Cough challenge in the assessment of cough reflex

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Introduction

Cough is the commonest respiratory symptom. Approximately four and a half million consultations per annum in UK primary care report cough as the main complaint. The use of over-the-counter cough remedies in the UK is estimated at 75 million sales per annum [1]. However, despite its prevalence, chronic cough is a difficult symptom to investigate and its successful management depends upon establishing the causative diagnosis. Current literature supports the view that the investigation of chronic cough in specialist centres provides both a definitive diagnosis and effective treatment in the majority of patients [2-4]. However, it is in the assessment of cough where the fundamental difficulty lies. Frequently, individual perception of cough differs between patients ranging from mild irritation to impeding a patient's quality of life [5]. The absence of a standardized approach to quantify cough and the fact that a number of centres employ different methods complicates the issue further.

In this review we will examine the principal surrogate method used to evaluate the therapeutic manipulation of cough reflex namely, inhalation cough challenge. The cough challenge shares methodological similarities to bronchial provocation tests. The difference lies in that the latter measures the response of the airways to bronchoconstricting substances whereas cough challenges assess the cough reflex response to tussigenic agents. In the investigation of bronchial hyperresponsiveness, standardized guidelines for bronchial challenge testing are generally applied [6]. Conversely, and despite cough challenges having been carried out for over 40 years [7], there are no agreed universal standards. Normal values of the cough response to particular tussive agents have not been established and methodologies used between the centres have frequently been difficult to compare.

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This review aims to detail the recognized physiological mechanisms of the cough reflex and then to proceed to describe the different methods of cough challenge and their application in the pharmacological testing of antitussive agents.

Cough reflex

Cough and bronchoconstriction are distinct, but interrelated reflexes. In fact, bronchial hyperresponsiveness is one of the commonest causes of chronic cough [2–4]. In animal studies, the inhalation of citric acid or capsaicin induces both cough and bronchoconstriction [8]. Tachykinin receptor antagonists result in the inhibition of both these reflexes because of the importance of the bronchoconstriction axon reflex in this species [9, 10]. Cough is recognized to be a reflex action with its sensory afferent mediated by the vagus nerve terminating in the airways [11, 12]. Both C fibres with nonmyelinated afferents and rapidly adapting receptors (RARs) with myelinated fibres are thought to mediate the reflex [11, 13, 14]. The difference between them reflects the speed of transmission of impulses within the vagus and not necessarily, the sensory pathways in the unmyelinated nerve terminals or putative 'cough receptor'. The afferent nerve fibres are thought to transmit impulses to the cough centre which is postulated to be situated in a diffuse area of the medulla and lower pons close to the nucleus solitarius [15-17]. The presence of a cough centre is supported by the fact that antitussive agents, which are thought to act centrally, decrease cough [18, 19]. The phrenic and other spinal nerves to respiratory muscles provide the efferent pathway of the cough reflex [20]. The sensory nerve endings of the afferent nerves can be triggered by a number of different stimuli and therefore a variety of chemicals can be used to investigate the cough reflex and the pharmacological effects of antitussive agents.

Cough challenge

The inhalation cough challenge facilitates the quantification of cough and the assessment of the antitussive effects of the specific therapies. In animal studies, it is possible to

examine a single vagal afferent fibre innervating the airways [21]. More importantly the experimental assessment of the cough reflex can also be performed in conscious animals using inhalation of capsaicin or citric acid [22-24]. However, in some animal species (particularly the guinea pig) bronchoconstriction, secondary to axonal reflexes is an important confounding feature and pretreatment with β-adrenoceptor agonist is required if only the cough reflex is to be stimulated. In addition, many small animals breathe nasally and have a poor cough response to distilled water. With the above provisos, inhalation challenges in animals are similar to human tussive responses. In fact, Laude *et al.* confirmed the viability of comparison between these two models by demonstrating the similarities between the human and the guinea pig in response to citric acid and capsaicin [24]. However, in this review, we will concentrate on discussing the assessment of the human cough reflex.

Delivery of cough stimulants

The cough challenge relies on the delivery of tussive agents as aerosols from jet or ultrasonic nebulizers. These nebulizers produce a high ratio of respirable aerosol particles at low volume and output. Jet nebulizers are employed more frequently, with ultrasonic nebulizers being reserved for distilled water challenge as they deliver a large volume of fog required to provoke cough.

The aerosol deposition is influenced by its particle size. The particles of 10 μm diameter being deposited mainly in the mouth and throat, 5–10 μm particles between the throat and the lungs and the particles of less than 5 μm mainly in the lungs [25]. Similarly, the cough response to inhaled tussigenic agents is also dependent on the diameter of aerosol particles produced [26]. Whilst large and small particles of capsaicin have similar deposition in the larynx the latter have better peripheral penetration and are more potent in inducing cough suggesting the more peripheral distribution of capsaicin sensitive nerves [26].

In addition, it is now recognized that the inspiratory flow rate can affect the cough response [27]. For these reasons, the dosimeter controlled jet nebulizers have become the preferred delivery systems for the inhalation cough challenge. The inhalation from the dosimeter generates a burst of compressed air that initiates a fixed duration of nebulization. This facilitates accurate calibration of the output although some variation in the velocity of inhalation might still occur [28, 29]. Whilst it is our opinion that the use of the dosimeter provides the best method for standardization of the delivery of tussigenic agents for the inhalation cough challenges we will also discuss other methodologies.

Methods of cough challenge

The inhalation cough challenge involves the delivery of tussive agents and the subsequent recording of the number of induced coughs. The results of cough challenge testing are most frequently expressed as D2 or D5 values, which are defined as the lowest concentrations generating two or five coughs per inhalation, respectively. In addition, data from cough challenges can also be expressed as dose-response curves, which are created by linear interpolation (Figure 1).

In essence, there are two main methods used for cough challenges; namely single dose and dose-response (Figure 2). Single dose inhalation challenge involves the administration of one concentration of the tussive agent. This method has been used for the screening of a large population of subjects to detect those with reproducible cough [30]. Furthermore, as it is less time consuming, the single breath method has acquired wide usage in the studies of the properties and the duration of antitussive activity of pharmacological agents [31-34]. Owing to the smaller number of inhalations required, this method has also been associated with a lower propensity for tachyphylaxis which has previously confounded dose-response inhalation cough testing with agents such as citric acid, distilled water and capsaicin [35, 36].

The second method is the dose-response cough challenge and involves the inhalation of incremental concentrations of tussive agent interspersed with inhalations containing placebo to increase challenge blindness. This method, depending on the length of inhalation, can be further subdivided into single breath or fixed-time inhalation challenges (usually between 15 and 60 s). The fixed-time

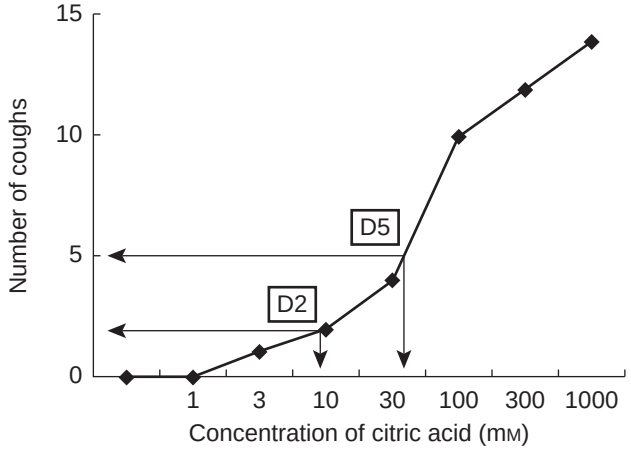

Figure 1 An example of a dose-cough response curve constructed by linear interpolation during citric acid inhalation cough challenge. D2 = concentration of citric acid causing two coughs per inhalation, D5 = concentration of citric acid causing five coughs per inhalation.

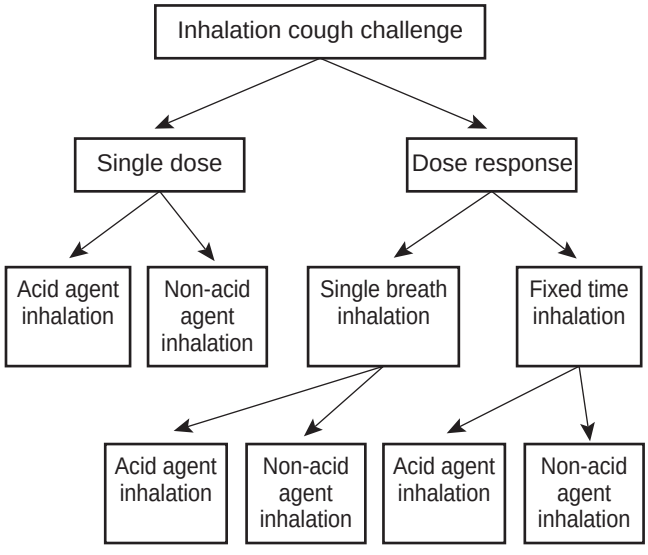

Figure 2 An overview of methodology of inhalation cough challenge.

1 min inhalation method has been used to test both capsaicin [36, 37] and citric acid [38, 39] induced cough responses. This method was reported by Auffarth *et al*. to have reproducibility similar to that of PC_{20} of histamine challenge [39]. However, as this type of cough challenge involves long inhalation periods, it has been associated with difficulties in the delivery of accurate amounts of tussigenic agents. This is partially due to the high inter-subject coefficient variation of the dose delivered from nebulizers [40, 41]. In addition, the amount of the tussigenic agent delivered with each inhalation can be affected by factors such as individual's breathing effort, tidal volume or type of nebulizer used. Since the one-minute inhalation method has been used infrequently and the reported studies utilized only a small number of subjects, we believe that this method should not now be employed in testing cough reflex sensitivity. Conversely, the 15 s inhalation method has been almost exclusively used by one centre [42-46] and whilst it appears to be reproducible by this particular group it is likely that there would be considerable technical difficulties in its wider usage.

For the reasons above-given, a more accurate single-breath method has acquired broader usage. There have been some variations to this method. The less reliable approach involving fixed simple inspiratory manoeuvres such as vital capacity volume inhalation has been employed infrequently [47]. Thus, easier and more accurate methods using single-breath dosimeter controlled nebulizers for the delivery of tussigenic agents have acquired wider usage. This particular methodology has been used to test the effects of gender [48], gastro-esophageal reflux [49] and upper respiratory infection [50] on the cough reflex. The single-breath method was also used to report increased cough sensitivity to citric acid [51] and capsaicin [52] in chronic obstructive pulmonary disease. However, the most relevant usage of this method has been in the investigations of the antitussive effects of pharmacological agents [32] and this will be discussed later.

There remain two further confounding issues associated with inhalation cough challenge testing. Firstly, the occurrence of diurnal variability of cough reflex [47]. Secondly, the fact that the cough can be consciously suppressed [19, 53]. To overcome the first problem most centres recommend the performance of cough challenge testing during the same time of the day. To reduce the effects of voluntary suppression of cough and to increase challenge blindness the use of placebo inhalation has been encouraged. As the learning effect is much lower during the repeated cough challenge, the effects of voluntary suppression can be minimized by performing the test twice [7, 35]. For example in our centre for the dose–response challenge we perform four repeated inhalations of each concentration separated by 30 s intervals with coughs being recorded within the first 10 s. The agents are administered in increasing concentrations with two randomly delivered placebo (normal saline) doses introduced as a control (Figure 3). For the single dose inhalation challenge, we perform five inhalations with 60 s intervals between each inhalation during which the number of coughs are counted [30, 32, 54].

In the next part of this review, we will discuss the use of different tussigenic agents employed for the cough challenge testing.

Tussigenic agents

While testing and developing the inhalational cough challenges a large number of tussive agents have been tried including, sulphur dioxide, ammonia, and cigarette smoke [55]. Although differing in their properties, only capsaicin and citric acid have stood the test of time probably as a result of their greater reproducibility. *In vitro* studies have shown that both capsaicin and citric acid act through the C fibres [21–23]. In addition, citric acid has also been reported to stimulate RARs within the larynx and the upper airways [56]. However, capsazepine a competitive inhibitor of capsaicin [22, 23, 57] also reduces the effects of acids [22, 58] suggesting that both of these agents may be stimulating the same pathway. This is consistent with an allosteric mechanism as shown by different individual sensitivities to these agents but the similar cough responses to acid tussigenic agents such as citric, acetic and phosphoric acids [59].

Acid tussigenic agents

In pharmacological practice amongst acid stimulants only, citric and tartaric acids have acquired common use

Equipment:
Mefar MB3 CE dosimeter (Mefar s.p.a. Bresia, Italy)

Solutions:
Citric acid is diluted in 0.9% sodium chloride to obtain concentrations of:
1 mM, 3 mM, 10 mM, 30 mM, 100 mM, 300 mM, 1000 mM
Capsaicin (stock solution made up in 100% ethanol) is diluted in 0.9% sodium chloride to obtain concentrations of:
0.1 μM, 0.3 μM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM

Procedure:
Capsaicin/citric acid is administered in incremental concentrations with two inhalations of normal saline solution randomly interspersed to increase challenge blindness.
Patients are instructed to exhale to functional residual capacity and then to inhale through the mouthpiece for 1 s (single breath inhalation).
The number of coughs in the first 10 seconds after each inhalation is recorded using Digital Audio Tape recorder.
There is a 30-second pause between each inhalation and each concentration of tussive agent is inhaled four times. Concentration response curves are constructed for each test.

Figure 3 The Hull method of single breath inhalation cough challenge.

in cough testing. Whilst tartaric acid has had only a limited usage in pharmacological experiments (such as the recent report of mexiletine induced reduction in cough response [60]) its main application has been in testing the physiology of the cough reflex [45, 61, 62]. In contrast, citric acid has been used to study pathology and the effects of pharmacological agents on the cough reflex for over 40 years [7]. Thus, opiates [63] and diphenhydramine [34] were reported to reduce citric acid cough sensitivity. In addition, Grattan *et al.* showed that dextromethorphan, a centrally acting codeine analogue devoid of opiate side-effects, reduced citric acid cough response when given orally but not as inhalation [33]. Others also reported significant antitussive effects of oral dextromethorphan but only at a higher dose [54]. The utility of citric acid in explaining the pharmacokinetic and pharmacodynamic relationship of antitussives was demonstrated by Wright *et al.* who found that dextromethorphan caused more prolonged inhibition of citric acid induced cough reflex compared with its metabolite dextrorphan [31]. Similarly, citric acid was used to show the pharmacodynamic response to menthol inhalation in healthy volunteers [32]. A further description of pharmacological studies with citric acid is provided in Table 1.

Non-acid tussigenic agents

Capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide) a pungent agent of hot pepper, has been the most commonly used nonacid tussigenic agents [14, 36, 64]. Capsaicin acts mainly on the afferent neurones of the nonmyelinated C-fibres by opening a nonselective cation channel of vanilloid receptor resulting in a flow of calcium and sodium down their concentration gradient [58, 64]. This leads to depolarization and associated neurotransmitter release. The capsaicin induced calcium flow and desensitization is inhibited by a specific antagonist; an inorganic dye ruthenium red [65, 66]. However, it is another vanilloid, resiniferatoxin an extract from *Euphorbia poisonae*, that is the most potent tussive substance known causing cough at nanomolar concentration [24]. Unfortunately it is unsuitable for challenge testing because of its 'all or none' response.

Owing to its direct effect on the sensory nerves, capsaicin has been frequently used in the testing of the antitussive properties of local anaesthetics. For example, Midgren *et al.* observed that inhaled lignocaine resulted in a dose dependent inhibition of capsaicin induced cough [36]. Similarly, others observed that inhaled lignocaine reduced capsaicin cough response [67]. The action of lignocaine was not affected by adrenaline [68]. In contrast, another local anaesthetic dyclonine failed to have any effect on cough response [67]. However, the major constraint of the above studies is associated with the small number of subjects tested. Despite the encouraging reports, inhaled local anaesthetics have acquired only a limited use in the treatment of intractable persistent cough probably because of concomitant laryngopharyngeal anaesthesia.

In contrast to local anaesthetics, the effects of opiates on capsaicin induced cough have proved difficult to demonstrate. For example, Fuller *et al.* reported that inhaled codeine and morphine had no effect on capsaicin induced cough response [69]. Similarly, Hutchings & Eccles observed that codeine did not affect capsaicin induced cough [19]. In addition, naltrexone an opioid antagonist failed to affect cough response to capsaicin [19]. Conversely, oral codeine and intravenous morphine resulted in cough suppression suggesting their central action. Unfortunately, these studies suffer from a number of methodological problems stemming from the variability of the cough response in un-selected subjects. Type 2 errors are likely if rigorous selection criteria are not used and this may explain the apparent lack of response rather than a problem with capsaicin itself.

When capsaicin challenge may be specifically altered is in angiotensin converting enzyme (ACE) inhibitor

Table 1 Pharmacological studies using different methodologies of inhalation cough challenge with citric acid or tartaric acid* as tussigenic agents.

Author	*Delivery system*	*Output (O) or volume (V)*	*Inh type*	*Dose or concentration*	*Recording method*	*Normal values*	*Agent tested*	*Number tested*
Lowry *et al.* [70, 100]	Neb (U)	1.8 ml min^{-1} (O)	1 min	0.68% in 0.79% NaCl	1 min	11.4 coughs min^{-1} [100]	Captopril [70]	21 [100] 16 [70]
Poundsford *et al.* [47, 63, 101]	Neb	833 ml (V)	5 s	0.5–32% [101] 0.125–2.0 × 10^{-1} mol l^{-1} [63]	C-Thresh [63] CI [101]	Total coughs and CI: 11.6 and 5.3 [101]	Salbutamol, ipratropium [101] Opiates [63]	16 [101]
Laude *et al.* [24]	Neb + D-meter	0.125 ml Inh^{-1} (O)	S-breath	10–1000.0 mM	10 s	D5 141.3 mM Capsaicin	Citric acid	10
Fujimura *et al.* * [46, 60, 61]	Neb	0.21 ml min^{-1} (O)	15 s	1.56–800.0 mg ml^{-1}	1 min	Geomean D5 1.03 mg l^{-1} (F) 2.62 mg l^{-1} (M) [61] 32.0 mg ml^{-1} [46, 60]	Mexiletine [60] Procaterol Methacholine [46]	14 [60] 28 [46]
Thompson *et al.* [30, 31]	Neb + D-meter	0.275 ml Inh^{-1} (O)	S-breath	500.0 mM	10 s	19.07 coughs (F) 11.97 coughs (M) [30]	Dextromethorphan dextrorphan [31]	160 [30] 19 [31]
Packman *et al.* [34]	–	–	S-breath	5%	1 min	–	Diphenhydramine	20
Morice *et al.* [32, 33]	Neb + D-meter	0.125 ml Inh^{-1} (O)	S-breath	33.0 μmol	1-min	9 coughs (baseline) [32]	Menthol [32] dextromethorphan [33]	20 [32]

Neb = Nebulizer (Jet unless stated otherwise), U = Ultrasonic, D-meter = Breath activated dosimeter, Inh = Inhalation, S-breath = Single breath, MMD = Mass median diameter, MMAD = Mass median aerodynamic diameter, CI = Cough index, i.e. number of coughs divided by the time of coughing, C-Thresh = Cough threshold, i.e. concentration causing one cough, Geomean = Geometric mean, ACEI = Angiotensin converting enzyme inhibitors.

induced cough. Captopril an ACE inhibitor when administered to healthy volunteers significantly enhanced capsaicin sensitivity [70]. Similarly, capsaicin sensitivity was raised in patients on regular ACE inhibitor therapy [71]. Furthermore, the increased cough reflex sensitivity to inhaled capsaicin was not affected by different doses of enalapril [72], suggesting that the individuals who develop ACE inhibitor cough might only represent the extreme of this phenomenon. Whilst, Yeo *et al.* observed that the sensitivity to inhaled capsaicin was reduced after 28 days from stopping enalapril [73] our experience in the Hull Cough Clinic is that the ACE inhibitor induced cough can take more than two months to resolve. However, the treatment of ACE inhibitor induced cough other then stopping the medication has been difficult with many agents such as inhaled sodium cromoglycate proving ineffective [74].

Capsaicin inhalation cough challenge has also been used to test antitussive properties of other agents. For example, cromoglycates such as sodium cromoglycate [37] and nedocromil sodium [75] failed to affect capsaicin induced cough response. Similarly, a β_2-adrenergic receptor agonist procaterol [43] was reported to have no effect on capsaicin induced cough. In contrast, sulindac a nonsteroidal anti-inflammatory drug [76] and baclofen a γ-aminobutyric acid (GABA) agonist [77] significantly increased capsaicin induced cough threshold. The postulated antitussive mechanisms of the latter agent were thought to be either due to its central action or possibly, through the suppression of pro-tussive peptides such as substance P [78]. However most of these studies were performed on a small number of subjects and the agents tested have not acquired any wider use in the treatment of cough.

Recently there has been an increasing interest in assessing the effects of different pharmacological agents in the treatment of cough associated with asthma [42, 79, 80]. For example, zafirlukast a leukotriene receptor antagonist was reported by Dicpinigaitis *et al.* to have no effects on cough in asthma [79]. In contrast, zafirlukast resulted in the suppression of capsaicin induced cough in the cough variant of asthma [80]. Other agents such as the cyclo-oxygenase inhibitor indomethacin and a selective thromboxane synthesis inhibitor OKY-046 increased capsaicin induced cough threshold in asthma [42]. Indomethacin also increased capsaicin induced cough threshold in patients with chronic bronchitis. However, both of these agents failed to affect cough in healthy volunteers. Further examples of pharmacological studies with capsaicin are given in Table 2.

To date, capsaicin has acquired the broadest usage in pharmacological studies. However, there have been other examples of nonacid cough stimulants such as the recently tested pelargonic acid vanyllyamide (PAVA) [81] and distilled water. The latter has been proposed to act mainly through the alteration of the ionic balance rather than as a direct effect on the cough receptors [82]. Whilst inhaled PAVA cough challenge was only employed in one non-pharmacological study [81] the distilled water cough challenge has found some application in the investigations of the ACE inhibitor induced cough [70, 83]. In addition, Lowry *et al.* reported that bronchodilators such as oxitropium bromide, ipratropium bromide and fenoterol hydrobromide significantly reduced ultrasonically nebulized distilled water induced cough in healthy volunteers and asthmatics [84, 85].

Recordings of cough

Symptom assessment in patients with lung disorders relies on subjective recording and therefore can be variable [86, 87]. Similarly, subjective measurements of cough are difficult to assess. In fact, one of the major difficulties in cough research has been our inability to accurately quantify clinically relevant cough. Part of the problem is the paroxysmal nature of cough, necessitating the recording of large amounts of data in order to generate an accurate picture of cough frequency. As cough is a variable phenomenon within patient groups, so large numbers of subjects have to be studied to generate statistically meaningful results.

The use of tools such as questionnaires or visual analogue scores frequently alienates assessment objectivity. However, there are a few reports suggesting that objective cough measurements correlate with subjective assessment of this symptom. For example Yeo *et al.* found a correlation between capsaicin cough response and subjective visual analogue score in ACE inhibitor induced cough [73]. Likewise, Hargreaves *et al.* found that sodium cromoglycate reduced both the severity of cough recorded in a cough diary and the sensitivity of cough response to capsaicin [74]. Furthermore, O'Connell *et al.* showed that the improvement of visual analogue score that was associated with successfully treated cough correlated also with the decrease of cough sensitivity to capsaicin [88].

Fortunately, the assessment of cough response during the inhalation cough challenge has been more straightforward. The simplest approach involves manual recording carried out by one investigator or, as is preferred to minimize error, by two independent examiners [44, 50, 61, 88, 89]. The more complex methods apply a wide range of instruments varying from fast running chart recorders [67], tape recorders [36] or pen recorders [53] to invasive techniques such as that involving a pressure transducer used by Matthys *et al.* [90]. Recently Freestone *et al.* used a more sophisticated system composed of a digital sound level meter to record cough sounds [91]. In this particular study, the authors confirmed lack of antitussive effects of codeine during upper respiratory tract

Table 2 Pharmacological studies using different methodologies of inhalation cough challenge with capsaicin as a tussigenic agent.

Author	*Delivery system*	*MMD*	*Output (O) or volume (V)*	*Inh type*	*Dose or concentration*	*Recording method*	*Normal values*	*Agent tested*	*Number tested*
Midgren *et al.* [36, 68]	Neb	3.0 μm	0.5 ml min^{-1} (O)	1 min	0–50.0 μM	1 min	D2 0.016–10 μM	Lignocaine + adrenaline [68]	26
Hargreaves *et al.* [74]	Neb + D-meter	3.5–4.0 μm	0.02 ml (V)	S-breath	0.4–12.5 nmol	–	Geomean D2–2.1 nmol D5–3.9 nmol	Sodium cromoglycate	10
Choudry *et al.* [67, 95, 102]	Neb + D-meter	3.5–4.0 μm	0.002 ml (V) [67, 102]	S-breath	0.4–100.0 nmol [67] 1.95–500.0 μM [95]	1 min [102]	Log C2 1.04 μM Log C5 1.81 μM [95]	Lignocaine + dyclonine [67] PGE_2, bradykinin [102]	10 [67] 6 [102]
Hansson *et al.* [68, 75]	Neb + D-meter	3.0 μm	0.5 ml min^{-1} (O)	S-breath	0.4–50.0 nmol	1 min	Geomean D2–3.2 nmol D5–17.7 nmol	Nedocromil sodium [75] Lignocaine + adrenaline [68]	6 [75] 10 [68]
Hutchings & Eccles [19]	Neb + D-meter	2.5 μm (MMAD)	–	S-breath	$0–3.33 \times 10^{-4}$ M	30 s	12 coughs (baseline)	Codeine Natrolexone	80
Yeo *et al.* [73]	Neb	3.5–4.0 μm	–	S-breath	0.05–3.2 nmol	1 min	–	ACEI	8
Dicpinigaitis *et al.* [48, 77, 79, 80]	Neb + D-meter	3.5–4.0 μm	1.007 ml min^{-1} (O) 0.02 ml (V)	S-breath	0.98–1000 μmol l^{-1}	1 min	Log C5 1.02 μM (F) 1.41 μM (M) [48]	Baclofen [77] Zafirlukast [79, 80]	20 [77] 16 [80]
Fujimura *et al.* [42, 43, 60, 89]	Neb	3.6 μm	0.21 ml min^{-1} (O)	15 s	0.49–1000 μM	1-min	D5 Geomean 8.22 μM (F) 45.0 μM (M) [89] 20.6 μM [42]	Procaterol [43] Indomethacin [42] Mexiletine [60]	35 [43] 28 [42]

Abbreviations: See Table 1.

infection, which was previously reported by Eccles *et al.* using inhalation cough challenge [92]. It is likely that the future of cough assessment will involve the usage of more elaborate systems with high quality digitized cough recordings suitable for examination by pattern recognition algorithms [93, 94].

Conclusions

The majority of the trials implemented to investigate antitussive agents were performed on a small sample size of un-selected healthy volunteers. Whilst antitussive medicines are primarily developed for the treatment of cough associated with acute upper respiratory tract infection (URTI), the relationship between induced and naturally occurring cough has not been widely investigated. It is not surprising that cough sensitivity to citric acid [95], capsaicin [50] and ultrasonically nebulized distilled water [96] is enhanced during URTI. However, the inhibition of induced cough might not necessarily be indicative of antitussive efficacy of medications against naturally occurring cough. For example, oxitropium bromide that was effective in reducing induced cough [84] did not affect URTI related cough [96]. The assessment of the antitussive effect against naturally occurring cough would therefore require the testing of cough medicines in subjects with URTI. Large numbers of subjects would be needed, necessitating therefore costly multicentre trials, to ensure adequate power because of the increased variability in cough response inherent in this type of study design.

As a consequence of the different methodologies used by separate groups of investigators, experimental results are frequently difficult to compare or reproduce, one example being the presence of confounding reports on the gender difference in cough response. Choudry & Fuller [95] and more recently Doherty *et al.* [97] failed to observe gender difference in cough response employing a single breath dosimeter capsaicin cough challenge. In contrast, Dicpinigaitis *et al.* using a similar methodology reported a lower cough threshold in female volunteers [48]. Fujimura *et al.* using a 15 s capsaicin inhalation method confirmed the presence of gender difference in cough response [89]. Similarly, we have recently reported on a marked cough gender difference with a single dose citric acid cough challenge [30] suggesting that this phenomenon remains unaffected by either the method or tussigenic agent used and that the previous negative studies were inadequately powered.

A wide range of inhalation cough challenge methods has been applied in the studies of cough reflex (Figure 2). It is our opinion that the more reproducible single breath dosimeter controlled method should be used. The choice of tussigenic agent will frequently depend on local experience. However, when both citric acid and capsaicin cough challenges are performed within a short period of time, the cough response can be diminished by a quarter when citric acid is inhaled after capsaicin and by a third when capsaicin inhalation follows the administration of citric acid [35].

The dose-response inhalation cough challenge is more appropriate for population studies as it provides a wide range of concentrations of tussive agent to take account of the wide individual variability of cough reflex sensitivity. The appropriate application of this method was demonstrated by Gordon *et al.* [98] while testing the effects of low-dose irritant fumes on cough reflex in a population of glass bottle workers. In contrast, the effects of antitussive medication on the sensitivity of cough reflex are more suitably measured with a single dose method in selected volunteers. Following the screening of a large number of subjects to select individuals with a clear, reproducible cough response, this method allows for the testing of the pharmacokinetics and pharmacodynamics of antitussive agents. The usefulness of the single dose method in pharmacological studies was demonstrated by Abdul Manap *et al.* [54] while measuring the antitussive effects of two doses of dextromethorphan in relation to inhibition of CYP2D6 activity by quinidine. In this study a power of 90% to detect 10% change in cough response and at least 80% power to detect a similar change for multiple treatments comparisons was provided by 22 subjects with reproducible cough sensitivity to inhaled citric acid. With a single dose method repeated cough challenge testing at different time points up to 12 h after dosing were performed thus, allowing for a calculation of the end point as an area under the total cough curve. However, in this type of study the effects of tachyphylaxis should always be considered. When cough testing is required to be repeated at short intervals, the citric acid inhalation challenge is preferred as the recovery from cough challenge to capsaicin is slower, resulting in greater long-term tachyphylaxis, particularly with higher doses [35]. Conversely, when the study design allows for longer periods between the cough sensitivity testing a dose response cough challenge with either citric acid or capsaicin may be appropriate. In the design of pharmacological studies employing the inhalation cough challenge one should also consider the placebo and gender effects. The placebo cough response is associated with a nonlinear increase in cough suppression, which is most pronounced at 4 h [99]. In addition, there are suggestions that females may cough more frequently and have more rapid adaptation of cough than males [99].

In this review article, we have discussed the various cough challenges implemented by the different centres. Regardless of the complexity or otherwise of the methods previously described the major confounding issue has

always been the absence of standardized protocols for cough challenge. This coupled with inadequate inter-centre comparisons has resulted on occasions in conflicting reports. We would like to suggest that standardized guidelines for cough challenge testing should be introduced allowing for greater accuracy and comparability in pharmacological and physiological research in the future.

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