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Inhibition of artificially induced cough in man by bronchodilators

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1 The antitussive properties of bronchodilators were evaluated in a total of 47 normal volunteers.
2 Cough was induced by inhalation of ultrasonically nebulized solutions of distilled water and hypotonic saline.
3 Inhaled fenoterol hydrobromide (360 μg; 20 volunteers) and inhaled ipratropium bromide (72 μg; 14 volunteers) both significantly reduced couch compared with placebo ($P < 0.01$). Oral salbutamol sulphate (4 mg; 11 volunteers) and oral pirenzepine hydrochloride (50 mg; 14 volunteers) had lesser effects.
4 Cough inhibition correlated with a small but statistically significant degree of bronchodilatation as measured by specific airway conductance (sGaw) and forced expiratory volume in one second (FEV_1) in six normal subjects studied with each treatment in a placebo controlled, double blind study ($r = 0.67$, $P < 0.001$).
5 Small reductions in airway tone are associated with a reduced cough response elicited by inhaled ultrasonically nebulized distilled water.

Keywords bronchodilator antitussive airway tone

Introduction

Cough is one of the commonest symptoms of respiratory disease and an important symptom of asthma (Irwin *et al.*, 1981). Treatment can be difficult, as witnessed by the diversity of therapeutic agents used as antitussives. There is a suggestion that bronchodilators may be effective. Recurrent cough in asthma can be successfully treated with bronchodilators, for example oral xanthines (Corrao *et al.*, 1979) and oral or inhaled β-adrenoceptor agonists (Ellul-Micallef, 1983). Furthermore the enhanced cough response to inhaled citric acid in normal volunteers following influenza infections is inhibited by pretreatment with nebulized isoprenaline (Empey *et al.*, 1976).

The problem with studying the efficacy of antitussives has been until recently the inability to induce cough artificially using a physiological stimulus. However, a cough reflex can be initiated by changing the ionic composition of the airway surface liquid (ASL) lining the airway epithelium (Banner *et al.*, 1984; Eschenbacher *et al.*, 1984; Godden *et al.*, 1986). Such changes can be artificially induced by the inhalation of ultrasonically nebulized aqueous solutions (Eschenbacher *et al.*, 1984; Godden *et al.*, 1986). Aqueous solutions low in chloride ions initiate cough and this appears analogous to the *in vivo* stimulation of vagal afferent receptors in the laryngeal epithelium which appear sensitive to falls in chloride ion concentrations (Boggs & Bartlett, 1982; Boushey *et al.*, 1974). In normal subjects this cough response is not associated with bronchoconstriction (Higenbottam, 1984). Cough may occur in asthmatics both associated with and

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without bronchoconstriction (Eschenbacher *et al.*, 1984).

This study reports the effects of pretreatment with β-adrenoceptor agonists and anticholinergic drugs, both orally administered and by inhalation upon this 'low chloride ion' induced cough. We have also investigated the relationship between bronchodilatation produced by these agents and the inhibition of cough.

Fenoterol (Heel *et al.*, 1978) and ipratropium (Pakes *et al.*, 1980) administered by metered dose aerosol were used as representative inhaled β-adrenoceptor stimulant and anticholinergic treatment. For oral treatment, salbutamol (Kennedy & Simpson, 1969) was used as the β-adrenoceptor stimulant and pirenzepine as the anticholinergic. The latter drug, chemically related to ipratropium, is a selective inhibitor of gastric acid secretion (Stockbrugger *et al.*, 1979).

This work was presented in part in a lecture at the 1984 SEPCR meeting in Barcelona.

Methods

Subjects

A total of 47 normal subjects were recruited from hospital staff. None had either a recent respiratory tract infection or gave a history of rhinitis or asthma. All gave informed consent and the studies had approval of the hospital ethics committee. Their mean age was 25 years (range 18 to 52 years); 17 were males and 30 females. There were 10 cigarette smokers. The experiments are shown in Table 1.

Cough challenge

The apparatus used has been previously described (Godden *et al.*, 1986). In brief, a DeVilbiss 65 ultrasonic nebulizer was used to nebulize aqueous solutions. This gives an output of 6.0 g min^{-1} of aerosol with a mean particle size of 4.3 μ. Approximately 60% of the aerosol from this nebulizer is believed to be deposited in the mouth, pharynx and central airways (Yeates *et al.*, 1981). The aerosol was inhaled through a low resistance two-way valve, with an expiratory port leading to a heated Fleisch pneumotachograph (P. K. Morgan Ltd, Rainham, Kent). The expiratory flow and volume signals were recorded on a hot pen recorder (Kontron 404, St Albans, England). Coughing can easily be distinguished from tidal breathing (Godden *et al.*, 1986) and the number of coughs during a 1 min inhalation of the aerosols was recorded. Subjects were unaware of the composition of the solutions to be inhaled. They inhaled, at 10 min intervals, aqueous solutions containing 0, 31, 75 and 150 mmol of sodium chloride (NaCl) administered in random order.

Experiment 1

For experiments 1–4, the treatments and placebo were tested on separate days, the order randomized and all were conducted double blind.

Twenty volunteers received a metered dose aerosol of fenoterol (360 μg) and an identical looking placebo aerosol (2 puffs) on separate days 30 min before a cough challenge. In addition to the solutions described above, an additional solution of 112 mmol NaCl was included.

Experiment 2

Eleven volunteers received salbutamol tablets (4 mg) and matched placebo tablet 2 h before cough challenge.

Experiment 3

Fourteen subjects inhaled ipratropium bromide (72 μg) from a metered dose aerosol and an identical placebo aerosol (4 puffs) 45 min before a cough challenge.

Experiment 4

Fourteen subjects were given pirenzepine tablets (50 mg) and matched placebo tablets 2 h before cough challenge.

Table 1 Studies undertaken by each subject

Study	*Subject number*	*Males*	*Females*
Experiment 1 Fenoterol	1, 2, 14, 18, 22–37	7	13
Experiment 2 Salbutamol	1, 3, 4, 7, 9, 16–21	4	7
Experiment 3 Ipratropium	2–15	8	6
Experiment 4 Pirenzepine	1–4, 8, 9, 18, 38–44	7	7
Experiment 5 Bronchodilatation	4, 21, 42, 45, 46, 47	1	5

Experiment 5

For this study six volunteers were recruited. Each underwent whole body plethysmograph measurement (Gould Autobox 2800, Coventry) of specific airway conductance (sGaw) followed by a flow-volume curve from which forced expired volume in one second (FEV_1) was derived. These measurement were performed in duplicate before and immediately after the cough challenge and this procedure was repeated after each treatment. Challenge for cough consisted of a single solution of distilled water, frequency of coughing being recorded during a one minute inhalation.

Treatments and challenges were undertaken on separate days and the order for administration of each treatment was randomized for each subject using a Latin square design balanced for 1st order carry over (residual) effects (Cochran & Cox, 1966).

The pretreatments were as follows: inhaled fenoterol (360 μg) 30 min before cough challenge; Oral salbutamol (4 mg) 2 h before cough challenge; inhaled ipratropium bromide (72 μg) 45 min before cough challenge; oral pirenzepine (50 mg) 2 h before cough challenge; placebo inhaler 30 min before cough challenge; placebo tablets 2 h before cough challenge.

Again the subjects were unaware of the contents of the treatments.

Statistical analysis

Experiments 1–4 Each active drug was compared with placebo for its effect on cough frequency at each concentration of saline. As the cough response to solutions with chloride concentration greater than 75 mmol l^{-1} was low these results were not included in the analysis. The justification for this was that to include them would have artificially reduced the residual variance.

The cough frequency values were transformed by taking square roots, which optimally stabilized the size of variance in each group. Three factor analysis of variance was performed where the factors were subjects, drugs (active/placebo) and concentration of chloride. This gave a mixed model analysis where the main effects of the drugs and concentration of chloride could be tested against the interaction term for subjects. The interaction between the three factors was used to compute the 95% confidence limits for the drug means at each chloride concentration. A probability level of $P = 0.05$ was set to test the main factors of the analysis and the interactions. Comparisons between means were however made using a least significant difference with a P value of 0.01 to reduce the risk of a Type I statistical error.

Experiment 5 In order to stabilize the variance within each group, cough frequency values were again transformed using square roots. For the values of FEV_1 and sGaw, a logarithmic transformation was performed for the same reason.

A three stage analysis of the data was undertaken. Firstly the FEV_1 and sGaw values were tested to determine whether they changed with the cough challenge using three-factor analysis of variance. The factors were: subjects, treatment, time of challenge before and after treatment. No change was detected.

Then we tested to see if FEV_1 and sGaw changed after treatment. A comparison was made between the values after the first cough challenge and values after treatment. In this we used analysis of variance incorporating both direct and first order residual effects of treatment (Cochran & Cox, 1966).

In the absence of any evidence for important residual effects the data were reanalysed including the cough frequency values as a Latin square design of order 3 (subject, periods, treatment). The five degrees of freedom between treatments were subdivided into three orthagonal contracts between tablet and inhaler (1 df); between placebo, β-adrenoceptor agonists and anticholinergics (2 df) and their interactions (2 df).

The least significant difference was calculated to enable comparisons to be made between oral and inhaled preparations for each of the types of drug, (placebo, β-adrenoceptor agonists and anticholinergics) and between the two presentations of each type of drug. Finally the two placebo groups were combined and compared with the oral and inhaled β-adrenoceptor agonists using Williams' test for ordered means (one-tailed) with respect to the mean values of FEV_1, sGaw and cough frequency. This was repeated for the anticholinergics.

The association between the changes in FEV_1 and cough frequency and sGaw and cough frequency were assessed with correlation analysis.

Results

Experiments 1–4

There was a significant ($P < 0.01$) reduction in cough frequency with both inhaled therapies compared with placebo when distilled water was inhaled (Table 2). Oral agents had a lesser and insignificant effect ($P > 0.05$). No cough occurred on any occasion in response to 150 mmol chloride

Table 2 The mean and 95% confidence interval of cough frequencies following placebo and active treatments. The cough frequencies were transformed by adding unity and taking square roots prior to analysis. The summary statistics are back-transformed means and back-transformed limits of the 95% confidence interval.

		Chloride ion concentration (mmol l^{-1})		
		0	*31*	*75*
Inhaled fenoterol	Placebo	11.6 (9.1, 14.4)	5.6 (3.4, 7.6)	1.2 (0.3, 2.5)
	Active	5.1 (4.2, 6.1)	3.0 (1.6, 4.6)	0.1 (0, 1.0)
Oral salbutamol	Placebo	11.5 (7.4, 16.3)	6.7 (3.5, 10.6)	0.4 (0, 2.3)
	Active	9.7 (6.0, 14.2)	3.8 (1.4, 7.0)	0.3 (0,2.2)
Inhaled ipratropium	Placebo	13.3 (10.5, 16.3)	3.4 (1.9, 5.1)	0.4 (0, 1.5)
	Active	3.4 (1.9, 5.1)	1.3 (0.3, 2.6)	0 (0, 0·9)
Oral pirenzepine	Placebo	12.8 (9.5, 16.5)	4.8 (2.7, 7.3)	0.2 (0, 1.4)
	Active	7.3 (4.8, 10.3)	4.1 (2.2, 6.5)	0.1 (0, 1.3)

and the solution was therefore excluded from the analyses. The dose-response relationship between decreasing chloride ion concentration and increasing cough frequency (Godden *et al.*, 1986) was confirmed ($P < 0.01$).

Experiment 5

All the subjects in this study had normal FEV_1 values.

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

For FEV_1, sGaw and cough frequency, the differences between treatments are significant ($P < 0.01$); the differences between subjects and times of measurement appeared unimportant ($P > 0.05$). Investigations of the subcomponents of the treatment effect revealed significant differences between presentation (oral *vs* inhaled) and between drugs (placebo *vs* β-adrenoceptor sympathomimetics and anticholinergic) for all three variables; in addition for sGaw there was a significant interaction between the two.

The means for FEV_1 and sGaw are plotted in Figures 1 and 2. There were no important differences between the two placebo preparations or placebo and oral anticholinergic.

Both inhaled treatments increased FEV_1 and sGaw, and decreased cough frequency.

The small but significant increases in FEV_1 and sGaw strongly correlated with the reduction in cough frequency (see Figures 3 and 4); correlation coefficients were respectively $r = 0.67$ ($P < 0.001$) and $r = 0.68$ ($P < 0.001$).

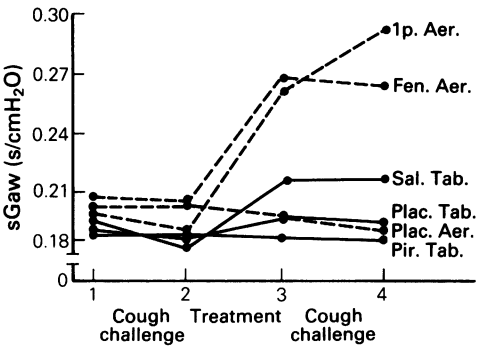

Figure 1 The back transformed means of sGaw taken before and after cough challenge and treatment. The cough challenges administered between measurements 1 and 2, and 3 and 4 have no effect on sGaw. Treatments were administered after measurement 2. Fenoterol and Ipratropium aerosols resulted in a significant increase in sGaw. Salbutamol caused a small increase. The abbreviations are as follows: Ip Aer—Ipratroprium aerosol, Fen Aer—Fenoterol aerosol, Sal Tab—Salbutamol tablet, Pir Tab—Pirenzepine tablet, Plac Aer—Placebo aerosol, Plac Tab—Placebo tablet.

Discussion

Anticholinergic and β- adrenoceptor stimulant metered dose aerosols appear equally effective in attenuating the cough response to aqueous solutions in normal subjects. The oral agents, salbutamol and pirenzepine, despite being taken at previously reported therapeutic doses, had little effect (Kennedy & Simpson, 1969; Stockbrugger *et al.*, 1979).

Before considering the mechanism underlying this attenuation of cough it is first necessary to compare the 'low choloride ion' induced cough with earlier methods of artificially inducing cough, for example the use of citric acid (Bickerman & Barach, 1954).

Citric acid aerosols on inhalation may provoke both cough and bronchoconstriction

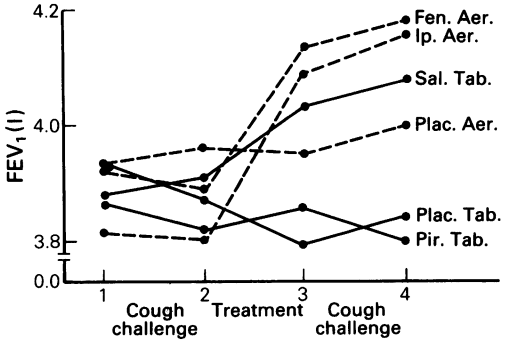

Figure 2 This graph shows the effect of cough challenges and treatment on forced expired volume in one second, FEV_1. Cough challenge has no significant effect on FEV_1 while the treatments, fenoterol and ipratropium aerosols resulted in a significant increase in FEV_1. The abbreviations are as described in Figure 1.

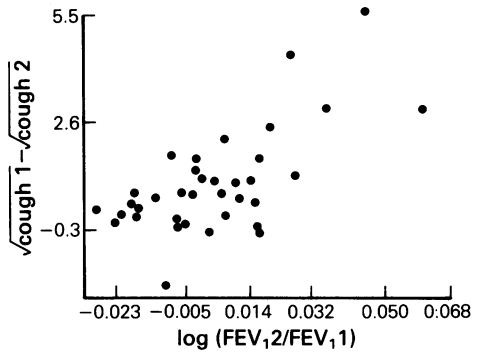

Figure 3 The relationship between cough suppression and increase in FEV_1 in response to treatment. The $\sqrt{\text{cough}}$ 1—$\sqrt{\text{cough}}$ 2 is the difference between the pre and post treatment values of transformed cough frequencies and the log (FEV_1 2 FEV_1 1) is the transformed ratio of pre to post treatment FEV_1. The degree of cough suppression appears to be associated with the increase in FEV_1.

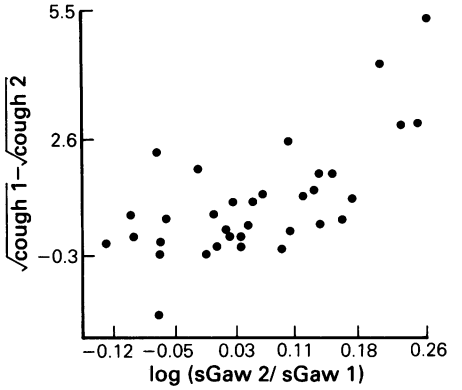

Figure 4 The relationship between cough suppression and the increase in specific airway conductance, sGaw in response to treatment. The $\sqrt{\text{cough}}$ 1—$\sqrt{\text{cough}}$ 2 is as in Figure 3 and log (sGaw 2/sGaw 1) is the transformed ratio of pre to post treatment sGaw. The degree of cough suppression appears to be associated with the increase in FEV_1.

(Simonsson *et al.*, 1967). Such aerosols are hypertonic solutions (Pounsford *et al.*, 1985) and are of low pH (pH = 2). Both factors are known to disrupt epithelial surfaces (Ferreira & Hill, 1982; Erlij & Martinez-Palomo, 1972; Wade *et al.*, 1973). Supporting the view that citric acid is possibly an injurious stimulus to the airways is the inability to tolerate inhalation of ultrasonically nebulized citric acid because of pain and discomfort (personal communication). Such an intense stimulus is likely to affect the lung 'nocioreceptors', 'C' fibre receptors which have a higher threshold (Boushey *et al.*, 1974). In this context a recent observation that sodium cromoglycate inhibits acetic acid induced cough (Mitsuhashi *et al.*, 1984) is consistent with the view that 'C' fibre receptor may be important with intense stimuli. Sodium cromoglycate may attenuate 'C' fibre activity in the lungs (Dixon *et al.*, 1980) although this observation remains disputed by other workers (Coleridge *et al.*, 1982). Our results differ from those observed with citric acid in that bronchodilators inhibit citric acid induced cough in asthmatics (Pounsford *et al.*, 1985) but not normals (Belcher & Rees, 1986). This we suspect may relate to the different and more intense stimulus offered by citric acid.

Rapidly adapting vagal receptors (RAR) of the larynx and upper airway are capable of responding to a reduction in chloride ions in the surface liquid covering the epithelium (Boggs & Bartlett, 1982; Boushey *et al.*, 1974). These receptors probably lie within the paracellular spaces between the epithelial cells (Jeffery & Reid, 1973). As airway epithelium is relatively 'leaky' (Frizzell *et al.*, 1979) the RAR in the paracellular spaces are in an optimal site to sample changes in composition of the surface liquid. Indeed the reflex responses initiated by their stimulation can be regarded as physiologically protective, preventing lung inundation (Boggs & Bartlett, 1982).

The surface liquid of the larynx and airways is susceptible to changes in composition, notably when the humidity of inspired air is altered (Man *et al.*, 1979). Ultrasonically nebulized aerosols offer a further method of altering surface liquid composition in view of their high volume delivery rates (Eschenbacher *et al.*, 1984; Godden *et al.*,

1986). Interestingly different reflex responses are observed when normals and asthmatics inhale aqueous aerosols. Asthmatics both cough and develop bronchoconstriction in response to distilled water whilst normal subjects just cough (Anderson & Schoeffel, 1984; Chadha *et al.*, 1984). It appears that the bronchoconstriction results from changes in osmolarity whereas coughing is a result of a reduction in permeant anion concentration, in this case chloride ion concentration (Eschenbacher *et al.*, 1984). Furthermore whilst cough is inhibited by lignocaine, only sodium cromoglycate inhibits bronchoconstriction (Sheppard *et al.*, 1983) suggesting separate mechanisms for the two responses.

It appears that with the low chloride induced cough, inhibition is associated strongly with physiologically small but statistically significant bronchodilatation. If it is indeed true that the stimulus is principally detected by RAR in the larynx and upper airway, for example the trachea, as conventional views suggest, then dilatation of intra-pulmonary airways appears to alter the threshold for cough. The bronchodilators are unlikely to have affected the musculature of the larynx as it has only striated musculature, and the trachea is well supported by cartilaginous rings, so limiting its ability to dilate. There are two possible explanations for the observed effect.

Slow phasic activity from RAR is seen during respiration when airway tone is increased (Widdicombe & Sterling, 1970). It is possible to argue that by conversely reducing airway tone the threshold for inducing cough is increased by inhibiting such respiratory activity of the RARs. Alternatively it is becoming recognized that the slowly adapting airway receptors (SAR or stretch receptors) influence the cough reflex (Hanacek *et al.*, 1984; Sant'Ambrogio *et al.*, 1984). Bronchoconstriction sensitizes the SARs (Widdicombe, 1961) which should reduce the threshold for cough: conversely bronchodilatation would attenuate SAR activity and so lessen cough. It is proposed that the action of the SARs is to regulate the threshold of the medullary 'cough neurones'.

It is unlikely that secretion of mucus or sol covering the airway influences the cough response to the aerosol. β-adrenoceptor stimulants should increase secretion whilst anticholinergics should inhibit secretion (Marin *et al.*, 1976; Al-Bazzaz & Cheng, 1979; Phipps *et al.*, 1982). However a topical effect upon the airway epithelium is possible. β-adrenoceptor stimulants could have increased intracellular cyclic AMP (Al-Bazzaz, 1981) which can reduce access to the epithelial paracellular spaces where the RARs reside (Duffey *et al.*, 1981). Anticholinergics could theoretically have a similar effect on epithelium (Shoemaker *et al.*, 1970). Such topical epithelial effects assume further importance in view of the increasing number of reports suggesting interaction between airway epithelium and bronchial smooth muscle in asthma (Barnes *et al.*, 1986; Flavahan *et al.*, 1985).

Our observations of the attenuation of the cough response to 'low chloride' with aerosols of β-adrenoceptor stimulant or anticholinergic agents have implications in treating cough in disease. β-adrenoceptor stimulants are effective in limiting recurrent cough in asthma (Ellul-Micallef, 1983) and nebulized isoprenaline can also reduce the enhanced cough response following influenza infection (Empey *et al.*, 1976). This raises the important question as to whether these metered dose aerosols could also be used to treat cough resulting from more common causes such as respiratory tract infections. As they have little effect on respiratory drive (unlike codeine containing oral treatment (Belville & Seed, 1968)) they may be safer.

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