was administered in a randomised, double blind fashion. One and 2 hours after the drug was ingested a capsaicin cough response was again measured.

b) Dextromethorphan.

Each subject attended the laboratory on two separate occasions at the same time of day. On arrival a baseline dose cough response was performed. The subjects then received a dose of either 20 ml dextromethorphan syrup 1.5 mg/ml or 20 ml of vehicle alone in a randomised double blind crossover manner. Within 60 seconds of taking the medication a dose cough response was again measured and thereafter at 25, 50, 75, 100 and 150 minutes.

3. Inhaled opiates.

a) Codeine

Six subjects were studied on two occasions. Baseline measurement of capsaicin cough responses were performed, then the subjects inhaled 1 ml of a solution containing either codeine (50 mg/ml) or normal saline in a randomized double blind fashion from a nebulizer controlled by a dosimeter (E.Jaeger, Hochberg, FRG). Five and thirty minutes after inhalation of the drug, the cough response, to the highest single dose of capsaicin given during the preliminary study, was recorded.

b) Morphine

Seven subjects, including the six subjects who had inhaled codeine, were observed. After baseline measurement of the capsaicin cough response, subjects inhaled 1 ml of a 10 mg/ml solution of morphine from the Jaeger dosimeter. Five minutes after inhalation a capsaicin cough response was performed and repeated 30 minutes thereafter.

c) 443C81

This study was performed on three separate occasions. On arrival the subjects had a baseline capsaicin cough response was performed for each subject. Using a randomised, double-blind, cross-over design the subjects were given either saline, 1 mg/ml 443C81 or 4 mg/ml 443C81 for 10 min from an ultrasonic nebuliser (Medix Electronic Nebuliser) each receiving a final volume of 10 ml. There was an interval of seven days between doses. A capsaicin cough response was performed thirty minutes after each treatment period.

Results

1. Intravenous morphine

The median capsaicin cough response was not altered by saline or naloxone (4mg) but was significantly reduced by morphine (15 mg/kg) ($P < 0.01$), an effect that was reversed by naloxone (4 mg; $P < 0.01$) (Figure 5.1). The geometric mean of D_2 was 3.9 (1.8-8.5) before, 5.0 (2.1-11.5) after saline, and 3.8 (1.2 -12.1) nmol after both saline and naloxone and 2.2 (1.2-4.0) before, 4.9 (2.5-9.8) after morphine, and 2.0 (0.9-4.6) nmol after both morphine and naloxone. The change in D_2 after morphine was significant ($P < 0.05$) as was its reversal by naloxone ($P < 0.05$). The geometric mean of D_5 was 19.8 (14.8-26.6) before, 19.8 (14.8-26.6) nmol after saline, and 23.2 (17.7-28.1) nmol after both saline and naloxone, and 19.8 (14.8-26.6) nmol after both morphine and naloxone (not significant).

2. Oral opiates

a) Codeine

Figure 5.2 shows that the capsaicin cough response was not altered by placebo, but there was a small statistically significant reduction in the total number of coughs 1 and 2 hours after oral codeine (60 mg) ($P<0.05$). The geometric mean for D_2 was 2.2 (0.9-5.6), 2.5 (1.3-4.9), and 2.6 (1.3-5.3) nmol before and 1 and 2 hours after placebo respectively and 1.8 (0.9-3.4), 3.1 (0.2-6.3), and 2.0 (0.9-4.3) nmol before and 1 and 2 hours after codeine respectively (not significant). The geometric mean of D_5 was 11.1 (5.2-23.6), 9.9 (5.0-19.7), and 12.5 (7.5-20.7) nmol before and 1 and 2 hours after placebo respectively and 7 (4.0-12.2), 11.1 (6.3-19.4), and 13.9 (9.0-21.4) nmol

before and 1 and 2 hours after codeine respectively (not significant).

b) Dextromethorphan

Immediately following dextromethorphan and the vehicle there was an increase in the D_2, from 4.2 nmol (2.1-8.3) to 6.8 nmol (2.9-8.3) for the vehicle and from 2.8 nmol (1.2-6.3) to 7.8 nmol (5.8-10.5) for dextromethorphan (Figure 5.3). The values were significantly different from baseline ($P < 0.05$) but not from each other. There was no significant difference between the two treatments throughout the full 150 minutes of the study (Figure 5.4).

Figure 5.1.

Left: median number of coughs caused by inhaling increasing doses of capsaicin before (●) and after saline (○) and after both saline and naloxone (□).

Right: median number of coughs caused by inhaling increasing doses of capsaicin before (●) and after morphine infusion (○) and after morphine and naloxone (□).

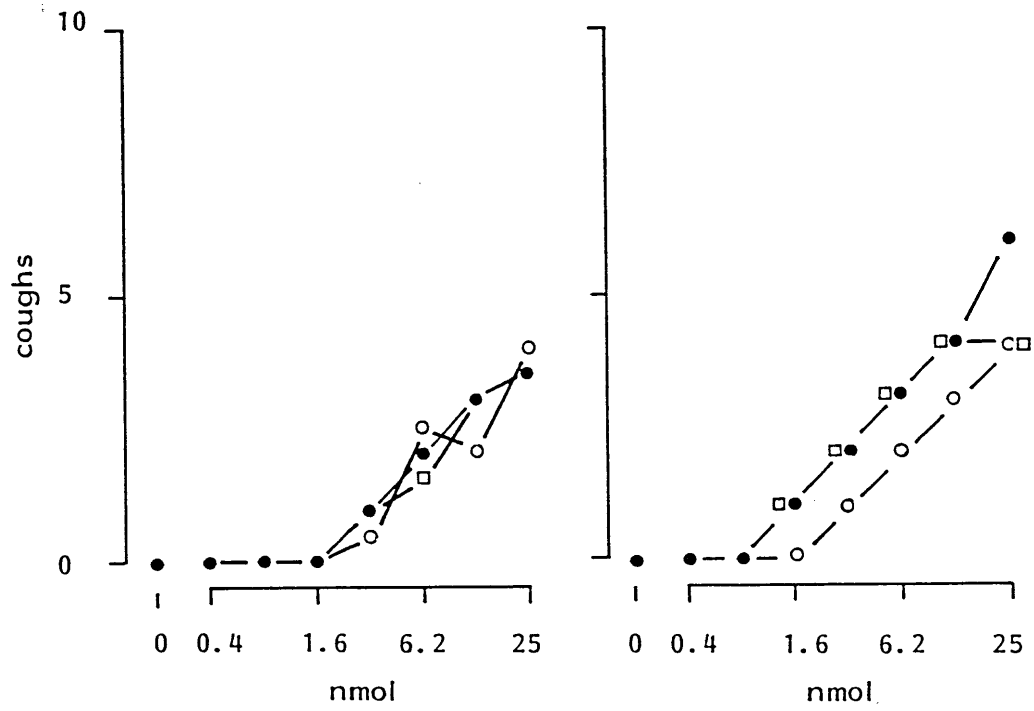


Figure 5.2.

Left: median number of coughs after inhalation of increasing doses of capsaicin given in random order before (●) and 1 hour (○) and 2 hours (□) after oral codeine.

Right: median number of coughs after inhalation of various doses of capsaicin before (●) and 1 hour (○) and 2 hours (□) after placebo. (n=6).

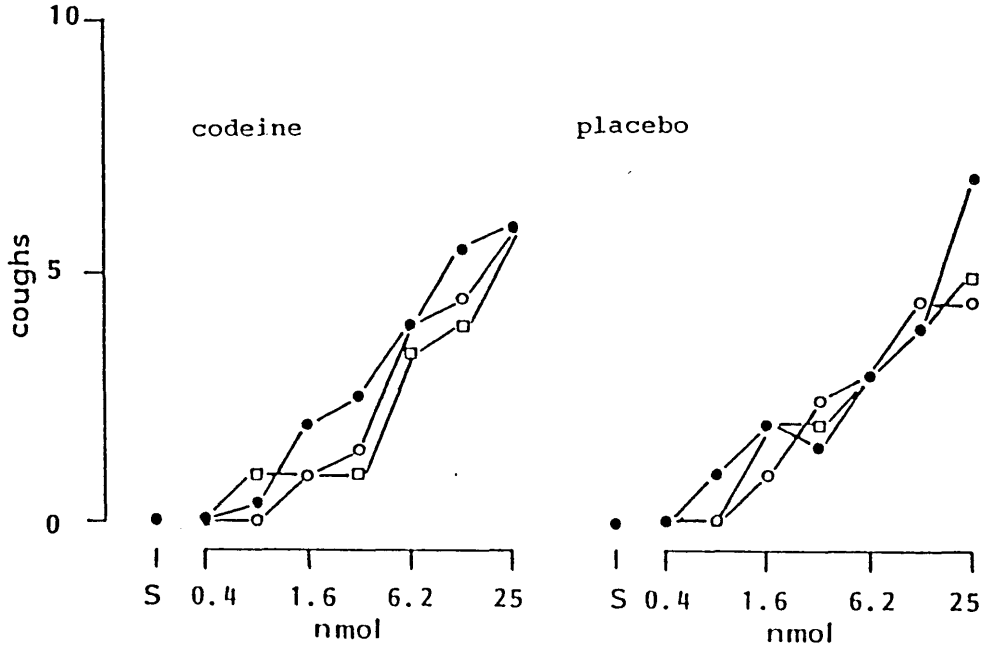
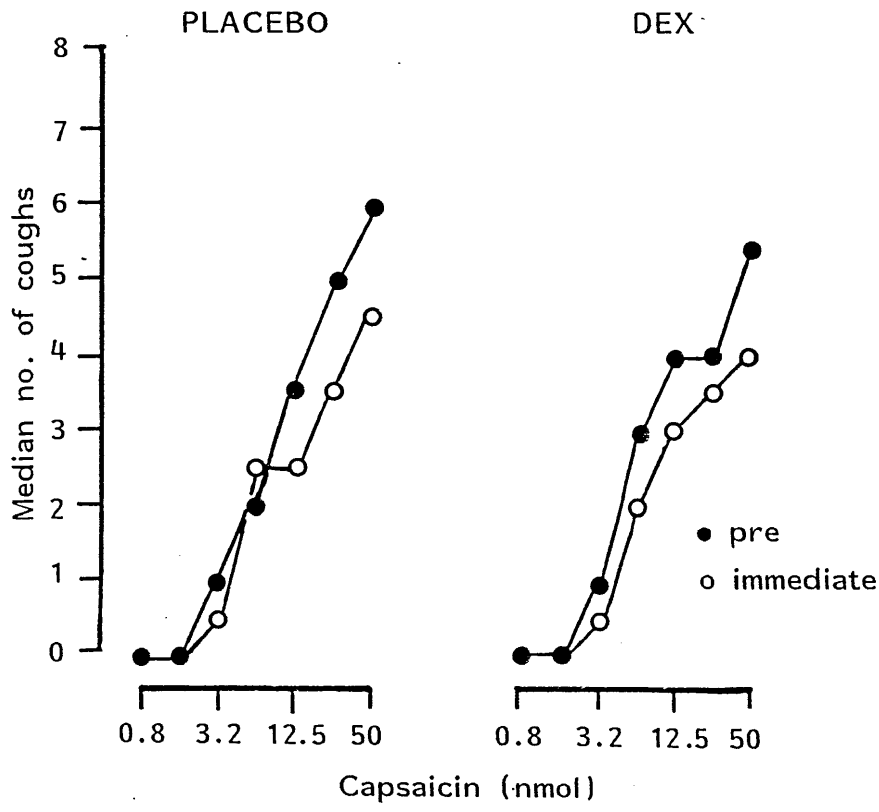


Figure 5.3.

Left: median number of coughs caused by increasing doses of capsaicin before (●) and immediately after (O) placebo.

Right: median number of coughs after increasing doses of inhaled capsaicin before (●) and immediately after (O) dextromethorphan.



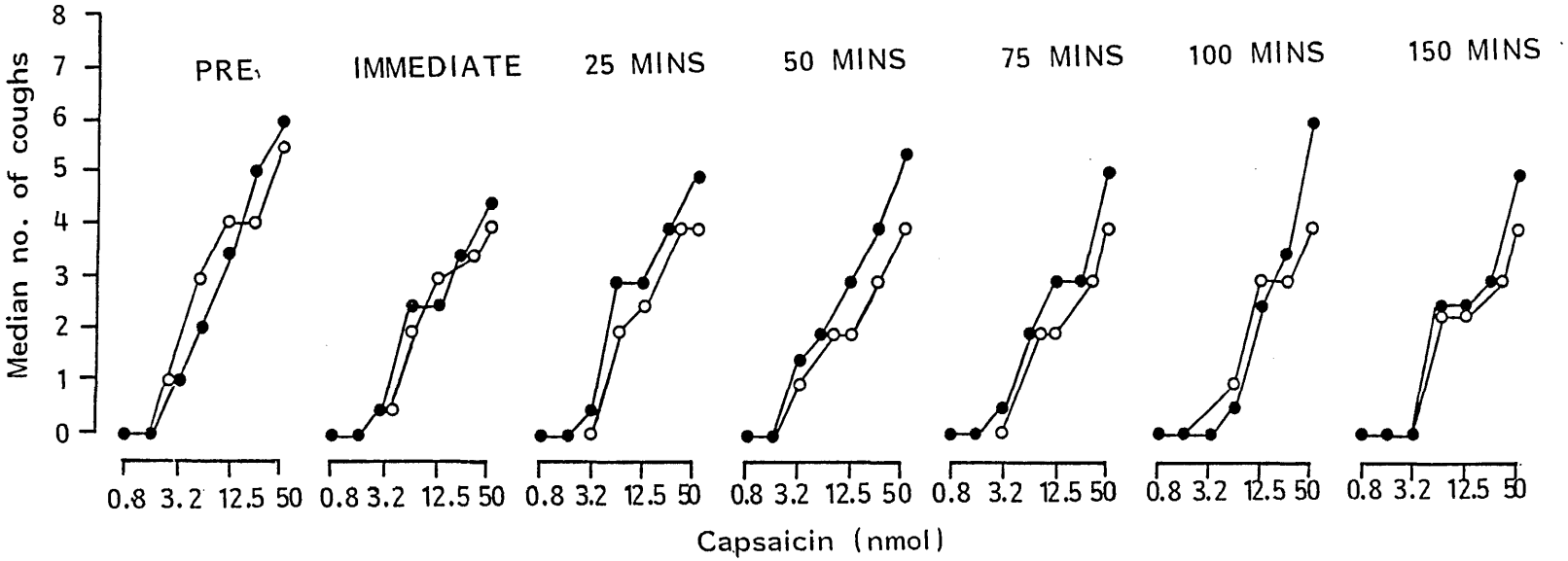


Figure 5.4.

The median number of coughs at various time points after placebo (●) and dextromethorphan (○).

3. Inhaled opiates.

a) Codeine

Inhaled codeine did not alter the number of coughs produced by inhalation of capsaicin. The median number of coughs after inhalation of a single dose of capsaicin 5 and 30 minutes after the inhalation of saline and codeine is shown in Figure 5.5.

b) Morphine

Figure 5.6 shows the median number of coughs before, 5 and 30 minutes after morphine was inhaled. The geometric mean (95 % CI) for D2 was 3.1 (2.3-4.2), 2.6 (1.3-5.2), and 2.3 (1.1-5.1) nmol before, 5 and 30 minutes after morphine respectively. The geometric mean for D_5 was 9.1 (5.1-16.8), 9.2 (5.1-16.8), and 10.2 (5.7-18.4) nmol before, 5 and 30 minutes after the drug respectively.

c) 443C81

There was no statistically significant difference in D_2 before and after inhalation of 1mg/ml and 4mg/ml 443C81 and saline. The D_2 (mean ± SE) prior to saline was 0.7 ± 0.11 and for 1mg and 4mg 443C81 was 0.43 ± 0.15 and 0.6 ± 0.16 respectively. Following saline, 1mg and 4mg 443C881, D_2 (mean ± SE) was 0.90 ± 0.13, 0.63 ± 0.22 and 0.9 ± 0.23 respectively (Figure 5.7).

Figure 5.5.

Median number of coughs after inhalation of a maximum dose of capsaicin before, 5 minutes and 30 minutes after inhalation of saline (S) and codeine (C) respectively (n=6).

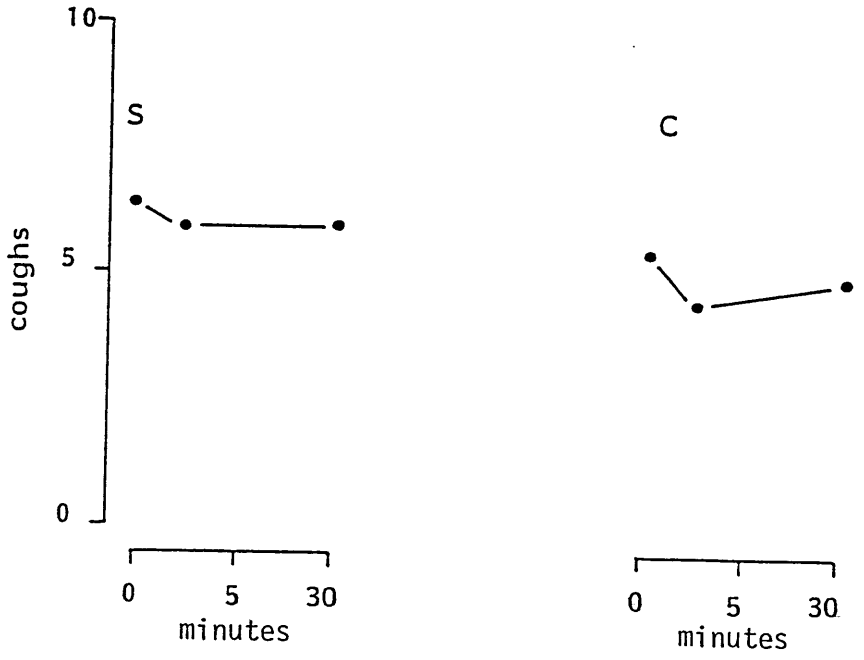
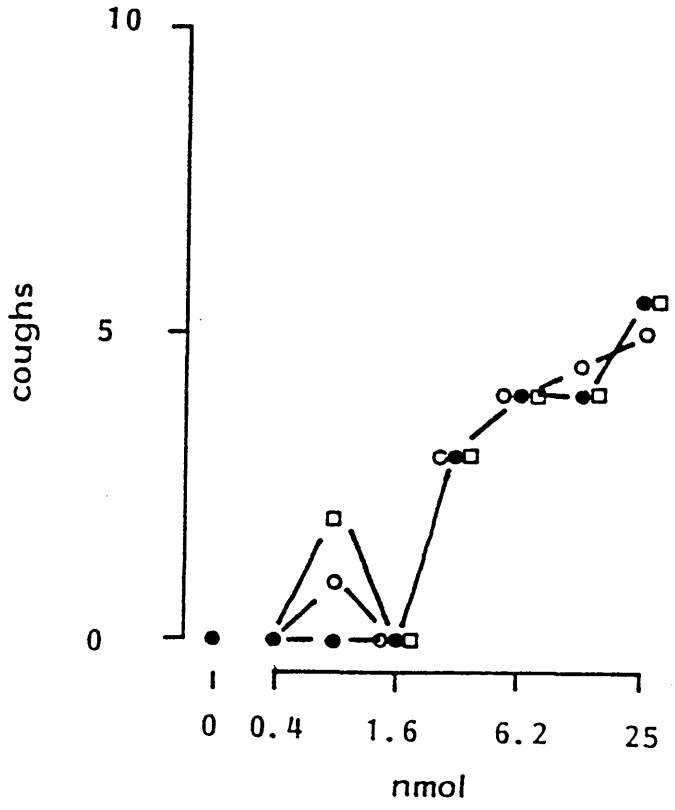


Figure 5.6.

Median number of coughs after inhalation of increasing doses of capsaicin in random order before (●), 5 minutes (○) and 30 minutes (□) after inhalation of morphine. (n=7).



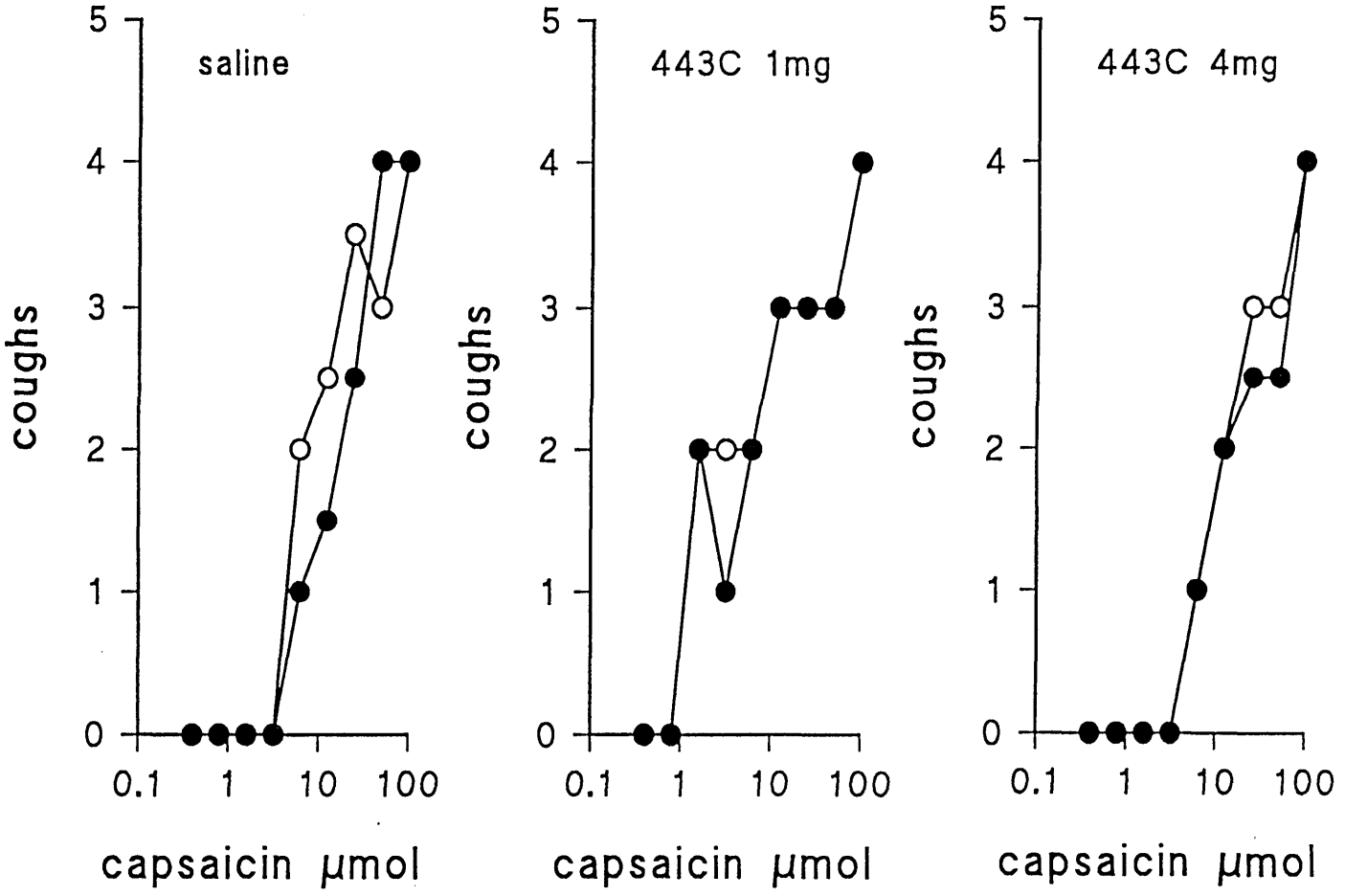


Figure 5.7.

The median number of coughs at each concentration of capsaicin before (○) and after (●) saline, 443C81 1 mg and 4mg.

Discussion

The study with intravenous morphine demonstrates that the capsaicin challenge is a valid model for the available antitussives, as the "gold standard" agent was effective. The inhibitory effect of naloxone demonstrates unequivocally that the effect of morphine is via μ opioid receptors which are probably found in the cough centre within the central nervous system, in view of the lack of effect of inhaled opiates.

The studies with orally administered opiates show that a high dose (60 mg codeine) has a marginal effect on the capsaicin induced cough. The less potent opiate, dextromethorphan had no effect other than that of a demulcent syrup.

The lack of inhibitory effect of inhaled opiates suggests that the hypothesis, that μ receptors in the lung could be used as a target for novel antitussives may not be correct. The lack of inhibition with codeine may be explained by lack of conversion to the active morphine in the lung and the dose of 443C81 may not have been sufficient. However the lack of effect of inhaled morphine also suggests either that the total dose was inadequate or it exerted its action outside the lung.

Thus this study has shown the usefulness of the capsaicin challenge with available antitussives. I have shown a minimal effect of oral opiates which is compatible with previous literature and the poor clinical efficacy. I was not able to obtain any evidence to support the presence of functional μ receptors in the lung. Additional work in this area might further clarify this issue.

SECTION 3

Local anaesthetics

In this section I report the effect of the inhaled local anaesthetics lignocaine, dyclonine and phenol.

Methods

Subjects

Twenty healthy, non-smoking volunteers aged 23-36 years (6 females) participated in this study. None of the subjects had a history of recent respiratory disease and none were taking any medication other than oral contraceptives. Only those individuals who responded with a minimum of three coughs to any single concentration were entered into the study.

Cough challenge

Performed as described in chapter 2 for protocol I. Protocol II varied slightly in that the doses of capsaicin used were 3.2, 6.4 and 12.5 nmols.

Protocol I - Lignocaine and dyclonine

Each subject attended the Clinical Laboratory at the same time of day on four separate occasions. A baseline capsaicin response was performed. In a randomised, double blind manner, 1 ml of solution, containing either 40 mg lignocaine, 4 or 8 mg dyclonine or 4 mg ascorbic acid (a control solution with a pH similar to that of the test solutions), were administered via the same nebuliser as used in the cough

challenge. This was driven by compressed air at 8 l/min until all the drug was inhaled. Ten minutes following inhalation of the test anaesthetic a capsaicin cough response was measured and the level of oropharyngeal anaesthesia, on a scale from 0 to 4 (0, 1 slight, 2 mild, 3 moderate and 4 complete anaesthesia) was subjectively reported by the subject.

Protocol II - Phenol

A double blind, crossover study was performed to compare the effects of 1.4 % phenol spray and its vehicle, on capsaicin induced cough. A baseline capsaicin cough dose response was performed, then 5 sprays of either phenol or vehicle spray were administered to the back of the throat after depressing the tongue with a tongue depressor. A dose cough response to capsaicin was repeated immediately, 10 minutes, 25 minutes and 60 minutes after dosing with the sprays.

Data analysis

Friedman's analysis of ranks was used for the anaesthesia score in protocol I and the D_3 was analyzed as described in Chapter 2.

Results

Protocol I

There was no difference between the anaesthetic score for lignocaine (40 mg) and dyclonine (8 mg) although both scores were significantly higher ($P < 0.01$) than the placebo or dyclonine (4 mg) (Table 5.1). Only lignocaine (40 mg) significantly shifted the capsaicin cough response to the right ($P < 0.05$) (Figure 5.8). There was

no difference in the concentration of capsaicin causing 3 or more coughs (D_3) before or after inhalation of either 4 or 8 mg dyclonine or 4 mg ascorbic acid (placebo) (Table 5.1). Lignocaine (40 mg), on the other hand, produced a 2.6 fold increase in D_3 ($P < 0.05$) (Table 5.1).

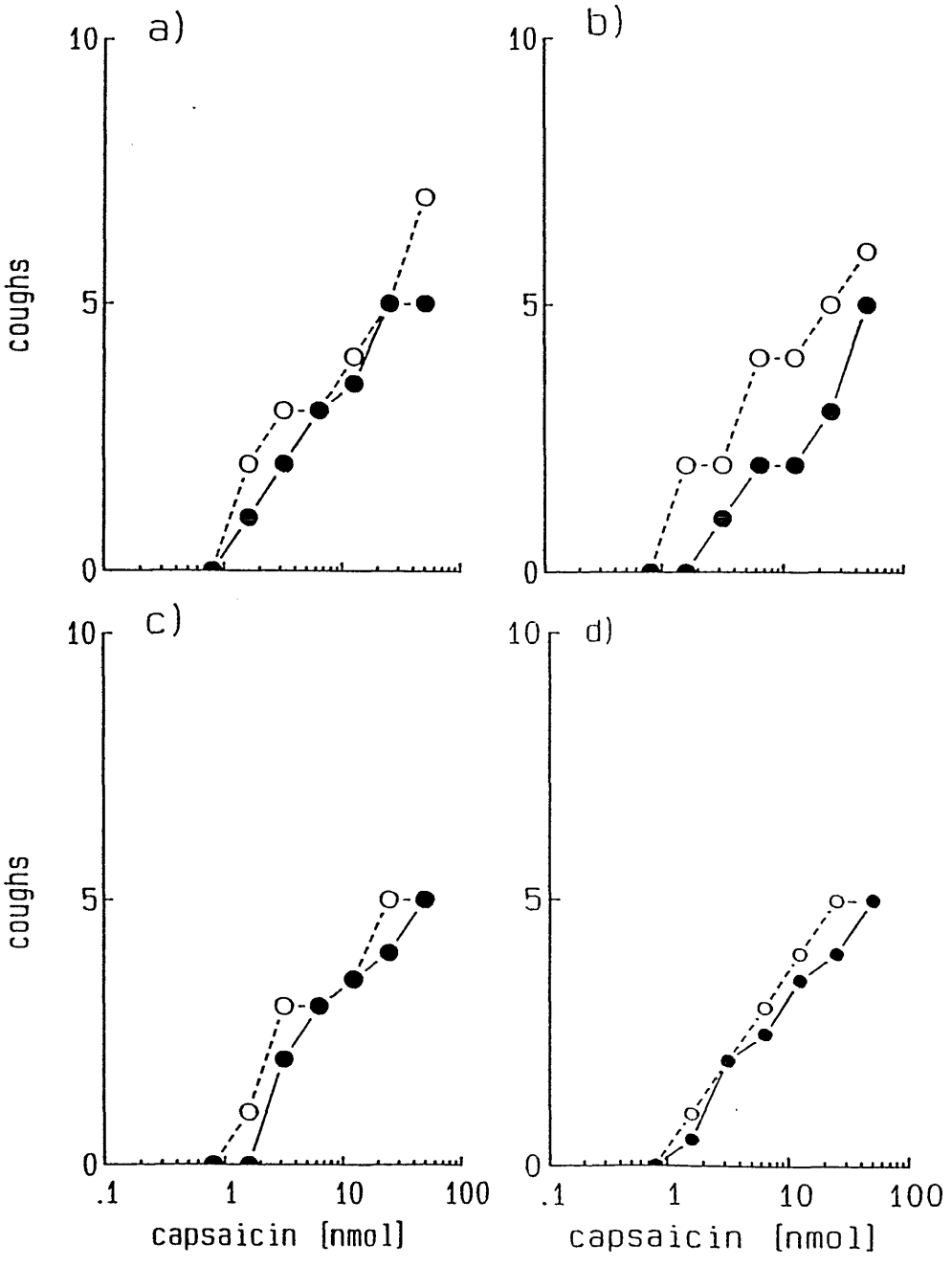
Table 5.1.

The median (range) anaesthesia score and geometric mean (95 % CI) for the capsaicin dose causing 3 or more coughs (D_3), before and after treatment of anaesthetic. (* $P < 0.05$, compared to post-placebo).

Treatment	Anaesthesia	D_3	
		Pre	Post
Placebo	0(0-3)	4.5(3.4-5.9)	7.8(4.8-12.5)
Lignocaine (40 mg)	3(1-4)*	4.8(3.5-6.6)	12.6(7.8-20.4)*
Dyclonine (4 mg)	1(0-2)	5.9(4.1-8.4)	8.9(5.1-15.7)
Dyclonine (8 mg)	2.5(1-4)*	4.0(2.9-5.1)	6.8(3.0-12.0)

Figure 5.8.

This shows the capsaicin cough dose response before (O) and after (●) either a) 4 mg ascorbic acid (placebo), b) lignocaine (40mg) c) dyclonine (4mg) or d) dyclonine (8mg).

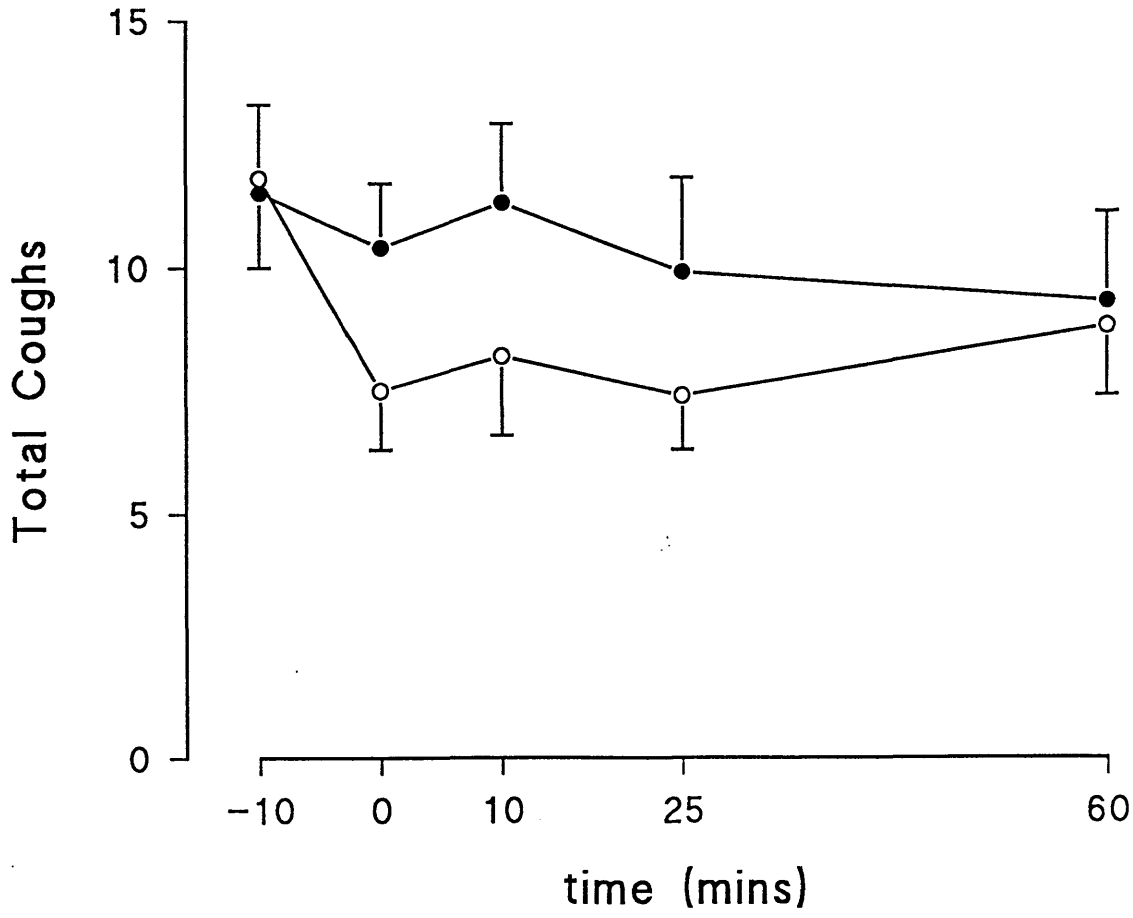


Protocol II

The phenol group showed reduced total cough counts in comparison with the vehicle group. The total number of baseline capsaicin-induced coughs (mean ± SE) was 11.8 ± 1.5 on the phenol day and 11.5 (± 1.5) on the vehicle day. Immediately after treatment with either phenol or vehicle, the total number of coughs was 8.0 ± 1.3 and 10.4 ± 1.7 respectively, the number of coughs after the phenol spray being significantly different from those at baseline and placebo ($P < 0.05$). This difference remained significant at 10 and 25 minutes. By 60 minutes the number of coughs in the phenol group had returned to basal levels (Figure 5.9).

Figure 5.9

Total mean (SE) cough counts summed across the different doses of capsaicin at various time points before and after phenol (○) and vehicle (●).



Discussion

In the present study, all three local anaesthetics, lignocaine, dyclonine and phenol caused moderate anaesthesia. Nebulised lignocaine (40 mg) and phenol sprayed directly on to the larynx, significantly suppressed the cough response to capsaicin, the change being greater than that found in previous studies for normal humans when using nebulised citric acid (Poulton & James 1979; Cross *et al.* 1976).

Unexpectedly, neither dose of dyclonine had an effect on the cough response. Earlier human studies, which used dyclonine to produce anaesthesia the mucosa of the tongue, suggested that it was 5-10 times more potent than lignocaine in producing anaesthesia (Adriani *et al.* 1964). In the present study, a similar degree of oropharyngeal anaesthesia was observed with 8 mg dyclonine and 40 mg lignocaine. Hence, the lack of effect of dyclonine on the sensitivity of the capsaicin-induced cough, is unlikely to be explained by chemical inactivation in the nebuliser. The most likely explanation for the differential effects of the two local anaesthetics on cough is a variation in their rate of penetration through the airway mucosa to the sensory nerve endings responsible for cough. Other factors, such as local metabolism and clearance of the anaesthetics from their site of action cannot be excluded (Karlsson 1988b).

Also local anaesthetics may have different effects on nerve conduction depending upon the type and size of nerve fibres as well as the physicochemical properties of the anaesthetic (Strichartz & Ritchie 1985). Animal studies (Jain *et al.* 1973) have shown that the cough reflex is inhibited at lower doses of local anaesthetics than the Hering-Breuer reflex, which regulates the rate and depth of breathing (Sant'Ambrogio 1982). This might be explained by the facilitated access

of the anaesthetic in question to nerves mediating cough whose endings are close to the airway epithelium, in contrast to those endings controlling the Hering-Breuer reflex, which lie within the bronchial smooth muscle (Karlsson 1988b).

The study implies that selecting local anaesthetics as putative antitussives has to be based upon experiments involving tussive challenge, rather than based on their topical potency as anaesthetics in other sites. It is also possible that local anaesthetics in a suitable delivery device, could be developed as therapies for cough, where cough is due to an abnormal increase in sensitivity of the reflex. This would therefore relieve the symptom but not put the patient at risk from aspiration.

SECTION 4

Effect of nedocromil sodium on capsaicin induced cough in normal volunteers and in patients with chronic and persistent cough.

Nedocromil sodium is a potent inhibitor of mucosal and connective tissue mast cells in primates and it inhibits antigen induced bronchoconstriction in man (Auty 1986). It is also a potent inhibitor of reflex bronchoconstriction provoked by inhaled sulphur dioxide (Dixon *et al.* 1987b) and bradykinin, both of which may act by stimulating airway C fibres. It is used as a prophylactic anti-asthma therapy as is sodium cromoglycate.

Although sodium cromoglycate does not affect capsaicin-induced cough (Collier & Fuller 1984), however, there is evidence to suggest that sodium cromoglycate and nedocromil sodium may have some antitussive properties. Leitch *et al* (1984) demonstrated that treatment for three weeks with sodium cromoglcate reduced cough in smokers. Lowry & Higenbottam (1988) demonstrated in normal volunteers pre-treated with nedocromil sodium, followed by a one minute inhalation of either capsaicin or ultrasonic nebulised distilled water (UNDW) or citric acid, that the cough frequency was significantly reduced compared to pretreatment with placebo in the capsaicin and UNDW group. No significant change was seen in the citric acid group.

Hence I investigated the effect of the more potent agent, nedocromil sodium, in two studies one with normal volunteers and the other in patients with chronic cough.

Methods

Subjects

Protocol I - Six healthy subjects (3 females) aged 23-34 years participated in the study.

Protocol II - Twelve patients (7 females) with an age range 29-80 years and with a history of persistent cough for greater than three months, but without a diagnosis of asthma, chronic bronchitis or any other mucus-producing condition were included in this investigation. Furthermore, each patient had an abnormal cough reflex on capsaicin challenge and a normal histamine bronchial challenge.

Cough challenge & Bronchial challenge

As described in chapter 2.

Diary cards (in protocol II)

Twice daily each patient subjectively assessed the degree of cough on a scale from 0 to 4. The definition being:

0 = No cough at all

1 = Occasional coughing - not troublesome.

2 = Frequent coughing but did not interfere with usual activities/sleep.

3 = Frequent coughing. Interfered to some extent with usual activities/sleep.

4 = Distressing cough most of the day/night.

Assessments were made upon awakening and last thing at night.

Cough questionnaire (in protocol II)

On each visit the subject was asked a series of questions, taken from 11 cards in a randomised order, relating to their cough. For each question the patient was asked which of the two statements most closely described the degree of cough and the response was recorded on a grid.

Protocol I

Volunteers were studied on two occasions. Capsaicin-induced baseline cough responses were measured, after which subjects inhaled two puffs of either nedocromil sodium (2 mg/puff) or a matched placebo in a randomised double blind fashion. Fifteen minutes later the capsaicin cough response was repeated.

Protocol II

The study consisted of four phases and was a randomised, double blind crossover study. Firstly a two week run-in period without treatment was performed followed by an initial period of treatment for four weeks with either nedocromil (4mg x 4) or placebo. This was followed by washout period, without treatment for three weeks, following the washout period the alternative treatment was administered for four weeks.

	Run-in	Treatment 4	Washout	Treatment 4
	2 weeks	4 weeks	3 weeks	4 weeks
Visit	1	2	3	4
Cough questionnaire	✓	✓	✓	✓
Cough challenge	✓	✓	✓	
Histamine challenge	✓			
Diary card	◄———	———	———	———►

Data analysis

The analysis of the diary cards in protocol II used the mean of the last week of run-in and washout and the mean of each two weeks of treatment. All variables were analyzed using non-parametric methods. The run-in and washout pre-treatment baseline was compared using the Wilcoxon Matched Pairs Signed Ranks test. Changes from pre-treatment baseline were used to compare the two treatments using the statistical method as above. The data analysis for protocol I was as described in Chapter 2.

Results

Protocol I

The effect of nedocromil sodium on the capsaicin cough dose response relationship is shown in figure 5.10. The D_2 was not altered by treatment with placebo or nedocromil sodium (4 mg). The geometric mean (95% confidence intervals) was 3.2 (3.2-3.2) nmol before and 1.8 (1.2-2.8) nmol after placebo and 1.6 (0.8-3.2) nmol before and 1.6 (1.12-2.1) nmol after nedocromil sodium. The D_5 was also unaffected by either treatment, being 17.7 (7.0-44.7) nmol before and 28.1 (22.3-35.4) nmol after placebo and 9.9 (4.6-21.4) nmol before and 9.9 (4.6-21.4) nmol after nedocromil sodium.

Protocol II

All patients had a positive capsaicin cough challenge and a negative histamine challenge. Each subject had at least a total diary card score, for day and night, of 14 during the last seven days of the run-in period.

Analysis of the cough scores from the questionnaire and diary cards, and the D_2 and D_5, using the Wilcoxon Matched Pairs Signed Ranks test were shown to be not significant. The results are shown in Table 5.2.

Figure 5.10.

Median number of coughs at each dose of capsaicin before (○) and after (●) placebo and before (□) and after (■) nedocromil sodium (n=6).

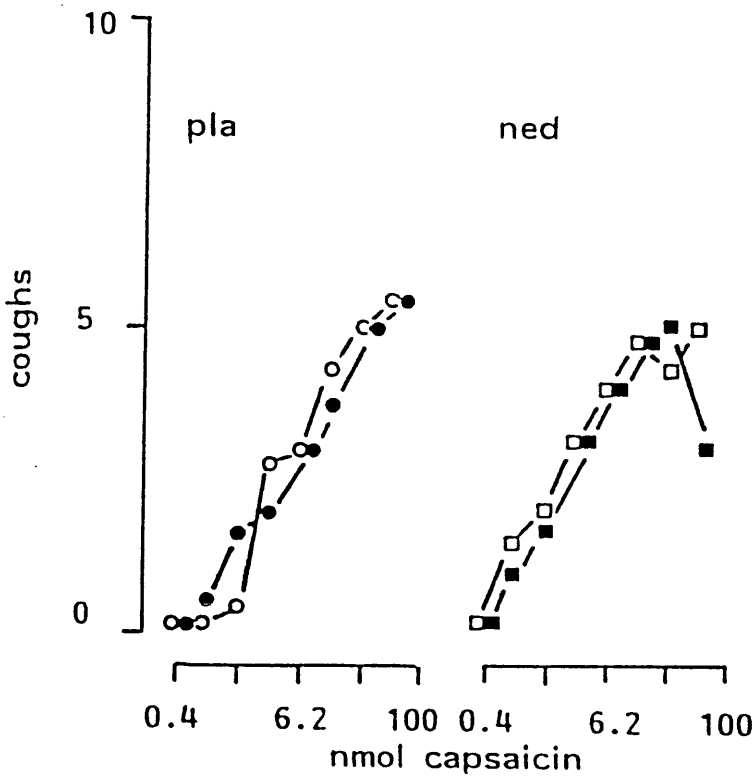


Table 5.2.

Cough scores from the questionnaire and diary cards and the log D_2 and log D_5 after treatment with nedocromil sodium and placebo.

	Nedocromil sodium Mean (Median)	Placebo Mean (Median)
Cough score from questionnaire	8.67 (9.00)	8.50 (8.50)
Diary card cough score:		
Daytime	13.60 (13.25)	13.46 (11.25)
Night-time	9.21 (8.50)	10.79 (10.00)
Capsaicin cough challenge:		
D_2	- 0.298 (-0.398)	-0.373 (-0.398)
D_5	0.027 (0.054)	-0.123 (-0.248)

Discussion

The basic pharmacology of nedocromil sodium suggests that it may have a role in the therapy of cough. In its early stages of development it was shown to reduce bronchial reactivity in asthmatic patients (Auty 1986). Jackson (1988) demonstrated in dogs, that nedocromil sodium but not sodium cromoglycate possessed antitussive properties, which may have been a result of inhibition of sensory nerve activity in the lung. A study on the effect of nedocromil sodium on viral upper respiratory tract infections, showed that there was a reduction in the symptoms to the viral infections (Barrow *et al.* 1990). Lowry and Higenbottam (1988) have also shown that nedocromil sodium causes a decrease in the cough induced by capsaicin and ultrasonic nebulised distilled water.

However, different results were obtained in these studies, where a single dose of inhaled nedocromil sodium (4 mg) in normals and treatment for four weeks with nedocromil sodium (4 mg x 4) did not alter the cough caused by capsaicin.
However these data do corroborate with previous results, where the cough, induced by one minute inhalation of capsaicin, was not affected by the prior inhalation of a similar drug sodium cromoglycate (Collier & Fuller 1984). Sodium cromoglycate inhibits both the reflex bronchoconstriction caused by sulphur dioxide (Allistone *et al.* 1985) and bradykinin (Fuller *et al.* 1987a), and nedocromil sodium inhibits reflex bronchoconstriction caused by sulphur dioxide (Dixon 1987b).

This result implies that capsaicin induces stimulation of cough via different sensory nerves than those through which inhaled sulphur dioxide and bradykinin act in man, despite the fact that all three substances stimulate C fibres in animals (Coleridge & Coleridge 1986). These differences require further investigations.

CHAPTER 6

GENERAL DISCUSSION

The primary purpose of this project as a whole was to develop a method for quantitating the sensitivity of the cough reflex in man and hence to investigate the reflex in patients with chronic cough and to evaluate antitussive therapies. The work described shows that the use of capsaicin for provoking cough, is safe and is tolerated well by subjects. Moreover, there is little variation in the baseline cough response to capsaicin, not only within and between subjects, but also on each experimental day, this shows that this method is reproducible. The normal population cough over a relatively small range of capsaicin doses demonstrating that the cough reflex is tightly controlled. The observation of the different sensitivities in the pateints with productive and non-productive cough, suggested the hypothesis that cough could be produced via either a mechanical cause ie. productive cough or through an abnormal increase in the sensitivity of the cough reflex ie. non-productive cough.

An increase in the sensitivity of the cough reflex was seen in a majority of patients with a non-productive cough, but this was not seen in the majority of patients with a productive cough. The abnormal cough reflex was first identified in those patients with angiotensin-converting enzyme inhibitor (ACE-I) cough whilst they were on ACE-I therapy. Not surprisingly removal of their therapy led to the disappearance of their cough. This was further investigated using two ACE-I enalapril and ramipril. No change in the cough sensitivity was observed in the majority of the patients, although there was a marked increase in the sensitivity to inhaled capsaicin in three of the patients. This phenomenon reflects that seen in the general population of patients taking ACE-I therapy. In the group of patients with a complaint of non-productive cough due to various aetiologies there was an abnormal increase in the

cough reflex. Some patients in this group with a history of postnasal drip, however, had a significantly lower sensitivity in the cough reflex. Patients with productive cough have in most cases a normal cough reflex, however those patients with bronchiectasis with active infection had an increased sensitivity in their reflex. Perhaps the inflammation associated with the infection causes epithelial damage, thus accounting for this increase. The presence of a normal cough reflex in patients with productive cough is predictable as their coughing is presumably due to mechanical stimulation of the cough reflex to initiate clearance of the mucus.

A large number of patients who present with chronic cough, have an abnormal cough reflex for which there is no obvious reason. It is not merely a reflection of asthmatic bronchial hyperactivity, since asthma per se does not alter the cough reflex; moreover the majority of patients had no asthma. Patients presenting with ACE-I cough have symptoms which may arise as a result of the increased presence of mediators such as bradykinin or prostaglandins.

The possible involvement of prostaglandins in producing the abnormal cough reflex was verified in this project, here prostaglandin E_2 was shown to increase the sensitivity of the capsaicin-induced cough, whereas bradykinin and histamine were without effect. A role for prostaglandins in ACE-I cough was suggested from the observation that sulindac reduced both the cough symptoms and the abnormal increase in the cough reflex in patients receiving ACE-I therapy, but not in those patients with other causes of their cough.

Investigation of the role of airway tone in the abnormal cough reflex using bronchoconstictor and bronchodilator agents, showed that despite changes in airway tone, no significant change was seen in the sensitivity of the cough reflex. This

is consistent with the lack of bronchodilator effect shown in non-asthmatic cough.

There were two initial reasons for understanding the mechanisms of cough, first to aid further knowledge of the disease and secondly to help in developing therapy. Of the treatments available for cough few are effective. Opiates are the most widely used antitussive agents: intravenous administration of morphine does infact shift the capsaicin cough dose response to reduce the sensitivity of the cough reflex, an effect reversed by naloxone. However, oral codeine and dextromethorphan were shown to have little effect on the cough reflex, thus explaining why these opiates are relatively ineffective in the normal daily treatment of cough. Recently the demonstration of peripheral opiate receptors in the lung of an animal model has led to the concept that cough may be inhibited by peripherally acting opiates. Contrary to this report an opioid μ receptor agonist 443C81, inhaled morphine and codeine were shown to have no significant effect on the cough reflex. Demulcents are widely used as antitussives and data from this study showed that syrup was able to effectively reduce the response of the cough reflex although it was short lived.

Local anaesthetics given by inhalation at relatively low doses will reduce, but not abolish, the cough reflex. A short-lived decrease in the sensitivity of the cough reflex was demonstrated using lignocaine or a spray containing phenol at a dose that caused a moderate oropharyngeal anaesthesia. In contrast, dyclonine, an ester-type anaesthetic was ineffective, implying that local anaesthetics have to penetrate the mucosa to inhibit the reflex, a property which undoubtedly varies with the drug. Taken together, this data supports the view that local anaesthetics do have a place in

chronic cough therapy although their duration of action will be short lived.

Nedocromil sodium, used as a prophylactic anti-asthma therapy was investigated, to determine its effect on cough unassociated with asthma. This study did not show any inhibitory effect on the capsaicin-induced cough in normal volunteers and patients with cough. Hence it is important to determine whether or not a cough is a secondary symptom of asthma, since the use of bronchodilators in patients with cough unassociated with asthma will be ineffective.

The demonstration of an abnormal cough reflex in a proportion of patients with non-productive cough may prove to be a diagnostic tool. More importantly the observation will help spur research into antitussives, to the potential benefit of all cough sufferers. The reason for this is that the aim will be to modify the abnormal reflex. Thus the antitussive will have therapeutic benefit without putting the patient at risk.

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