Understanding of the complexity of the cough reflex using experimental models and randomised clinical trials

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Abstract

My contribution to understanding the cough reflex spans experimental models of rodent airways, patient and volunteer responses to inhaled tussive agents, and randomised placebo-controlled drug trials. Through studying sensory pathways, I aimed to understand how menthol may relieve a cough. My early work focused on menthol's potential bronchodilator effects. In anaesthetised guinea pigs, menthol reduced capsaicin and neurokinin-A induced increases in airway resistance and caused dose-dependent relaxation of pre-constricted bronchi. This Dose dependency supported a drug-receptor interaction, confirmed by ligand-binding studies, identifying an L-menthol binding site in guinea pig lung membranes. Since then, TRPM8 (cold and menthol receptor 1) has been characterised as a therapeutic target for chronic cough (CC). My early work contributed to the current development of AX8, a synthetic cooling compound shown to reduce cough in patients with recalcitrant CC, likely via TRPM8-mediated sensory nerve modulation.

To explore a central pathway in cough, I conducted the first randomised placebo-controlled study of slow-release morphine in refractory and unexplained cough. Morphine significantly improved daily diary scores and Leicester cough questionnaire outcomes compared to placebo, with post hoc analysis indicating that sedation does not underlie its antitussive effects. This work informed subsequent trials of morphine in Idiopathic pulmonary fibrosis (IPF)-related cough, and supported repurposing of the opiate nalbuphine for treatment of cough associated with IPF and refractory chronic cough, both studies showing clinically meaningful cough reductions.

Progress in novel antitussives requires rigorous cough challenge methodologies. I validated the European Respiratory Society standard citric acid cough challenge, improving reproducibility by adopting a doubling dose protocol and physiological thresholds (C2 and C5). This standardisation confirmed the efficacy of Gefapixant, a first-in-class P2X3 antagonist, which significantly reduced ATP- and distilled water-induced coughs in both healthy volunteers and patients with chronic cough, indicating peripheral target engagement. My work contributes to the licensing of Gefapixant in the European Union, the first licensed antitussive since 1953.

Together, these studies provide valuable insight into cough mechanisms spanning peripheral receptor activity through to central nervous system modulation. They highlight the importance of standardised human cough methodology to advance our understanding and facilitate the development of effective antitussive therapies.
This body of work highlights the complexities of chronic cough and the need for integrated approaches targeting peripheral receptors, central modulation and sensory nerve pathways.

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Preface

Following a long career in clinical research, my first drug study testing a cough treatment was in 1997, testing a drug called Levodropropizine. I recall it as if it were only yesterday, as managing that study was the biggest learning curve of my life. I had no clue on how to orchestrate a clinical trial, the paperwork required, the precision; it was all new to me coming from a laboratory background. It felt like going back to school as the monitor looked disapprovingly at me, knowing I was a complete rookie.

Since then, I have been involved in well over 50 cough studies, from the use of cough challenges in healthy volunteers, through to testing novel therapies and discovering new pathways in cough.

It's been a labour of love for 28 years, and in that time, most treatments I have tested have failed. You see the same patients attending the clinic or getting involved in the research as their cough persists. Over the last decade, I would say real strides have been seen in the cough world with the use of nalbuphine and Gefapixant. From my personal experience, these treatments either work profoundly on a patient or have a minimal effect or may work but can't be tolerated due to side effects.

This thesis depicts part of my journey working with chronic cough from early experimental models through to studies on Gefapixant and looking to the future and alternative treatments.

Contribution Statements

Chapter 2 paper: Capsaicin and neurokinin A-induced bronchoconstriction in the anaesthetised guinea-pig: Evidence for a direct action of menthol on isolated bronchial smooth muscle. Wright C, Laude E, Grattan T, Morice A. (1997). *British Journal of Pharmacology*, 121 (8), pp.1645–1650. Available at: doi:10.1038/SJ.BJP.0701319

I was the first and corresponding author for this article and was primarily responsible for the conception, design, conduct of research, analysis of data, drafting, revision, and final approval of the manuscript. Dr Laude and Professor Morice contributed to the critical review and final approval of the paper.

Chapter 2 paper: Identification of the L-menthol binding site in guinea-pig lung membranes. Wright C, Bowen W, Grattan T, Morice A. (1998). *British journal of pharmacology*, 123 (3), pp.481–486. Available at: doi:10.1038/SJ.BJP.0701642

I was the first and corresponding author for this article and was primarily responsible for the conception, design, conduct of research, analysis of data, drafting, revision, and final approval of the manuscript. Dr Grattan and Professor Morice contributed to the critical review, and final approval of the paper.

Chapter 3 paper: Validation of the ERS standard citric acid cough challenge in healthy adult volunteers. Wright C, Jackson J, Thompson R, Morice A. (2010). *Cough (London, England)*, 6 (1). Available at: doi:10.1186/1745-9974-6-8

I was the first and corresponding author for this article and was primarily responsible for the conception, design, conduct of research, analysis of data, drafting, revision, and final approval of the manuscript. Rachel Thompson and Jennifer Jackson helped with the recruitment of patients and the conduct of the cough challenges. Professor Morice contributed to the critical review and final approval of the paper.

Chapter 4 paper: Opiate therapy in chronic cough. Morice A, Menon M, Mulrennan S, Everett C, Wright C, Jackson J, Thompson R. (2007). *American Journal of Respiratory and Critical Care Medicine*, 175 (4), pp.312–315. Available at: doi:10.1164/RCCM.200607-892OC,

I was the fifth author on this paper; however, I was involved in the design, recruited and conducted the study alongside Jennifer Jackson and Rachel Thompson. I also analysed all the data and presented this work at the cough symposium in Italy. The Paper was written by Professor Morice, but I contributed to the critical review.

Chapter 4 paper: Is opiate action in cough due to sedation? Dickinson R, Morjaria J, Wright C, Morice A. (2014). *Therapeutic Advances in Chronic Disease*, 5 (5), pp.200–205.

I was the third author on this paper; I was involved in mainly the analysis of all the data. The paper was written by Rebecca Dickinson, but Professor Morice and I contributed to the critical review.

Chapter 5 paper: The effect of Gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study. Morice A. Kitt M, Ford A, Tershakovec A, WU W, Brindle K, Thompson R, Thackray-Nocera S, Wright C (2019). *European Respiratory Journal*, 54 (1). Available at: doi:10.1183/13993003.00439-2019

I was the co-author for this article and was primarily responsible for the conception, design, conduct of research, and analysis of data. Rachel Thompson, Kayleigh Brindle, and Susannah Thackray-Nocera helped with the recruitment of patients and performing the cough challenges. Professor Morice wrote the paper, and I contributed to the critical review and approval of the paper.

Acknowledgements

Writing a PhD has been a long time coming. My first animal experimentation was in 1996 at Sheffield University Medical School, under the supervision of Professor Morice. During this time, I was taught specialised techniques in animal surgery and got to grips with myography. Dr Liz Laude was a fantastic mentor, and I owe so much gratitude to her for the support she gave me while doing the animal experimentation and advice when I wrote my first paper.

Professor Alyn Morice has been that constant throughout my career, supporting me in this endeavour and keeping me interested and stimulated in the field.

I would also like to thank Tim Grattan for giving me the opportunity to use the facilities in Harlow and learn how to perform the ligand binding techniques.

Also, a big thanks goes out to my work colleagues, Jennifer Jackson, Rachel Thompson, and Susannah Thackray-Nocera, for the help in recruiting patients and the hundreds and hundreds of cough challenges performed.

To the patients and healthy volunteers who took part in the studies, you were so generous with your time and commitment. I will be eternally grateful for this.

Professor Simon Hart, I appreciate you being my supervisor and the guidance you have given me. Many a time I have come to your office and chewed off your ear, and you are so polite about it.

Finally, I would like to say a huge thank you to my family and friends. Matthew Wright, Joanne Taylor, Brian Taylor, Rachel Thompson, Tanya Cavany and my son Alex for supporting me throughout, giving me the boosts I needed, and never giving up on me. I'm pretty sure they all thought this day would never come.

Dedicated to my mum Marie Angelica Dignam.

Author's declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources, these are clearly identified using quotation marks and the reference(s) is fully cited.

I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations.

I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources.

I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised

Summary of abbreviations and their meanings

ACh	Acetyl Choline
ACOS	Adverse Cough Outcome Survey
AD	After Death
ATP	Adenosine Triphosphate
BCST	Behavioural Cough Suppression Therapy
BID	Twice a day
BSM	Bronchial Smooth Muscle
C1	Concentration Eliciting One Cough
C2	Concentration Eliciting Two Coughs
C5	Concentration Eliciting Five Coughs
CA	Citric Acid
CAA	Citric Acid Aerosol
CC	Chronic Cough
CGRP	Calcitonin Gene Related Peptide
CHS	Cough Hypersensitivity Syndrome
CMR1	Cold and Menthol Receptor
COVID	Coronavirus Disease
CYP2D6	Cytochrome P450 2D6
EMA	European Medicines Agency
ERS	European Respiratory Society
FDA	Food and Drug Administration
FMRI	Functional Magnetic Residual Imaging
GP	Guinea Pig
hr	Hour
HV	Healthy Volunteers
HYMS	Hull York Medical School
IBS	Irritable Bowel Syndrome
KD	Koko Digidoser
KCL	Potassium Chloride
LCQ	Leicester Cough Questionnaire

MD	Mefar Dosimeter
MG	Milligrams
MHRA	Medicines Health Research Authority
MST	Morphine Slow-Release Tablet
MOR	Mu Opiod Receptors
NEP	Neutral Endopeptidase
NICE	National Institute for Health and Care Excellence
NKA	Neurokinin A
NTS	Nucleus Tractus Solitarius
OAA	Observers' Assessment of Awareness
OCCS	Opioid Containing Cough Suppressant
OTC	Over the Counter
P2X3	P2X Purinoceptor 3
RAR'S	Rapidly Adapting Receptors
RAW	Airways Resistance
RCC	Refractory Chronic Cough
SAR'S	Slowly Adapting Receptors
SIP	Sickness Impact Profile
SLN	Superior Laryngeal Nerve
SP	Substance P
SS	Sedation Scale
TDS	Three Times a Day
TRP	Transient Receptor Potential
TRPM8	Transient Receptor Potential Melastatin Subfamily 8
TRPV4	Transient Receptor Potential cation channel Subfamily V Member 4
TRPV1	Transient Receptor Potential cation channel Subfamily V Member 1
UCC	Unexplained Chronic Cough
UTC	Urge To Cough
VAS	Visual Analogue Scale
Ve	Minute Ventilation

Index of Appendices

The following is a list of the publications forming the basis of each chapter around which this thesis by published work is built:

Chapter 1 Cough Reflex

Cough is a vital, protective reflex that clears inhaled particles and removes excess secretions. However, this reflex can become either hypoactive or hyperactive due to dysfunction. When the cough reflex is impaired, debris accumulates in the airway, leading to atelectasis, bronchiectasis, and infection (Madison and Irwin, 2010). The risk of aspiration also increases, particularly in the elderly (Miles et al., 2013). Among stroke patients with an abnormal reflex cough test, there is a higher prevalence of aspiration pneumonia and an increased risk of death (Addington et al, 1999). When the cough reflex becomes exaggerated or persistent, it transitions from a protective mechanism to a pathological response, contributing to morbidity and reduced quality of life.

Chronic cough (CC) is defined as a cough lasting 8 weeks or longer. Its peak incidence occurs in individuals aged 50 to 60 years, and it's twice as common in women as in men (Dicpinigaitis and Bauf, 1998), placing a substantial burden on both patients and the healthcare system. The recent Rotterdam Study reported that in the general population, 10% of adults aged 45 years and older have CC, with prevalence increasing with age (Arinze et al., 2020). A systematic review of common presentations in GP surgeries globally identified cough as one of the ten most frequently reported symptoms (Finley et al.2018). In a longitudinal community-based study of public service employees self-reporting a CC, 80.5% still had a cough a year later (Lätti, Pekkanen and Koskela, 2019). Even after multiple consultations, only half of patients receive a clear diagnosis for their cough (Chamberlain et al., 2015). In secondary care specialist cough clinics, a longstanding cough persists in 50-60% of patients five to seven years after their first clinic appointment (Koskela, Lätti and Purokivi; 2019).

Although the first referenced cough treatment using opium was reported in 1025 AD, by the Persian physician, Avicenna (Stein and Thiel, 2017), CC remains challenging to treat both in the community and in specialist cough clinics. In Great Britain, sales of over-the-counter (OTC) cough, cold and sore throat treatments exceeded £583 million in 2023. The second-highest value among all OTC drug categories that year (Matej Mikulic, 2024), highlighting the ongoing need for more effective therapies. Despite this burden, no novel pharmaceutical agent for cough has received NICE approval in

over 50 years. The lack of diagnosis for the cause of CC for so many patients and unresponsiveness to targeted treatments has driven a re-evaluation of its classification. Because many patients have heightened cough sensitivity to low levels of chemical, thermal and mechanical stimuli, CC is now conceptualised as cough hypersensitivity syndrome (CHS) (Morice et al., 2014).

CC results in a significant loss of quality of life and is associated with numerous physical and psychological complications. Zoglmann et al (2015) reported that 65.5% of women with a CC have urinary incontinence, while 70% of patients experience impaired sleep (Kubo et al., 2021; Everett et al., 2007). Patients also report vomiting, nausea, and retching (French et al., 1998), alongside high rates of anxiety and depression. The detrimental impact of CC on quality of life was first reported by French et al., (1998), who found that baseline scores for socialisation, rest, work, recreation, and pastimes in CC patients were comparable to those they had previously observed in disabled patients with chronic obstructive pulmonary disease.

The overall burden of CC on the patient is substantial. In an internet-based survey hosted by the European Lung Foundation, 96% of respondents stated that cough affected their quality of life, 91% felt fed up or depressed, and 81% said it limited their ability to undertake activities (Chamberlain et al., 2015). More recently, patients with refractory or unexplained CC in Spanish outpatient clinics identified the most significant impacts as being on mood and emotion, followed by everyday activities, sleep, and leisure time (Puente-Maestu et al., 2023).

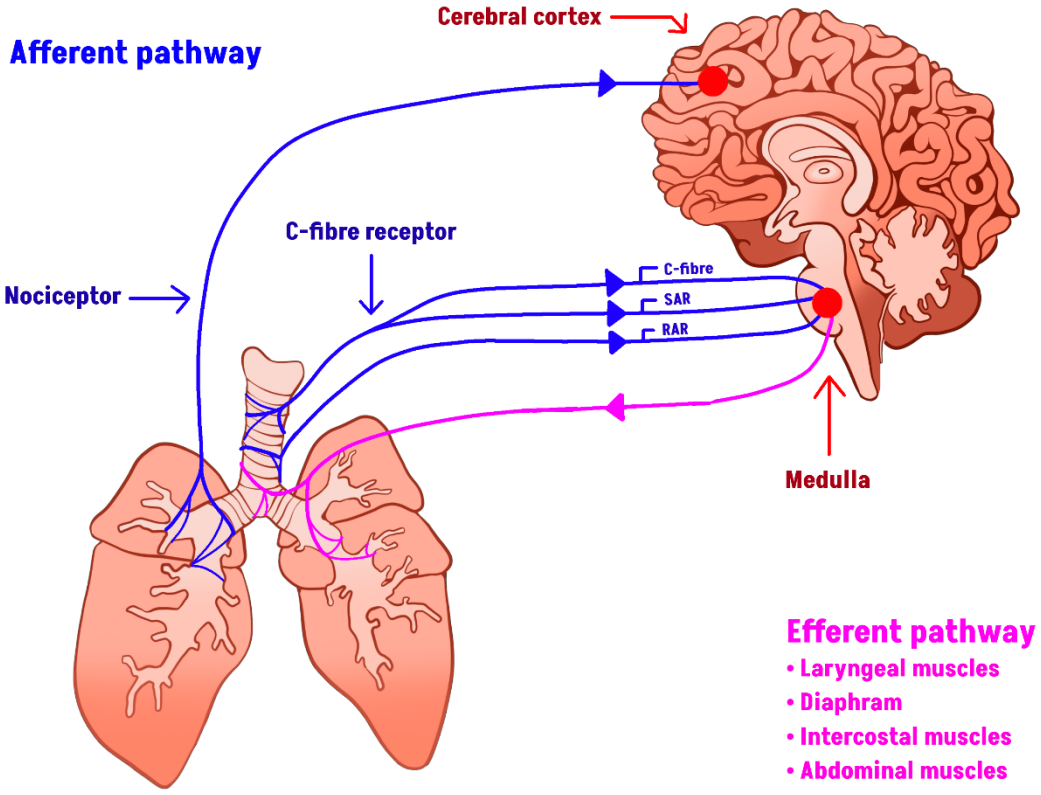
At present, there remains an unmet need for safe and effective therapeutic agents to treat unexplained or refractory chronic cough (UCC/RCC), as well as a clearer understanding of the mechanisms underlying the heightened cough reflex.

1.1 Cough Reflex Overview

Cough involves a complex reflex arc originating from the stimulation of sensory nerves within the airways. Cough can be initiated voluntarily or reflexively. It contains three components:

1. Afferent limb- consisting of the vagal afferent fibres such as rapidly adapting receptors (RARs) and C fibres.
2. Central processing centre- consisting of the nucleus tractus solitarius (NTS) in the medulla oblongata.
3. Efferent limb- involving motor nerves such as the vagus, spinal and phrenic nerves carrying signals from the cough centre to the respiratory muscles involved in coughing (Irwin, Rosen and Braman, 1977) (Figure 1).

Figure 1. Cough reflex arc illustrated by Alexander Gilbert (2025).



1.2 Early Studies of Cough Reflex and Receptors

Early foundational studies demonstrated that the cough reflex is highly dependent upon the two principal branches of the vagus, the superior and recurrent laryngeal nerves, which innervate the larynx. Vagotomy significantly reduces or abolishes cough in humans and animals (Klassen, Morton and Curtis, 1951), indicating that non-vagal pathways play only a minor role. Work in anaesthetised, spontaneously breathing cats further differentiated the pathways involved in mechanical versus chemical activation of cough. Mechanical stimulation reliably evoked cough when applied to the larynx and upper trachea, but became progressively harder to elicit in the Lower respiratory tract,

and almost impossible at the level of the small airways and alveoli (Berguland,1980). In contrast, chemically induced cough showed the opposite distribution; responses were amplified when delivered to more distal airways rather than to the larynx or larger proximal airways (Widdicombe, 1954b). Notably, chemically evoked cough persisted even after vagotomy, suggesting the involvement of vagal fibres resistant to surgical interruption or alternative chemosensitivie pathways. Overall, these findings suggest that cough can be evoked by both chemical and mechanical stimulation but through distinct and regionally distributed populations of cough receptors located in the larynx, trachea, proximal airways and throughout the bronchial tree.

1.2.1 Larynx

The larynx is an important reflexogenic site and has been extensively studied in relation to airway defence. The density of innervation to the larynx exceeds that of all other respiratory segments. In the cat, the superior laryngeal nerve (SLN) contains approximately 3,000 myelinated fibres, of which 2,200 supply the larynx (Dubois and Foley, 1936). Overall, about one-third of all vagal afferents innervate the larynx, with the remainder distributed across the tracheo-bronchial tree, lungs, heart, and gastrointestinal tract.

Sensory innervation of the larynx is conveyed primarily by the SLN. This nerve joins the vagus at the nodose ganglia and travels alongside it, terminating in the subnuclei of the NTS. The SLN contains specialised receptors sensitive to transmural pressure, temperature variations, or laryngeal motion (Sant'Ambrogio et al., 1983). Although bilateral sectioning of the SLN has little effect on breathing patterns, in unanaesthetised animals (Citterio, Mortola and Agostoni, 1985), these receptors are highly active during airway challenges. Chemical, mechanical and direct nerve stimulation can activate them and initiate protective reflexes (Iscoe, Feldman and Cohen, 1979). More recent work suggests that abnormalities in these pathways contribute to cough hypersensitivity and laryngeal dysfunction in chronic cough (Sundar, Stark and Morris, 2024).

The internal branch of SLN, composed primarily of afferents from the upper larynx, appears to be the dominant afferent source, as sectioning it abolishes most of the reflex responses elicited from the larynx (Mathew, Abu-Osba and Thach, 1982). It

plays a central role in respiratory modulation (Sant'Ambrogio et al., 1983), and electrical stimulation elicits several reflexes, such as swallowing (Kitagawa et al., 2009), coughing (Gestreau, Bianchi and Grélot, 1997) and respiratory suppression (Miller and Loizzi, 1974). Contemporary clinical studies show that targeted SLN interventions, such as SLN block, can modulate cough responses in patients with neurogenic or refractory chronic cough, reinforcing the SLN's central role in cough regulation (Vrapciu et al., 2025; Binhazzaa, 2025;Peachman et al., 2026).

1.2.2 Airway Receptors

Three distinct phenotypes of sensory nerve endings in the lung have been described:

- Aδ-myelinated fibres- Rapidly adapting receptors (RARs),
- Aδ-myelinated fibres-Slowly adapting stretch receptors (SARs)
- unmyelinated C-fibres

These are summarised in *Table 1* (Ganong, Chapter 37).

Table 1. Nonchemical influences on respiration: responses mediated by receptors in airways and lung. Ganong review of Medical Physiology: Chapter 37.

Vagal Innervation	Type	Location in the interstitium	Stimulus	Response
myelinated	Slowly adapting	Airway smooth muscle cells?	Lung inflation	Inspiratory time shortening
				Hering-Breuer Inflation, dilation relexes
				Bronchodilation
				Tachycardia
				hyperpnea
	Rapidly adapting	Airway epithelial cells	Lung hyperinflation	Cough
			Exogenous and endogenous substance (e.g histamine, prostaglandins)	Bronchoconstriction
				Mucus secretion
Unmyelinated C fibres	Bronchial C-fibres	Close to blood Vessels	Lung hyperinflation Exogenous and	Apnea followed by rapid breathing
			endogenous substance (e.g., capsaicin, bradykinin, serotonin)	Bronchoconstriction
				Bradycardia
				Hypotension
				Mucus secretion

1.2.2.1 Slowly Adapting Receptors (SARs)

SARs are fast conducting mechanoreceptors (Nasra and Belvisi, 2009), stimulated primarily by lung inflation, and they adapt to changes in the length of the airways' smooth muscle, particularly in peripheral airways, and are thought to mediate the Hering-Breuer inflation reflex (Berguland E, 1980). They are insensitive to most mechanical and chemical stimuli, but control cycle-by-cycle breathing by reducing efferent vagal discharge (Schelegle and Green, 2001). Although SARs don't directly trigger cough, they can modify its strength through central mechanisms (Hanáček, Davies and Widdicombe, 1984).

1.2.2.2 Rapidly Adapting Receptors (RARs)

RARs are concentrated in key cough -sensitive sites, including the larynx, carina, proximal bronchi and deeper intrapulmonary airways (Widdicombe, 1998). Their vagal afferents respond to light mechanical stimulation, and show irregular discharge during normal breathing with bursts during inspiration (Boushey et al., 1974; Sant'Ambrogio et al., 1984). They react to a wide range of irritants, including cigarette smoke (Lu-Yuan Lee and Morton, 1988), distilled water (Boggs and Bartlett, 1982), acid, alkaline, low chloride solutions (Anderson et al., 1990) and mediators such as histamine and prostaglandins (Coleridge et al., 1978). In humans, RAR activation produces glottic closure and a brief pause in inspiration and with stronger stimulation, a laryngeal cough. Their responsiveness increases during respiratory infection (Dixon, Jackson and Richards, 1979) paralleling a heightened cough during viral respiratory infections (Empey et al., 1976).

1.2.2.3 C-fibres

C-fibres are unmyelinated and constitute the majority of airway sensory nerves (Jammes, Barthelemy and Delpierre, 1983). They terminate throughout the upper and lower airways and are activated primarily by chemical stimuli, most notably Capsaicin (Karlsson, Sant'Ambrogio and Widdicombe, 1988). Capsaicin, pre-treatment in guinea-pigs, selectively inhibits C-fibre-mediated cough to capsaicin and citric acid (CA) while leaving RAR-mediated cough (e.g., nicotine-evoked) intact, demonstrating that multiple afferent pathways can mediate cough (Bergren and Sampson, 1982; Forsberg et al., 1988).

Bronchial C-fibres: Respond to many of the same irritants that activate RARs, such as capsaicin, phenylbiguanide, ammonia, sulphur dioxide, lobeline, inflammatory mediators- bradykinin and histamine and some prostaglandins (Coleridge and Coleridge, 1984). Their primary role is to alter the respiratory pattern in response to chemicals by initiating apnoea and rapid shallow breaths.

Pulmonary C-fibres: Located in alveolar and bronchiolar walls. These respond similarly but with greater mechano-sensitivity and notably do not respond to histamine or bradykinin (Kaufman *et al.,* 1982; Coleridge & Coleridge, 1994).

Direct stimulation alters breathing, producing rapid shallow breaths or apnoea, but does not initiate cough. The involvement of non-myelinated fibres in cough is conflicting, as they mediate changes in respiratory pattern. It was suggested that the strength of the stimulus is important; this may influence CNS gating mechanisms, resulting in distinct physiological reflexes (Karlsson, 1996). Pulmonary C-fibre activation appears more inhibitory than excitatory in relation to the cough reflex.

More recently, C-fibres have become strongly implicated in cough hypersensitivity syndrome, where structural and functional neuroplasticity leads to heightened responsiveness to previously innocuous stimuli (Chung et al., 2022; Lee and Pisarri, 2001; Carr, 2004;).

1.2.3 Further Understanding of Afferent Cough Receptors

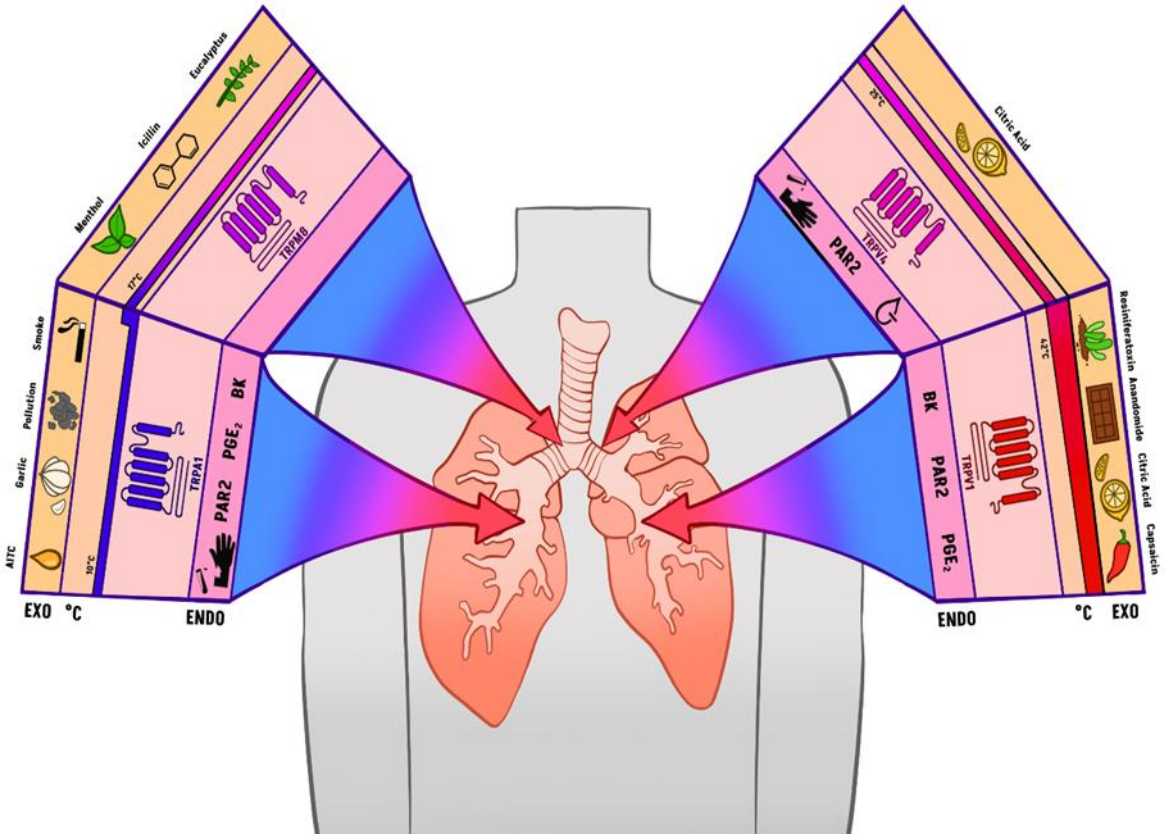
Historically, there has been significant debate regarding which vagal afferent subtypes initiate cough, with conflicting evidence supporting roles for both RARs and C-fibres. Most recently, a distinct subset of vagal afferents was identified that responds to mechanical and acid stimulation but not to capsaicin and were termed “true cough receptors”. They are non-peptidergic, but rather express glutamatergic neuron markers, and uniquely express alpha3-containing Na+/K+ ATPase isozymes at their terminals, suggesting a potential therapeutic target (Mazzone et al., 2009).

This has led to a dual afferent model proposing two distinct vagal pathways: - nodose ganglion-derived Aδ mechanosensors (true 'cough receptors') and jugular ganglion-derived C-fibres, which are more chemosensitivie (Canning et al., 2004).

C-fibres are now strongly implicated in airway irritation, urge-to-cough, and chronic cough hypersensitivity, consistent with modern evidence showing sensitisation of vagal pathways and altered central processing in chronic cough. Recent studies demonstrate enhanced brainstem and midbrain responsivity to airway sensory stimuli in chronic cough, supporting the concept of central and peripheral hypersensitivity in C-fibre pathways (Moe et al., 2024).

C fibres express multiple transient receptor potential (TRP) channels, which are chemo, mechano, and thermosensitive cation channels. Multiple subtypes have been identified and are expressed in many types of cells (Figure 2). They act as key detectors of irritants in the airway and play a major role in the initiation and amplification of the cough reflex. TRP channels function as molecular sensors; some are related to cough, including TRP vanilloid 1 (TRPV1), which is widely expressed in C fibres and activated by capsaicin acid and heat (Kollarik and Undem, 2004). TRP ankyrin 1 (TRPA1), also expressed in C-fibre afferents, responds to irritants, such as mustard oils and certain cannabinoids, also eliciting cough (Jordt et al., 2004). Recent therapeutic research continues to target TRP channels, purinergic (P2X3) receptors and other peripheral mechanisms as potential treatments for chronic cough, reflecting the evolving understanding of these afferent pathways (Guilleminault, Grassin-Delyle and Mazzone, 2024).

Figure 2. Overview of the role of TRP receptors and their agonists adapted from (Grace et al., 2014), illustrated by Alexander Gilbert (2025).



1.2.4 Human Cough Reflex Studies

Cough challenges have long been used to investigate different aspects of the cough reflex in both health and disease. The earliest reported human cough challenge was described by Bickerman (1957) who used aerosolised CA in healthy volunteers (HV). Subsequent work in patients with pulmonary disease tested a variety of inhalation challenges (CA, histamine, charcoal dust and cold air) to provoke cough and measure associated changes in airway resistance (Simonsson, Jacobs and Nadel, 1967). These studies showed increased airway resistance in many patients and first suggested that cough receptors were “sensitised” in disease.

Capsaicin-induced cough in humans was later described in 1984 (Collier and Fuller, 1984) where the effects of sodium cromoglycate on capsaicin-induced cough in HV and asthmatics were examined.

The cough challenge has since been used to identify interindividual and group differences, such as heightened cough reflex sensitivity in women compared to men

(Dicpinigaitis and Bauf, 1998) and in individuals with respiratory diseases compared to HV.

1.2.5 Bronchoconstriction and Cough

Airway protective reflexes, including cough and bronchoconstriction, are closely linked, leading to early speculation that one may initiate the other. However, evidence indicates that these are separate reflexes. Both CA and capsaicin stimulate cough and bronchoconstriction through capsaicin-sensitive neurones (Forsberg et al., 1988). Chronically capsaicin-treated guinea pigs exhibited neither cough nor bronchoconstriction in response to either CA or capsaicin but coughing induced by nicotine and mechanical stimulation was unchanged, demonstrating that reflexes can be independently triggered. A further study confirmed this separation, in CA-induced bronchoconstriction, ipratropium bromide inhibited bronchoconstriction but not the associated cough; a similar effect was seen with Cromoglycate (Forsberg et al., 1992). There is also evidence of separate pathways in humans. Inhaled codeine and morphine did not inhibit capsaicin-induced cough; however, they did reduce upper airway bronchoconstriction. When delivered systemically, they reduced cough without affecting bronchoconstriction. The rapid onset of action and lack of oral effect suggest a local action in the lung, which may be due to inhibition of the reflex bronchoconstriction induced by capsaicin (Fuller et al., 1988).

1.2.6 Airway Axon Reflexes

Airway axon reflexes result from stimulation of peripheral C-fibres, bypassing the central pathways. The cell bodies of these nerves contain three principal neuropeptides- Substance P (SP), Neurokinin A (NKA), and Calcitonin gene-related peptide (Holzer, 1988) found in several species, including man (Barnes, 1986). The transmitters can be released both centrally and peripherally. When released peripherally in the guinea-pig, they produce a local axon-reflex characterised by bronchoconstriction, vasodilation, mucus secretion, neurogenic inflammation, and mast cell activation, releasing histamine.

Capsaicin-induced bronchoconstriction occurs via a peripheral axon-reflex mechanism (Holzer P., 1991). The tachykinins released are subject to degradation by a variety of enzymes, including neutral endopeptidase (NEP). Inhibition of NEP potentiates

capsaicin-induced cough (Nadel, 1992). Lundberg and Saria (1987) were the first to demonstrate axon reflex bronchoconstriction and increased vascular permeability when C-fibres were stimulated with tachykinins in rodents. Tachykinins have since been shown to evoke cough in this species (Advenier et al., 1993).
The role of axon reflex in man is questionable, but it is thought to play a part in the pathophysiology of asthma.

1.2.7 Central Actions

Early research reported a possible central action for cough as early as 1954 by Widdicombe, (1954a) who showed that morphine, codeine, and pholcodine inhibited mechanical and chemical initiation of cough in anaesthetised cats through what was thought to be a central mechanism.
Subsequent work demonstrated that codeine and morphine given systemically suppress capsaicin-induced cough in humans (Fuller et al., 1988). Anaesthesia further supports the central involvement (Nishino, Hiraga and Yokokawa, 1990). Chronic cough is now understood to involve central hypersensitivity, with central pathways becoming dysregulated in refractory cough. This central dysregulation is very similar to sensitisation observed in chronic pain disorders and contributes to persistent coughing even after the stimulus has abated (Singh et al., 2020).
Cough can also be initiated voluntarily, although relatively little is known about the cortical pathways responsible; they likely share similarities with those pathways responsible for voluntary breath holding and other conscious modifications of respiration.

At the time my physiological studies in this thesis were undertaken (1997–1998), understanding of cough mechanisms was limited by an incomplete appreciation of how airway irritants produced bronchoconstriction and cough, and whether these responses were driven primarily by vagal afferent pathways or by direct actions on airway tissues. Although capsaicin-sensitive C-fibres and neuropeptide release were recognised as important, it was not clear how these pathways interacted with airway smooth muscle, nor whether agents such as menthol acted through sensory modulation or through direct pharmacological effects.

Chapter 2 Physiological In vivo and in vitro experimental models

There are two related papers in this chapter. The first paper examined whether menthol directly affects the bronchial smooth muscle and whether this contributes to its antitussive properties in guinea pigs and humans.

The second paper is a deeper dive into whether menthol's actions are via a specific pharmacological receptor.

2.1 Abstract 1. Capsaicin and neurokinin A-induced bronchoconstriction in the anaesthetised guinea-pig: Evidence for a direct action of menthol on isolated bronchial smooth muscle. Wright C, Laude E, Grattan T, Morice A (1997). *British Journal of Pharmacology*, 121 (8), pp.1645–1650. Available at: doi:10.1038/SJ.BJP.0701319

1 *For many years, menthol has been used in the treatment of respiratory disorders although, bronchodilator effect of menthol has yet to be described. Using the bronchoconstrictors capsaicin (acting via stimulating the release of neuropeptides from sensory afferents) and neurokinin A (NKA) we have raised airways resistance in the guinea-pig (GP) and studied the effect of menthol on both capsaicin and NKA-induced bronchoconstriction in vivo. In vitro, the effect of menthol on acetylcholine (ACh) and KCl precontracted GP bronchi was also studied.*

2 *GP (n=13) were anaesthetized (urethane 1.5 g kg71 , i.p.) and a bolus injection of capsaicin (7.5 mg ml^{-1}, i.v.) or infusion of NKA (1 mg min^{-1}, i.v.) was given either in the presence of air (0.81 min^{-1}) or air impregnated with menthol vapour (7.5 mg $^{-1}$) freely breathed from a tracheal cannula via a T-piece. Airways resistance (Raw) and ventilation were measured throughout. Bronchi of mean internal diameter (1029+73.6 mm; n=24) were removed from GP (n=16) and mounted in the Cambustion myograph. Bronchial rings were maximally precontracted with 80 mM KC1 or 2 mM ACh. Relaxation due to a cumulative dose of menthol (1 ± 3000 mM) was measured.*

3 *Menthol produced a significant (P<0.05) 51.3% reversal of the capsaicin-induced increase in Raw and also inhibited the significant (P<0.05) reduction in minute ventilation (Ve) associated with the capsaicin-induced increase in Raw. Menthol also caused a significant (P<0.05) 41% reversal of the NKA-induced increase in Raw. The NKA-induced decrease in Ve was again significantly (P<0.05) reversed with menthol inhalation. Menthol caused a significant (P<0.001) dose-dependent relaxation of KCl and ACh precontracted bronchi.*

4 *We have shown that menthol attenuates both capsaicin and NKA-induced bronchoconstriction invivo and relaxes KCl and ACh preconstricted bronchi in vitro. Menthol inhibition of NKA and capsaicin-induced bronchoconstriction could be, in part, explained by a direct action of menthol on bronchial smooth muscle.*

2.2 Abstract 2. Identification of the L-menthol binding site in guinea-pig lung membranes. Wright C, Bowen W, Grattan T, Morice A. (1998). *British journal of pharmacology*, 123 (3), pp.481–486. Available at: doi:10.1038/SJ.BJP.0701642

1 *L-Menthol inhibits both neurokinin A and capsaicin-induced bronchoconstriction in the guinea-pig and relaxes pre-constricted guinea-pig isolated bronchi. Structure-activity relationships have been defined for the action of (-)-menthol and related compounds on cold receptors, suggesting an action of L-menthol at a pharmacological receptor. We have performed radioligand binding studies to characterize the binding sites for [3H]-L-menthol in whole cell membranes prepared from guinea-pig lung tissue.*

2 *In kinetic studies, [3H]-L-menthol was found to bind rapidly and reversibly. Binding of [3H]-L-menthol to lung membranes was found to be time-dependant becoming fully associated to its site within 40 min, and half-maximum association occurred within 8 min (t1/2=8 min). [3H]-L-menthol was fully dissociated from its binding site within 8 min, (t1/2=2 min).*

3 *Inhibition studies presented a pharmacological profile of the `L-menthol site'. Capsaicin, capsazepine, D-menthol, eugenol, SCH23390 and camphor were all found to displace [3H]-L-menthol binding. In contrast WS3, noradrenaline, 5-hydroxytryptamine, spiperone, ¯unarazine, bepridil and nicardipine were without effect.*

4 *We have identified a L-menthol binding site in the guinea-pig, which may represent a site common to a variety of compounds.*

2.3 Targeting cold receptors in the treatment of cough

Menthol is known for its cooling sensation, and it was thought that it possibly exerted this effect by an action directly on “cold receptors” located on a subset of nerve fibres specific to cold, mainly C-fibres and Aδ primary afferent fibres (Hensel and Zotterman, 1951), but a pharmacological explanation for menthol's airway actions had not yet been characterised.

My early work addressed this gap and demonstrated a dose-dependent relaxation of bronchial smooth muscle (BSM) (Wright et al., 1997) and identified a specific reversible menthol -binding site in guinea pig BSM (Wright et al., 1998). These findings provided early evidence that menthol exerted direct receptor-mediated effects on airway smooth muscle, a concept not yet established at the time.

Subsequent research confirmed and characterised a menthol-sensitive receptor (McKemy, Neuhausser and Julius, 2002; Peier et al., 2002), the cold and menthol receptor (CMR1), also known as the transient receptor potential melastatin subfamily member 8 (TRPM8). My research effectively formed a bridge between early physiological observation of cold sensitivity and the later molecular identification of the cold and menthol receptor (TRPM8). My studies anticipated this receptor's existence and supported the concept of a pharmacologically meaningful menthol target within airway tissue. The menthol receptor was initially found in trigeminal sensory neurons and approximately 15% of total bronchopulmonary C-fibres (Bonvini & Belvisi, 2017; Mazzone & Undem, 2016). As well as several non-neuronal tissues, including the prostate, bladder, the male genital tract (Stein et al., 2004), and human airway epithelium (Sabnis et al., 2012). This helped clarify mechanisms through which menthol's antitussive effects might occur.

At the time of my original papers, the clinical effects of menthol on cough were incompletely characterised. Although menthol had been used in topical rubs since 1890s to treat whooping cough. Early controlled studies, such as those by Packman and London (1980), testing aromatics contained within chest rubs on the CA-induced cough in HV, provided only indirect evidence that menthol reduced cough. More recently, the antitussive activity of menthol has been demonstrated in both HV’s (Morice et al., 1994), patients with CC (Millqvist, Ternesten-Hasséus and Bende, 2013)

and in guinea pigs (Laude, Morice and Grattan, 1994) using the CA and capsaicin-induced cough models. These studies demonstrated that menthol had reproducible antitussive effects in a dose-dependent manner, supporting a pharmacological receptor mechanism- evidence that aligned with my early works (Wright et al., 1997, 1998).

Based on indirect evidence of menthol's antitussive properties, TRPM8 sensory neurons could be a good target for treating cough. TRPM8 is activated by Cold (<26°C) and other natural cooling compounds, such as eucalyptol (McKemy, Neuhausser and Julius, 2002) and camphor (Selescu et al., 2013), as well as synthetic "super cooling" agents such as icillin (Wei and Seid, 2011) and WS-12 (Sherkheli et al., 2010). Modern research into TRPM8 agonists, such as the menthol analogue AX8, builds upon these foundations. Like menthol, it reduces capsaicin-induced cough in the guinea pig (Dong et al., 2016). A randomised, placebo-controlled, crossover study investigated the efficacy and safety of AX8 40 mg sublingual tablet BD against a placebo on RCC/UCC. Results showed AX8 significantly reduced cough frequency at 2 and 4 hrs post-dose compared with placebo. This response was potentiated in those patients who reported a throat discomfort visual analogue scale (VAS) score $\geq$ 55 mm at baseline (Smith et al., 2023), suggesting a sensory nerve-mediated mechanism.
It is postulated that the effects of AX8 on cough sensitivity are via targeting the TRPM8 receptors on sensory nerves implicated in cough. However, the mechanisms of antitussive action of AX8 are unknown and could be in part due to bronchodilation (Wright et al., 1997). Sublingual AX8-003 tablet dissolves in the mouth and may exert a local analgesic or sensory modulating action within the larynx (Liu et al., 2013). Potentially explaining why patients with higher scores for "throat discomfort" had a greater response to AX8-003. This is consistent with a study by Plevkova (2013), CA-induced cough in anaesthetised guinea pigs was only suppressed by menthol when delivered as a vapour to the upper airway, not when topically applied, and not when bypassing the upper airway and delivered to lower airways. These effects were mimicked by cold air and attributed to stimulation of a subset of TRPM8 neurons within the nasal passage.
Laryngeal cooling reduces ventilation by increasing expiratory time. It is thought that specific cold receptors present within both the larynx and the nasal passage may be an

afferent pathway responsible for the reflex (Mathew *et al*., 1990). L-menthol reduces respiration when delivered in warm air to the larynx of the guinea pig (Orani et al., 1991). The depressant effect of menthol upon respiration following cold receptor stimulation is thought to prevent the initiation of cough, possibly through reducing the amount of irritant inhaled. Menthol may also be acting via a central action (Pan et al., 2012), as the respiratory and cough centres lie in proximity within the brain.

It is now apparent that menthol's antitussive effects are not solely dependent upon TRPM8, indicating that a mechanistic gap exists. Studies by Maher (2014), demonstrated vagal ganglia of mouse and guinea pig expressing TRPM8 were stimulated by both menthol and TRPM8 agonist WS3. Preincubation of the ganglia with menthol inhibited nerve activation to capsaicin, but this wasn't blocked by the TRPM8 antagonist JNJ41876666. Both WS3 and menthol were shown to relax human, guinea pig, and mouse trachea; again, this wasn't blocked by the TRPM8 antagonist. This suggests that menthol and WS3, can relax airway smooth muscle and inhibit vagal sensory nerve activity independently of TRPM8. This reinforces my early hypothesis that menthol may also modulate intracellular calcium efflux, as supported by evidence from ileal smooth muscle (Amato, Liotta and Mulè, 2014), and neuronal preparations (Hawthorn et al., 1988).

In summary, although the molecular identification of TRPM8 represented a major advance, my early work helped establish the pharmacological groundwork that made this discovery meaningful in the airway context. At the time, there was no identified menthol receptor, and no clear mechanism linking menthol, cold sensitivity, and airway reflex control. By demonstrating receptor-like binding and functional airway responses to menthol, my research provided an important conceptual bridge that has since supported the development of TRPM8-targeted therapeutics and guided new approaches to treating cough and airway disease.

Chapter 3 : Experimentally induced cough in humans

This chapter examines the usefulness of CA cough challenge along with other cough provocation methods and considers how these approaches are used today and what potential they hold for the future.

The chapter contains one paper which evaluates the European Respiratory Society (ERS) standard CA cough challenge and emphasises the importance of methodological standardisation across scientific groups to allow true comparisons of data.

3.1 *Abstract 3.* Validation of the ERS standard citric acid cough challenge in healthy adult volunteers. Wright C, Jackson J, Thompson R, Morice A. (2010). *Cough (London, England)*, 6 (1). Available at: doi:10.1186/1745-9974-6-8

Protocols for measuring cough sensitivity that can vary in terms of nebuliser, tussive agent, single and dose response. A definitive method for measuring cough sensitivity needs to be established. The ERS guidelines recommend the KoKo DigiDoser (KD) delivery system. Study aim was to compare the reproducibility of this citric acid (CA) cough challenge and previously established Mefar dosimeter (MD) protocol. 39 (female 26) volunteers mean age (40.4 yrs) were randomised to either KD or MD. Intra-day and inter-day reproducibility was compared. We calculated the concentration of citric acid evoking 2 coughs (C2). The geometric mean C2 (95%CI) was similar for both KD and MD, of 263 (200,339) mM and 209 (151,288) mM, respectively. The mean KD C2 was not significantly different. ($F = 0.807$, $p = 0.93$) from baseline over 1, 2, and 4 hrs however, the MD demonstrated significant variability ($F = 7.85$, $P < 0.001$) Measuring mean log C2 at baseline and at 2 weeks, the KD demonstrated a stronger intraclass correlation of log C2 at baseline with 2 week log C2, ICC = 0.70 than was shown with the Mefar, ICC = 0.41Administering CA from KD offers a reproducible cough challenge in healthy volunteers. The results correlate well with the MD challenge but offer greater intra-day and inter-day reproducibility.

3.2 Cough provocation testing in humans

3.2.1 Historical and Physiological Foundations

CA is one of the most widely used tussive agents in human cough challenge testing, first introduced over 60 years ago (Bickerman et al., 1957). Historically, the CA challenge was developed to quantify cough reflex sensitivity and to assess emerging antitussive therapies. CA induces cough primarily via C-fibre activation (Canning et al., 2004; Nurmi et al., 2019) and is also believed to stimulate RARs in the larynx and upper airway (Morice, Kastelik, & Thompson, 2001).

Over the years CA challenge has helped demonstrate the efficacy of several antitussive treatments, including opiates (Morice et al., 2007b) diphenhydramine (Packman et al., 1991) and dextromethorphan (Grattan et al., 1995). Although the methodologies used across studies varied considerably, with inconsistencies in delivery devices, inhalation Protocols, dosing schedules, and endpoints limiting comparability across studies.

3.2.2 Translating Animal Physiology to Human Challenge Methods

Much of the early understanding of cough reflex physiology came from animal models. Animal models, particularly guinea pigs, have been fundamental in developing and validating physiological cough challenge techniques in humans (Belvisi and Bolser, 2002). These models demonstrated:

1. *Efficacy of tussive agents*: Citric acid and capsaicin reliably induce cough in animals and are the standard tools for human testing.
2. *Dose-dependent reflex responses*: The cough reflex follows a quantifiable dose-response relationship, with definable thresholds.
3. *Sigmoidal dose-response behaviour:* Increasing stimulant concentration produces a characteristic sigmoid curve, with threshold, ascending slope and maximal response.

These findings directly shaped expectations for human challenge testing, by establishing the need to measure both threshold sensitivity and the slope of the dose–response relationship. They also highlighted that any human method must be sensitive enough to detect small physiological shifts in this curve, as seen in disease and following pharmacological intervention.

The main concern with animal modelling is that no model of spontaneous cough has been developed; thus, antitussives are being tested on the protective cough reflex and not the augmented cough as seen in disease (Plevkova et al., 2021).

When my work began in 2010, there was no standardised, reproducible human methodology for conducting a CA cough challenge. Centres utilised different nebulisers, inhalation patterns, dosing strategies, and endpoints. This variability made it difficult to compare data across studies or reliably determine whether an intervention genuinely affected cough reflex sensitivity.
Our own experience highlighted these limitations during a randomised trial of dextromethorphan versus codeine (Morjaria Jaymin et al., 2013), which used a single concentration of CA (10%) delivered over 5 inhalations. Strict screening criteria (7-20 coughs, reproducible within ±20%) resulted in 96 volunteers screened to recruit 50 participants; we found the existing CA challenge methodology to be labour-intensive, biased, and highly variable (unpublished data). This methodology generated substantial variability in the cough response even in the absence of any drug or placebo intervention, which may obscure potential therapeutic effects. These limitations directly informed and motivated our efforts to refine and optimise CA cough-challenge methodology.

3.2.3 Developing a More Standardised Physiological Method

The European Respiratory Society (ERS) task force highlighted the need for a robust, standardised method for measuring cough sensitivity to be established to allow comparison of results from different groups (Morice et al., 2007a).
My work introduced several key modifications aligned with the established capsaicin challenge methodology (Dicpinigaitis, 2003).

- *Single inhalations of doubling concentrations* of CA replaced the single-dose, multi-breath method.
- *C2 and C5 thresholds* (lowest concentrations evoking ≥2 and ≥5 coughs) were adopted as standardised, physiologically meaningful endpoints.
- *Delivery system upgrade* from the Mefar Dosimeter to the KoKo DigiDoser, incorporating an airflow limiter to ensure consistent inhalation.

My study (Wright et al., 2010) was the first to systematically compare reproducibility between CA cough challenge methodologies. Incorporating the above modifications to the challenge identified that CA delivered via the KoKo Digidoser gave overall improved intra-day and inter-day reproducibility.

3.2.3.1 Refining Concentration Ranges

One outcome of my research was identifying that the top concentration of CA 1M was insufficient to elicit C5 in 25% of HV tested, and therefore only C2 was analysed. Our protocol has now been updated to include concentrations of CA up to 4M, enabling more complete profiling.

3.2.4 Limitations of C2 and C5

Both C2 and C5 measure cough reflex sensitivity and not cough frequency, so although reproducible and useful in research for target engagement, they have poor diagnostic utility in clinical practice. Some limitations include:

1. *Ceiling effects*: Many HV's fail to reach a C5, even at the highest concentrations of tussive agent.
2. *Weak correlation with daily cough frequency:* C5 correlates only moderately with 24-hour objective cough counts (Decalmer et al., 2007).
3. *Poor diagnostic discrimination*: Overlap in C5 values between healthy individuals and patients with chronic cough reduces diagnostic utility (Birring et al., 2006) .

From a physiological perspective, thresholds alone cannot fully describe the behaviour of the cough reflex because they capture only a single point on what animal studies have shown to be a complex sigmoid curve. A more complete assessment would quantify the threshold to start coughing, the response intensity and the maximal responsiveness. This could be achieved by measuring the area under the curve (AUC) for the full response profile. This negates the artificial ceiling of response and improves the detection of subtle pharmacological effects.

Hilton (2013) measured maximum cough response to capsaicin (Emax) and the dose inducing half maximal response (ED50), as a novel way of quantifying cough reflex

sensitivity. Although time-consuming and potentially subject to tachyphylaxis (Morice A.H, Higgins KS and Yeo WW, 1992), Emax demonstrated better discriminatory ability between health and disease.

Despite its limitations, cough challenge testing is still a current utility in understanding and assessing cough reflex sensitivity (Ebihara et al., 2020; Rai et al., 2018; Qiu et al., 2011), and evaluating the efficacy of novel pharmacological therapies, including P2X3 antagonists such as gefapixant (Morice et al., 2019). Recently, new cough challenges have emerged, including ATP and AMP (Fowles et al., 2017). The ATP challenge was invaluable in demonstrating target engagement of P2x3 antagonist Gefapixant (Morice et al., 2019).

3.2.5 Factors Influencing Cough Challenge Outcomes

A range of physiological and psychological factors can alter cough challenge performance:

- *Voluntary cough suppression*: Healthy volunteers can suppress their cough during challenge testing, which can significantly influence C2, whereas patients with RCC cannot, suggesting altered central processing in these patients (Cho et al., 2019).
- *Participant perception:* In the study by Janssens (2015) Volunteers were told that inhaling CA could be harmful, and on a separate occasion, they were informed that they were inhaling a natural substance. Knowing the inhalation was harmful significantly increased the perceived urge to cough (UTC).
- *The testing environment:* Patients attending a hospital environment, knowing their cough sensitivity was being tested due to asthma, had a significantly greater tendency to cough, and UTC, compared to being in a neutral environment where asthma wasn't mentioned (Rietveld, Van Beest and Everaerd, 2000).

3.2.6 Summary and Future Directions

The human cough challenge has value in both understanding cough airway reflex physiology and the pharmacodynamic effects of antitussives. They allow assessment of whether a new therapy modulates cough reflex sensitivity and offers indications of the mechanism. However, cough challenge response doesn't always correlate with subjective observations, such as in our study of morphine on RCC/UCC. Morphine significantly improved the mean Leicester cough questionnaire (LCQ) score and daily cough severity scores compared to placebo, but did not affect cough reflex sensitivity to CA (Morice et al., 2007b).

There is still a lack of standardisation of cough challenges between centres, and poor reporting in papers of the precise methodology used (Wallace et al., 2019). Caution still needs to be taken when comparing cough challenge data.

3.2.6.1 Potential Improvements to the Human Cough Challenge Methodology

Although human cough challenges remain central to understanding cough reflex physiology and assessing target engagement, several methodological alterations could significantly improve reproducibility, interpretability, and translational value.

1. *Full Dose Response Modelling:* Adopt AUC: Characterising the entire dose response curve would enhance physiological precision, provide a more comprehensive understanding of both sensitivity and response magnitude. These measures eliminate ceiling effects, incorporate gain and maximal responsiveness, and may be more sensitive to subtle pharmacological shifts.
2. *Standardisation of Delivery Systems and Reporting*: Establishing **i**nternationally standardised delivery systems, alongside detailed reporting checklists like spirometry guidelines, would markedly improve cross-study comparability, including but not limited to nebuliser type, output, and particle size; stimulus volume and inhalation control; exact concentration range and doubling protocol; environmental conditions and instructions.

3. *Control of Environmental and Cognitive Influences:* Standardise testing environment, measure UTC as well as cough frequency, assess cough suppression capability and consider cognitive modulation.
4. *Enhanced Physiological Profiling:* Incorporate AUC rather than threshold-only endpoints, Phenotype patients (e.g., hypersensitive vs hyposensitive reflex phenotypes). Complement peripheral challenges with central mechanistic assessment, i.e., fMRI
5. *Methodological Innovations*: Introduce learning or familiarisation challenges to reduce novelty effects, combine peripheral and central acting therapeutics to probe reflex pathways and explore newer tussive agents (ATP, AMP) for targeted assessment of specific pathways.

Taken together, tussive challenges remain a powerful physiological tool capable of quantifying reflex sensitivity, demonstrating target engagement, and translating key principles established in animal models into measurable human responses. Although they are not diagnostic and correlate poorly with daily cough frequency and patient-reported outcomes, their contribution to the field has been substantial. Tussive challenges have enabled mechanistic understanding of the human cough reflex, provided a reproducible platform for evaluating antitussive therapies, and played a central role in validating major pharmacological targets such as P2X3. They continue to be an essential component of early-phase drug development and serve as a bridge between animal physiology and clinical therapeutics.

Chapter 4 Translational Research-Randomised Clinical Trials of Central and Peripheral Cough Suppressants.

This final research chapter looks at both central and peripheral neuromodulators studied in the treatment of RCC and their current application. There are two related papers, one determining whether low-dose opiates, specifically slow-release morphine (MST) are effective in the management of chronic refractory cough (CRC) and unexplained chronic cough (UCC). The second paper investigates whether the beneficial effects of morphine on CC patients could be attributed to a non-specific sedative effect rather than direct action on the cough reflex pathway.

The third paper examines peripheral neuromodulators, specifically P2X3 receptor antagonists and considers current and potential clinical applications. This paper investigated the effects of Gefapixant (100 mg) on cough responses induced by a range of standardised cough challenge agents**,** including adenosine triphosphate (ATP)**,** citric acid (CA)**,** capsaicin, and distilled water**,** in both healthy volunteers (HV) and patients with chronic cough (CC)**.** This study represented the first evaluation of the antitussive properties of Gefapixant within controlled cough challenge models.

4.1 Abstract 4 Opiate therapy in chronic cough.

Rationale: *Cough is the most common complaint for which medical attention is sought, and chronic cough can be both physically and mentally debilitating. There is currently no evidence supporting the use of antitussives in chronic treatment-resistant cough.*

Objectives: *We tested the hypothesis that morphine sulfate in the dose of 5 mg twice daily would bring about a reduction in cough frequency and severity in patients failing to respond to specific measures. Methods: Patients recruited from the Hull Cough Clinic were enrolled into a randomized double-blind placebo-controlled study using 4 weeks of slow-release morphine sulfate and a corresponding period of matched placebo. An open-labeled extension of the core study allowed dose escalation to 10 mg twice daily. Cough was assessed using the Leicester Cough Questionnaire, daily symptom diary, and citric acid cough challenge.*

Results: *Twenty-seven patients completed the core study. A significant improvement of 3.2 points over baseline was noted on the Leicester Cough Questionnaire ($p < 0.01$). A rapid and highly significant reduction by 40% in daily cough scores was noted among patients on slow-release morphine sulfate ($p < 0.01$). Objective testing of the cough reflex using citric acid cough challenge tests did not show any significant changes. Eighteen patients continued into the extension study. Two-thirds of these patients opted to increase the morphine to 10 mg twice daily. At the end of 3 months, there was a similar improvement in cough between the 5- and 10-mg groups.*

Conclusion: *Morphine sulfate is an effective antitussive in intractable chronic cough at the doses of 5 to 10 mg twice daily*

4.2 Abstract 5 Is opiate action in cough due to sedation

Methods: *We performed a post hoc analysis of two published trials with three opioids. In study one, patients with chronic cough were treated with 4 weeks of modified release morphine sulphate (5 mg twice daily) or placebo in a double-blinded placebo-controlled fashion. Cough suppression was assessed subjectively by the Leicester Cough Questionnaire and objectively by citric acid aerosol (CAA) induced cough challenge. In study 2, normal volunteers were given single doses of placebo, codeine 30 mg or dextromethorphan 50 mg and cough suppression assessed using the CAA-induced cough challenge. Sedation was contemporaneously assessed by direct questioning.*

Results: *There were 14 episodes of patient-reported sedation; 2 with modified release morphine sulphate, 9 with codeine and 3 with dextromethorphan. There was no correlation between change in the Leicester Cough Questionnaire or the CAA-induced cough challenge and reported sedation.*

Conclusion: *This observational study suggests that sedation is unlikely to underlie the antitussive properties of these opioids. Eliciting the mechanism of these medications in cough may be a target for future tailored drug development.*

4.3 Abstract 6 The effect of Gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study

We evaluated the effect of gefapixant on cough reflex sensitivity to evoked tussive challenge. In this phase 2, double-blind, two-period study, patients with chronic cough (CC) and healthy volunteers (HV) were randomised to single-dose gefapixant 100 mg or placebo in a crossover fashion. Sequential inhalational challenges with ATP, citric acid, capsaicin and distilled water were performed 1, 3 and 5 h after dosing. Mean concentrations evoking ≥2 coughs (C2) and ≥5 coughs (C5) post-dose versus baseline were co-primary endpoints. Objective cough frequency (coughs·h^{-1}) over 24 h and a cough severity visual analogue scale (VAS) were assessed in CC patients. Adverse events were monitored.

24 CC patients and 12 HV were randomised (mean age 61 and 38 years, respectively). The cough challenge threshold increased for ATP by 4.7-fold (C2, p≤0.001) and 3.7-fold (C5, p=0.007) for gefapixant versus placebo in CC patients; in HV, C2 and C5 increased 2.4-fold (C2, p=0.113; C5, p=0.003). The distilled water C2 and C5 thresholds increased significantly (p<0.001) by a factor of 1.4 and 1.3, respectively, in CC patients. Gefapixant had no effect on capsaicin or citric acid challenge. Median cough frequency was reduced by 42% and the least squares mean cough severity VAS was 18.0 mm lower for gefapixant versus placebo in CC patients. Dysgeusia was the most frequent adverse event (75% of HV and 67% of CC patients).

ATP-evoked cough was significantly inhibited by gefapixant 100 mg, demonstrating peripheral target engagement. Cough count and severity were reduced in CC patients. Distilled water may also evoke cough through a purinergic pathway.

4.4 Opioids: Central neuromodulators?

Morphine was discovered in 1803 by Friedrich Serturner, who named it after the Greek god of dreams, Morpheus. Morphine was first sold in 1827 to treat pain. In 1898, Merck marketed the ethyl ether of morphine called "dionin", which was the first semi-synthetic morphine derivative to serve as a "cough sedative". Morphine is thought to depress the cough reflex through direct action on the cough centre in the medulla. It has an affinity for delta (δ) kappa k and mu (μ) opioid receptors (MOR) found on neuronal cell membranes in neurons responsible for pain. Morphine is a full agonist of MOR, which is found in both central and peripheral nervous systems (Rachinger-Adam, Conzen and Azad, 2011) accounting for its strong analgesic effects.

The first paper reporting the central action of drugs on the cough reflex was studied in anaesthetised cats (Widdicombe, 1954a). Morphine inhibited both chemically and mechanically induced cough, with a more potent effect on chemically evoked responses. A direct action of morphine on cough receptors could not be ruled out, but since it blocked two distinct afferent nervous pathways, this suggested a central mechanism. Further supporting a central action, Fuller (1988) studied inhaled and systemic opioids on capsaicin-induced cough and respiratory resistance in HV. Inhaled codeine and morphine did not affect cough sensitivity to capsaicin, but oral codeine and morphine IV significantly reduced cough.

4.4.1 Morphine and cough

At the time of our research, there was very little literature supporting a role for opiates in the relief of clinical cough. Most studies found no difference in opiates compared to a placebo (Freestone and Eccles, 1997; Eccles, Morris and Jawad, 1992; Taylor et al., 1993).

Published data from our cough clinic using a probability-based ERS-aligned diagnostic algorithm (Morice et al., 2004) demonstrated that we were able to diagnose 93% of our patients, but 7% -10% remained undiagnosed or refractory to treatment, despite therapeutic trials of anti-tussive therapy (Kastelik et al., 2005). Before conducting our randomised placebo-controlled trial, we prescribed MST 5mg to 10 mg BD to RCC/UCC in the clinic. Of the 1189 Patients seen in the clinic, 94 (8%) (69 (73%) female) had treatment with MST. Patients were individually assessed and received 5mg MST BD, which was increased to 10 mg BD if symptoms were uncontrolled at 5mg. 50% of the

patients treated reported a significant improvement in their cough. Encouraged by these observations, we performed the first randomised placebo-controlled trial of MST treatment in UCC and treatment-resistant chronic cough.

MST significantly improved LCQ total scores and significantly reduced daily diary cough severity. Although we saw a dose-dependent trend towards inhibition of CA cough sensitivity, this did not quite reach significance. This is consistent with trials of codeine on both UTC and cough sensitivity to capsaicin (Davenport et al., 2007) and further corroborated with a study of MST in refractory CC in patients known to be responders to MST. MST was found to significantly reduce tickle and irritation associated with CA-induced cough challenge but did not affect either UTC or cough sensitivity (Mitchell et al., 2017).

We demonstrated that MST is an effective antitussive in RCC at doses of 5 to 10 mg twice daily. The Morphine was well tolerated, with the most common side effects reported of constipation (40%) and drowsiness (25%).

Although MST is believed to be centrally acting, a purely central focus likely oversimplifies the complex network of cough regulation, at present the precise mechanism of action and effect on cough remains incompletely understood. Opioid receptors have not only been identified in the brainstem but also on the sensory afferent arm of the vagus nerve, including bronchopulmonary C-fibres. Activation of these receptors inhibits neurotransmitter release at the synapse through Ca channel inhibition (Heinke, Gingl and Sandkühler, 2011). The same mechanism of action for menthol's antitussive properties as postulated in my early works (Wright et al., 1997, 1998). This provides a plausible mechanism for modulation of peripheral afferent signalling before impulses even reach central cough networks. The effects of morphine can still be interpreted within the framework of cough physiology established in earlier chapters. The animal literature shows that the cough reflex operates as a dose-dependent, modifiable neural circuit. MST appears to shift the excitability of this reflex arc. The improvements in quality of life and perceived cough severity strongly suggest that morphine is altering the functional behaviour of the cough reflex rather than merely sedating patients. This aligns with the physiological principle that both peripheral and central modulation can alter the reflex threshold and responsiveness.

Sedation was specifically sought in this study and was present only transiently, whereas antitussive efficacy continued throughout the trial, so unlikely that cough suppression was due to sedation. Investigating the potential of sedation influencing the effect of morphine on CC further. My colleagues and I did a post hoc analysis across our trials, testing the combined therapy of dextromethorphan and codeine against dextromethorphan and codeine monotherapy on CA-induced cough sensitivity (Morjaria Jaymin et al., 2013). Post hoc analysis showed that those patients demonstrating significant sedation did not have the greatest efficacy in terms of cough suppression (Dickinson et al., 2014). Further evidence supporting sedation doesn't influence cough sensitivity comes from the work with anaesthetics. The effects of anaesthetics on the cough reflex during the recovery period from surgery were assessed using Observer's Assessment of Awareness/Sedation Scale. Although slight sedation was recorded during the recovery period, cough sensitivity to CA did not change (Guglielminotti et al., 2005).

My work was the first controlled evidence demonstrating that low-dose morphine produced clinically meaningful improvements in refractory cough (Morice et al., 2007b). This helped establish opioids as a legitimate therapeutic option and directly informed later investigations, including testing MST in patients with RCC who had responded favourably to MST in a clinic setting. This study measured objective cough frequency for the first time. MST was found to reduce objective cough count by 71.8% over 24 hrs, as well as subjective cough severity, and there was a significant improvement in cough quality of life questionnaire (Mohammed et al., 2017). Real-world effectiveness and tolerability of low-dose opioids were also successful in 60% of patients treated in a clinic setting, including patients with RCC associated with airway disease, gastroesophageal disease, and nasal disease, as well as unexplained CC (King et al., 2022). Establishing that in patients with RCC or UCC, using MST, you can improve quality of life and cough severity through targeting the symptom rather than the cause (Morice et al., 2007b) led to Morphine being tested for the first time in cough associated with Idiopathic pulmonary fibrosis (IPF), the "PACIFY COUGH" study. This was a randomised, placebo-controlled, crossover study measuring the change in cough counts/hr from baseline following 14 days of treatment. Morphine reduced the

mean awake cough frequency by 39.4% compared to placebo (Wu et al., 2024), extending the application of opioids beyond RCC and into disease-associated cough. Thus, the translational pathway from physiology to MST clinic data, then to a randomised trial and then application in IPF represents a clear trajectory emerging from this thesis work.

4.4.2 Future alternatives to morphine

A possibly safer alternative to morphine, nalbuphine, is currently being tested for its antitussive properties. An opioid analgesic, acting as an antagonist on mu receptors and an agonist at Kappa opioid receptors, nalbuphine has mainly been used in the past for pain management in pre-hospital settings such as the ambulance services. The fact that nalbuphine is not an agonist of mu receptors may give it an improved safety profile. The first study reporting the potential antitussive properties of nalbuphine was that by Wang (2020) where pretreatment with nalbuphine was found to abolish the cough induced by sufentanil during anaesthesia.

Trevi Therapeutics has now developed an oral extended-release (ER) form of nalbuphine, investigating the antitussive potential and adverse event profile in cough associated with IPF. This was a randomised, placebo-controlled crossover study with an escalating dose of nalbuphine (27 mg OD to 162 mg BD) over 21 days, to help define the optimal dose for future studies. Nalbuphine significantly reduced objective cough frequency in a dose-dependent manner in patients with IPF, with a maximum 53.4% change compared with placebo, and the 2 higher doses also significantly improved patient-reported outcomes of cough frequency and severity. 31% of patients discontinued treatment prematurely as unable to tolerate nalbuphine side effects (Maher et al., 2023).

The most recent study with nalbuphine reported this year was in refractory CC patients. Top line results are that nalbuphine reduced 24 hr cough counts by 55% compared with placebo, and there were improvements in patient-reported outcomes by day 7 of treatment -press release 2024.

Not unlike morphine, nalbuphine not only acts on central opioid receptors but also acts on kappa opioid receptors located on peripheral nerve endings of A delta and C -fibres. Thus, having a dual action on cough with the potential to control cough hypersensitivity, whether caused through peripheral or central mechanisms (Birring et

al., 2025). A peripheral and centrally acting antitussive is promising for achieving clinically meaningful efficacy, especially if the potential for abuse can be limited.

Opioid treatment for cough is still profound, according to a study by Weiner (2024) of 23,210 CC patients, around a fifth received an opioid containing cough suppressant (OCCS) prescription, and the odds of an opioid containing prescription were twice as likely in CC as compared to non-CC. This suggests that opioids have an important role in the treatment of CC, but due to the side effects and the possible addiction profile associated with opioids, caution needs to be taken with prescribing.
Further research needs to be undertaken in moulding effective but safer treatments for CC. Perhaps new opioid derivatives with improved properties are needed, with dual action both centrally and peripherally, which have low sedation and a low potential for drug abuse.

4.5 Peripheral Neuromodulator

Due to the potential side effects of opioids licensed for cough, and possible addiction (Antoniou and Juurlink, 2014), new strategies to treat RCC have concentrated on drugs working peripherally, such as corticosteroids, antibiotics (Krishnan et al., 2019), and P2X3 antagonists. P2X3 and P2X2/3 receptors are ATP-gated ion channels expressed by C and Aδ afferents, including those that innervate the upper and lower airways. ATP is released in viral infections (Ledderose et al., 2023), in response to irritant gases (Chan et al., 2024), and during inflammation (Okada et al., 2013). Studies have shown the expression of P2X3 in central and peripheral neurons (Chen et al., 1995; Llewellyn-Smith and Burnstock, 1998; Dunn, Zhong and Burnstock, 2001). ATP is thought to bind to P2X3 receptors and stimulate C-fibres, contributing to neural excitability and hypersensitisation of nerves. This is seen in CC, where nerves become more sensitive to environmental irritants and can be more responsive to pain (North, 2003). ATP also interacts with TRPV1, leading to increased pain sensitivity. In models of lung inflammation, sensory nerves have increased expression of ion channels and receptors, which modifies sensitivity to chemical stimuli (Mazzone and Undem, 2016). Expression of TRPV1 has also been found to be increased in CC, compared to HV, which may account for the heightened cough sensitivity (Groneberg et al., 2004).

The first exploration for a P2X3 antagonist to test the potential anti-tussive effect in refractory CC (RCC) was a proof-of-concept trial. Assessing the efficacy and safety of AF-219 (also known as Gefapixant and MK-7264), a first-in-class P2X3 receptor antagonist, in subjects with RCC (Abdulqawi et al., 2015). Patients were dosed with 600 mg twice a day for 2 weeks. AF-219 had a 75% reduction in daytime cough frequency compared to placebo; the number of coughs/hr was reduced by a mean of 26 and 21, respectively. All patients in the AF219 arm of the study reported taste disturbance; six withdrew because of this adverse event.

We studied the effects of Gefapixant on evoked tussive responses to ATP, CA, capsaicin, and distilled water compared to placebo in HV and patients with RCC. All tussive challenges evoked a cough in both CC patients and HV; there was a distinct difference in cough hypersensitivity between the two groups, with CC patients having heightened sensitivity in all challenges. Gefapixant significantly reduced cough in both patient groups in response to ATP (Figure 3), indicating successful peripheral target engagement by the compound. A significant reduction in cough was also seen with the distilled water challenge (Figure 4), which could be because hypo-osmolar solutions activate TRPV4 and TRPM3, which are thought to release ATP, which in turn activates P2X3 (Bonvini et al., 2017). No effect was seen on either CA or capsaicin-induced cough. In the CC patients, a single dose of 100 mg Gefapixant also significantly reduced observed cough frequency by 42%. Still, its efficacy wasn't without its side effects, with 75% of HV and 66.7% of CC patients experiencing dysgeusia.

Further research into Gefapixant antitussive properties and confirming its therapeutic properties, "Cough-1" study, demonstrated a 62% reduction with 45 mg Gefapixant BID at 12 weeks of treatment compared to a 53% reduction with placebo. In the placebo arm, cough frequency was reduced to 9.1 coughs/hr as opposed to 7.18 coughs/ hr in the treatment arm. In "Cough-2", there was a 63% reduction in 24 hr cough for 45 mg Gefapixant BID at 24 weeks of treatment as opposed to 57% to placebo (McGarvey et al., 2022b).

Figure 3. Concentration of ATP inducing 2 coughs after administration of MK-7264 or placebo in HV and CC patients

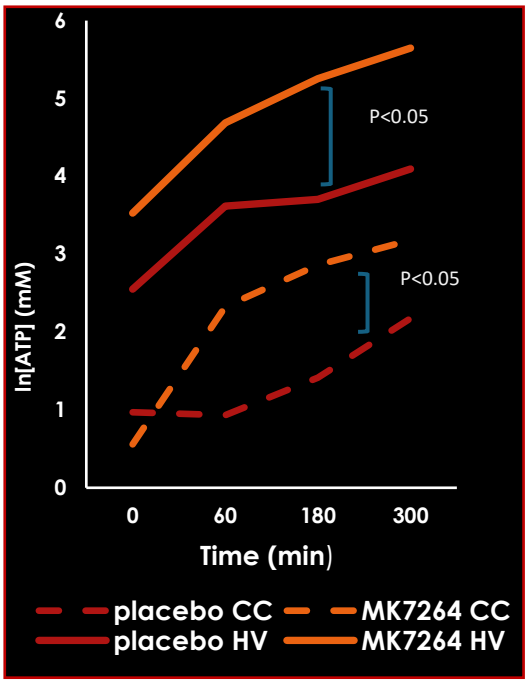
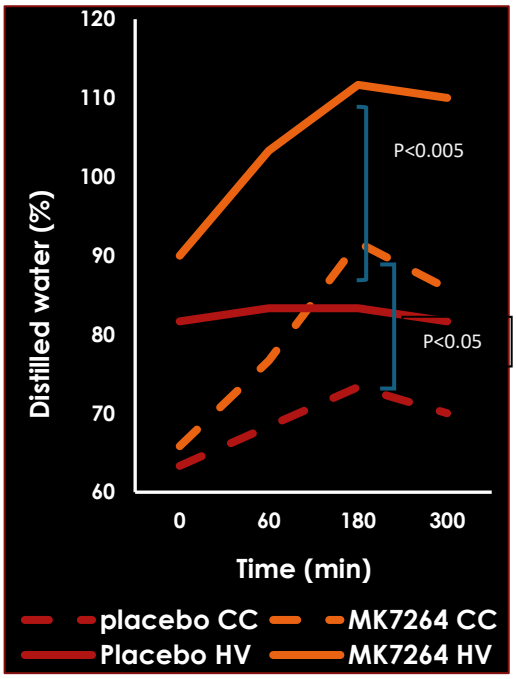


Figure 4 % distilled water inducing 2 coughs after administering 100 mg MK-7264 or placebo in HV and CC patients



Gefapixant is the first drug to be licensed for RCC in more than 60 years. Although gaining a licence, it suffers from significant drug-related adverse effects, including taste disturbance. This has brought about further research into more specific antagonists, such as BAY-1817080, which, in ascending doses (10-800mg) in healthy males, had minimal effect on taste disturbance (Klein et al., 2022). This compound significantly reduced cough in RCC patients at doses ≥ 50 mg, and only 21% of patients reported taste disturbance, but it had weaker clinical efficacy than Gefapixant. The same is also the case for other P2X3 antagonists, such as BLU-5739 (Smith et al., 2022), and Sivopixant (Niimi et al., 2022). Therefore, there is still a need to develop small-molecule drugs targeting P2X3 based on new allosteric mechanisms for the treatment of RCC.

Chapter 5 Discussion

This body of work provides a translational framework for understanding and quantifying the cough reflex, integrating airway pharmacology, sensory neurobiology, human experimental methodology, and early-phase therapeutic development. Across six publications, the research demonstrates a clear progression from mechanistic insight to clinically meaningful application, illustrating how targeted investigation at multiple levels of the reflex pathway can advance our understanding of cough pathophysiology.

Early studies using capsaicin, neurokinin A, and menthol focused on the peripheral airways as a key site for antitussive intervention. Demonstrating that menthol acts directly on bronchial smooth muscle and identifying its specific binding site within lung tissue provided some of the first evidence for receptor-level modulation of airway sensory processing. These publications laid essential groundwork for defining the peripheral contribution to cough hypersensitivity. Establishing that alterations in afferent nerve signalling can significantly influence cough reflex sensitivity.

Subsequent investigations expanded this framework to include central mechanisms. The finding that low-dose morphine suppresses cough independently of sedation was particularly significant, providing evidence that central neuromodulatory pathways can be selectively targeted without compromising alertness. Together, these studies pre-dated and supported the now-established concept of cough hypersensitivity as a disorder of altered central and peripheral pathways. This work established central pathways as a viable target for therapeutic intervention.

A methodological contribution of equal importance was the validation of the ERS citric acid cough challenge. By establishing reproducible protocols and normative sensitivity data, this work contributed to internationally standardising human cough challenge methodology. This contribution continues to influence the field as reliable and comparable challenge protocols are essential for both mechanistic studies and early-phase drug development.

The translational impact of this programme was further demonstrated in the evaluation of a P2X3 antagonist, which provided human evidence that reducing peripheral afferent hypersensitivity leads to meaningful reductions in cough reflex sensitivity. This study bridged receptor-level biology with therapeutic efficacy, and

contributed to the establishment of P2X3 antagonists as a new class of antitussive agents.

These papers show the complexity of chronic cough, and understanding its pathophysiology requires the study of peripheral receptor pharmacology, central neural modulation, and carefully standardised human testing. The findings suggest avenues for future refinement of tussive challenge methodology. Incorporating both peripheral and central challenge modalities, stratifying participants by sensitivity phenotypes, and integrating receptor-specific measures would all increase the power of human models. Moreover, mathematical modelling of full dose–response curves allows quantifying cough sensitivity with greater physiological precision. Modelling could capture changes in threshold, gain, and maximal response—parameters that map directly onto peripheral versus central mechanisms and could serve as useful biomarkers in therapeutic development.

5.1 RCC, UCC, and the Challenge of Treatment

RCC and UCC represent a significant proportion of referrals to specialist cough clinics (Dávila et al., 2023; Haque, Usmani and Barnes, 2005). RCC reflects pathological hypersensitivity within cough neuronal pathways, CHS (Chung et al., 2022; Morice et al., 2014). This condition shares key characteristics with hyperalgesia and allodynia. Individuals who report sensitivity to environmental irritants exhibit heightened responses to capsaicin cough challenge compared with asymptomatic individuals (Ternesten-Hasséus, Bende and Millqvist, 2002).

OTC remedies are ineffective for RCC/UCC, and no approved treatments exist. Current guidelines therefore recommend off-label use of centrally acting agents, such as pregabalin, gabapentin, and low-dose Morphine (Morice et al., 2020) these therapies are limited by adverse effects and the risk of dependency.

5.1.1 Placebo Response in Cough Studies

The first randomised study I managed in CC evaluated antitussive properties of Levodropropizine on CA-induced cough in HV, measuring total cough counts across 5 challenges over 12 hours. A subsequent investigation of DF1012, a novel antitussive, employed the same methodology. Neither agent reduced cough sensitivity to CA.

However, sub-analysis of DF1012 revealed a gender-specific placebo response ($p<0.0005$), unpublished data. A smaller but similar response was reported with dextromethorphan in HV (Rostami-Hodjegan et al., 2001). Stratification into placebo responders (≥5 coughs reduction from baseline) and non-responders (≤5 coughs) showed that antitussive effects were detectable only in placebo non- responders (Figure 5), with no measurable benefit above placebo in the responders (Figure 6), unpublished data. Approximately 50% of HV appeared to be placebo responders; a pattern mirrored in RCC clinical trials.

Figure 5 Placebo responders- % change in cough frequency to CA following placebo, Dextromethorphan 60 mg, codeine 60 mg, and combined Dextromethorphan 60 mg and codeine 60 mg.

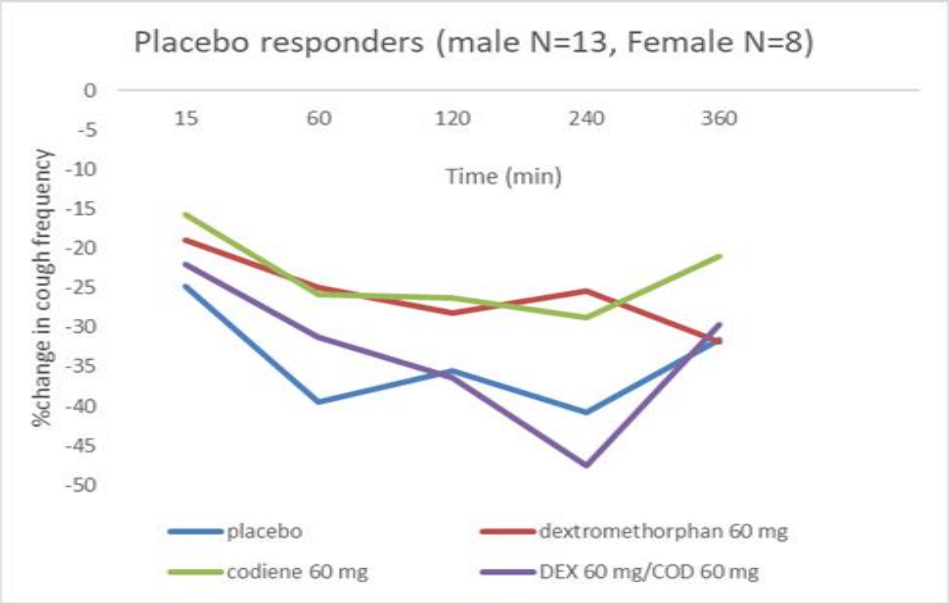
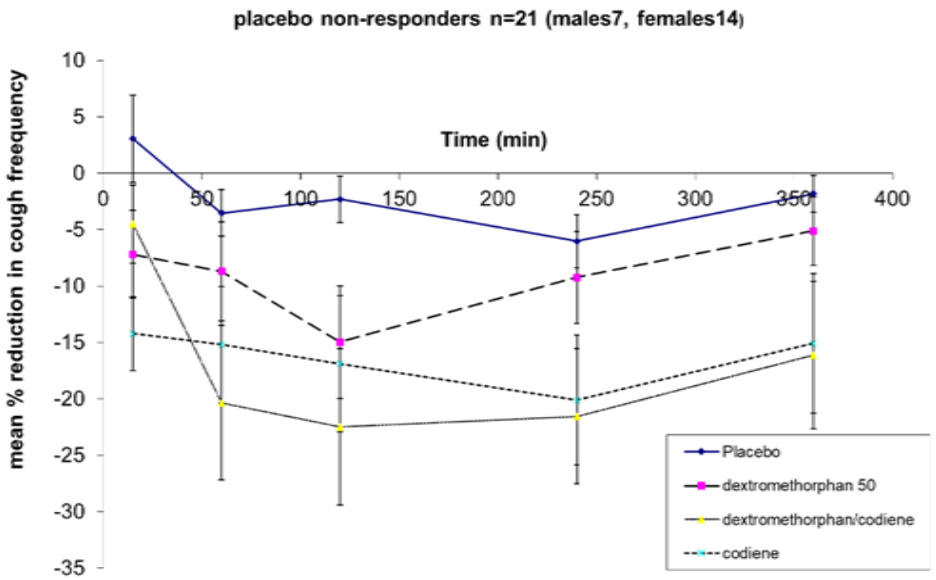


Figure 6 Placebo non-responders; % change in cough frequency to CA following placebo, dextromethorphan 60 mg, codeine 60 mg, and combined dextromethorphan 60 mg and codeine 60 mg.



Large placebo responses are consistently reported: Sivopixant, for example, showed a 60% reduction in 24 hr cough in placebo-treated patients (McGarvey et al., 2022a). and Gabapentin research found that nearly half (46.2%) of placebo-treated patients had achieved a clinically meaningful change in the LCQ score (Ryan, Birring and Gibson, 2012). Similar findings have been reported in the Gefapixant trials (McGarvey et al., 2022b).

This suggests large proportions of antitussive trial participants are placebo responders; For these individuals, active treatment effects may be equal or smaller than the placebo response, potentially underestimating true therapeutic efficacy.

Industry responses to this include placebo run-ins (Smith et al., 2025) to identify and exclude placebo responders prior to randomisation. While this may reduce variability, it increases screen failure rates and denies a subset of patients access to potentially beneficial new therapies.

5.1.2 Psychological Modulation of the Cough Reflex

Placebo response likely reflects expectation-driven modulation of the reflex; Chronic Cough is a biopsychosocial disease, involving dynamic interactions among physiological, psychological, and social factors. The reflex can be voluntarily

modulated, indicating significant behavioural and conscious control over the cough reflex pathway.

In pain research, placebo analgesia induced by strong expectation can be blocked by naloxone, indicating involvement of endogenous opioids (Levine, Gordon and Fields, 1978). Non-opioid mechanisms can also mediate placebo responses, depending on expectation levels (Amanzio and Benedetti, 1999; Colloca and Benedetti, 2005). A similar phenomenon appears in cough; expectations that CA is noxious significantly increased the UTC (Janssens et al., 2015), and Conditioning can reduce the UTC responses (Leech, Mazzone and Farrell, 2012).

Cough and pain share substantial neurobiological homology. Functional magnetic resonance imaging (FMRI) has revealed overlapping higher-order brain networks involved in cough control and chronic pain (Mazzone et al., 2009a). Neuroimaging studies in CC highlight structural and functional changes in brain regions associated with cough suppression and emotional regulation (Namgung et al., 2022).

Chronic cough, like chronic pain, is associated with depression (Berna et al., 2010), particularly in women (Heo et al., 2021). Anticipation alone can activate pain-related brain areas even in the absence of a physical pain stimulus (Apkarian et al., 2005). Chronic pain can also produce long-lasting emotional disturbances, often referred to as "secondary pain affect" (Price, 2000). This sets up a vicious circle where secondary pain affect aggravates pain, which then fuels worrying, and the cycle repeats. Similar processes are evident in anxiety -related or habitual cough, as supported by patient narratives (Brindle et al., 2023).

5.1.3 Behavioural and Digital Therapeutic Opportunities

Because the brain remains plastic, behavioural and psychological therapies can modify these processes. Behavioural cough suppression therapy (BCST) improves cough symptoms and overall quality of life (Yi et al., 2024). But access is limited by long waiting lists, shortages of trained therapists, and difficulty maintaining techniques post-therapy. Group-based cognitive/behavioural modulation therapies delivered virtually have shown encouraging results (Kear et al., 2025;Kear et al., 2025; Lillie et al., 2014), improving LCQ scores in RCC patients.

To broaden access the development of a digital BSCT application is strongly justified. Digital therapeutics have shown success in other related conditions such as IBS and chronic pain (Brenner et al., 2024; MacPherson et al., 2022).

Cough is a complex disease with limited effective treatments. Many of the therapies discussed in this thesis overlap mechanistically with analgesia, such as menthol (Li et al., 2022) Morphine and nalbuphine. Understanding CC as a neuropathic condition has guided the development of emerging sensory nerve-targeted therapeutics, including charged sodium channel blockers, NK1 receptor antagonists, and P2X3 antagonists. However, many have shown limited efficacy in randomised controlled trials, partly due to high placebo responsiveness.

Patients with CC exhibit elevated emotional distress (Hulme et al., 2017; McGarvey et al., 2006), Illness perceptions steer how they recognise and handle their disease and have been shown to predict reduced quality of life in IBS (Rutter and Rutter, 2002). These psychological factors likely influence outcomes in RCC. Harnessing psychological mechanisms offers a valuable non-pharmacological alternative, especially in RCC patients whose symptoms are driven or amplified by expectations. A digital BCST app would provide widely accessible evidence-based support, offering education, improving inhibitory control, and helping patients modify emotional and cognitive contributors to cough.

Conclusion

Overall, this programme of research advances understanding from peripheral pharmacology to central neuromodulation, human challenge methodology and therapeutic evaluation. Collectively, these studies demonstrate that chronic cough arises from an interaction of peripheral hypersensitivity, central processing, expectation, and behavioural control. While pharmacological agents targeting these pathways offer value, the consistently high proportion of placebo responsiveness, the influence of emotional stress, and the role of voluntary control underline the need for therapeutic approaches that engage the cognitive and emotional dimensions of the disorder. The evidence presented here supports both targeted drug development and the integration of behavioural and psychological therapies, including digitally delivered therapies, into the future landscape of chronic cough management.

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Appendices

APPENDIX 1

British Journal of Pharmacology (1997) 121, 1645–1650

Capsaicin and neurokinin A-induced bronchoconstriction in the anaesthetised guinea-pig: evidence for a direct action of menthol on isolated bronchial smooth muscle

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1 For many years menthol has been used in the treatment of respiratory disorders although, a bronchodilator effect of menthol has yet to be described. Using the bronchoconstrictors capsaicin (acting via stimulating the release of neuropeptides from sensory afferents) and neurokinin A (NKA) we have raised airways resistance in the guinea-pig (GP) and studied the effect of menthol on both capsaicin and NKA-induced bronchoconstriction *in vivo*. *In vitro* the effect of menthol on acetylcholine (ACh) and KCl precontracted GP bronchi was also studied.

2 GP ($n=13$) were anaesthetized (urethane 1.5 g kg^{-1}, i.p.) and a bolus injection of capsaicin (7.5 μg ml^{-1}, i.v.) or infusion of NKA (1 μg min^{-1}, i.v.) was given either in the presence of air (0.81 min^{-1}) or air impregnated with menthol vapour (7.5 μg l^{-1}) freely breathed from a tracheal cannula via a T-piece. Airways resistance (R_{aw}) and ventilation were measured throughout. Bronchi of mean internal diameter (1029 + 73.6 μm; $n=24$) were removed from GP ($n=16$) and mounted in the Cambustion myograph. Bronchial rings were maximally precontracted with 80 mM KCl or 2 mM ACh. Relaxation due to a cumulative dose of menthol (1–3000 μM) was measured.

3 Menthol produced a significant ($P<0.05$) 51.3% reversal of the capsaicin-induced increase in R_{aw}, and also inhibited the significant ($P<0.05$) reduction in minute ventilation (V_e) associated with the capsaicin-induced increased in R_{aw}. Menthol also caused a significant ($P<0.05$) 41% reversal of the NKA-induced increase in R_{aw}. The NKA-induced decrease in V_e was again significantly ($P<0.05$) reversed with menthol inhalation. Menthol caused a significant ($P<0.001$) dose-dependent relaxation of KCl and ACh precontracted bronchi.

4 We have shown that menthol attenuates both capsaicin and NKA-induced bronchoconstriction *in vivo* and relaxes KCl and ACh preconstricted bronchi *in vitro*. Menthol inhibition of NKA and capsaicin-induced bronchoconstriction could be, in part, explained by a direct action of menthol on bronchial smooth muscle.

Keywords: Menthol; capsaicin; neurokinin A; airways resistance; bronchodilatation

Introduction

Menthol has been used for many years in a wide range of over the counter medications. As a medicine, its most popular application is in the relief of common cold symptoms such as cough and chest congestion, although there is very little objective clinical evidence to show that menthol has any beneficial effects at the levels used in proprietary cough and cold products.

Antitussive activity of menthol has recently been demonstrated in both healthy volunteers (Morice *et al*., 1994) and in guinea-pigs (Laude *et al.*, 1994) with the citric acid-induced cough model. It was suggested by Laude that the antitussive properties of menthol in the guinea-pig may be due to a direct inhibitory action on the cough reflex, although an action on other respiratory mechanisms associated with cough, such as bronchoconstriction and airways secretion could not be ruled out.

Evidence of an effect of menthol on pulmonary function is very limited. Cohen & Dressler (1982) have studied the effects of a mixture of aromatic vapours (including menthol) on the calibre of airways in volunteers suffering from the common cold. By measuring forced expiratory volumes, peak expiratory flow rate and lower as well as total airways resistance, an improvement in airway's calibre with a 20–60 min aromatic vapour inhalation was found. However, since a mixture of aromatic vapours was used it is hard to attribute any effects of the mixture to menthol alone. Tamaoki *et al.* (1995) have more recently demonstrated a reduction in airway hyperresponsiveness, measured as a shift in the methacholine dose-response curve, in patients with mild asthma following long-term treatment with menthol. However, this group did not find any improvement in measures of forced expiratory volume in 1 s (FEV_1) following menthol treatment, which suggests that menthol had no effect on airway's calibre in these patients.

Thus evidence of bronchodilatation following menthol inhalation has yet to be obtained, furthermore there is no evidence of a direct action of menthol on bronchial smooth muscle. However, menthol has been shown to relax ileal smooth muscle in guinea-pigs by Hawthorn *et al*. (1988) and a specific action of menthol on airways epithelium has been demonstrated by Chiyotani *et al*. (1994), in dog, where menthol application *in vitro* increased Cl^- secretion.

In the present study, we have examined the effect of prior menthol inhalation on caspsaicin-induced increase in airways resistance (R_{aw}) in the guinea-pig. In view of the complex mechanism whereby capsaicin induced bronchoconstriction, involving sensory nerve stimulation and neuropeptide release, further studies have been designed to investigate the possible sites of action of menthol by use of exogenous neurokinin A *in vivo* and in preparations of isolated bronchi preconstricted with potassium chloride (KCl) and acetylcholine (ACh).

Methods

Airways resistance

Airways resistance was determined in Dunkin-Hartley guinea-pigs (420–645 g; $n=13$) anaesthetized with intraperitoneal

APPENDIX 2

British Journal of Pharmacology (1998) 123, 481–486

Identification of the L-menthol binding site in guinea-pig lung membranes

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1 L-Menthol inhibits both neurokinin A and capsaicin-induced bronchoconstriction in the guinea-pig and relaxes pre-constricted guinea-pig isolated bronchi. Structure-activity relationships have been defined for the action of (−)-menthol and related compounds on cold receptors, suggesting an action of L-menthol at a pharmacological receptor. We have performed radioligand binding studies to characterize the binding sites for [^{3}H]-L-menthol in whole cell membranes prepared from guinea-pig lung tissue.

2 In kinetic studies, [^{3}H]-L-menthol was found to bind rapidly and reversibly. Binding of [^{3}H]-L-menthol to lung membranes was found to be time-dependant becoming fully associated to its site within 40 min, and half-maximum association occurred within 8 min ($t_{1/2}$=8 min). [^{3}H]-L-menthol was fully dissociated from its binding site within 8 min, ($t_{1/2}$=2 min).

3 Inhibition studies presented a pharmacological profile of the 'L-menthol site'. Capsaicin, capsazepine, D-menthol, eugenol, SCH23390 and camphor were all found to displace [^{3}H]-L-menthol binding. In contrast WS3, noradrenaline, 5-hydroxytryptamine, spiperone, flunarazine, bepridil and nicardipine were without effect.

4 We have identified a L-menthol binding site in the guinea-pig, which may represent a site common to a variety of compounds.

Keywords: L-Menthol; binding site; lung membranes

Introduction

L-Menthol is a cyclic terpene alcohol, naturally occurring in the volatile oils of various species of mentha. L-Menthol has been used for many years in a wide variety of pharmaceutical preparations, generally utilizing its familiar fragrance and flavour, with little knowledge as to its pharmacological properties or medicinal benefit. Recent research into this compound has shown L-menthol to be of great interest with many specific effects. However, the mechanisms of action of L-menthol are yet to be elucidated.

The most commonly known and exploited property of L-menthol is its apparent cooling activity, which is thought to be mediated by the specific stimulation of peripheral cold fibres (Hensel & Zotterman, 1951; Hellekant, 1969). This effect is not limited to L-menthol as over 1200 compounds have been found to possess the same property. These compounds fall into a particular category which has four molecular requirements including, a hydrogen bonding group, hydrocarbon skeleton, the correct hydrophilic/hydrophobic balance and a molecular weight range of 150–350 g (Watson *et al*., 1978). These were prerequisites for intrinsic activity which suggests a specific drug-receptor interaction on peripheral cold receptors. Eight stereoisomers of L-menthol were tested by the Watson group, and L-menthol was found to have the greatest cooling activity with D-menthol being 45 times less active. Further studies on the epithelial and trigeminal responses of cold fibres of rat and dog lingual nerves have also shown L-menthol to behave very differently in comparison with a range of other related alcohols tested, indicating that the effects of menthol are through a specific receptor or channel (Simon & Sostman, 1991).

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Evidence of a possible drug-receptor interaction of menthol comes from studies on Ca^{2+} channels. Menthol has been found to reduce specifically Ca^{2+}-stimulated outward current in cat lingual fibres (Shafer *et al*., 1986).

A similar action of menthol on calcium channels has also been observed in other tissues. In molluscan neurones and vertebrate dorsal root ganglion cells Swandulla *et al.* (1986), observed that L-menthol application caused a reversible reduction in Ca^{2+} current when applied extracellulary, but had no effect on Ca^{2+} current when instilled intracellularly, indicating that the site for menthol may be situated on the cell membrane. Closely related compounds cyclohexanol (cyclic alcohol from which menthol derived), thymol, and menthone showed little or no activity, D-menthol was half as active as its stereoisomer indicating a stereochemically selective effect of L-menthol on Ca^{2+} current. Chiyotani *et al*. (1994) also demonstrated a specific action of menthol in cultured epithelium cells. The effect of L-menthol on Ca^{2+} channels has not only been found to be specific, but reversible and stereochemically selective as well.

The most significant evidence of a specific action of L-menthol at the site of a pharmacological receptor comes from the work of Hawthorn *et al.* (1988). Using a receptor binding assay, this group demonstrated competitive antagonism with L-menthol of [^{3}H]-nitrendipine and the dihydropyridine radioligand [^{3}H]-(+)-PN200-110 binding to cardiac, neuronal and intestinal smooth muscle. L-Menthol inhibition of [^{3}H]-nitrendipine and [^{3}H]-(+)-PN200-110 binding was more potent in intestinal smooth muscle compared with cardiac muscle and neuronal preparations.

That the effects of L-menthol are fully reversible, and stereochemically selective, together with the fact that L-menthol has been demonstrated to displace [^{3}H]-nitrendipine and [^{3}H]-(+)-PN200-110 binding, indicate that its effects could

APPENDIX 3

Wright et al. Cough 2010, 6:8
http://www.coughjournal.com/content/6/1/8

RESEARCH Open Access

Validation of the ERS standard citric acid cough challenge in healthy adult volunteers

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Abstract

Protocols measuring cough sensitivity can vary in terms of nebuliser, tussive agent, single and dose response. A definitive method for measuring cough sensitivity needs to be established.
The ERS guidelines recommend the KoKo DigiDoser (KD) delivery system. Study aim, was to compare the reproducibility of this citric acid (CA) cough challenge and previously established Mefar dosimeter (MD) protocol.
39 (female 26) volunteers mean age (40.4 yrs) were randomised to either KD or MD. Intra-day and inter-day reproducibility was compared.
We calculated the concentration of citric acid evoking 2 coughs (C2).
The geometric mean C2 (95%CI) was similar for both KD and MD, of 263 (200,339) mM and 209 (151,288) mM respectively.
The mean KD C2 was not significantly different. ($F = 0.807$, $p = 0.93$) from baseline over 1, 2, and 4 hrs however, the MD demonstrated significant variability ($F = 7.85$, $P < 0.001$)
Measuring mean log C2 at baseline and at 2 weeks, the KD demonstrated a stronger intraclass correlation of log C2 at baseline with 2 week log C2, ICC = 0.70 than was shown with the Mefar, ICC = 0.41
Administering CA from KD offers a reproducible cough challenge in healthy volunteers. The results correlate well with the MD challenge but offer greater intra-day and inter-day reproducibility.

Trial Registration: Current controlled trials ISRCTN98385033

Background

The methodology of citric acid cough challenge was first reported in humans over 50 yrs ago [1]. It was developed to allow for the quantification of cough reflex sensitivity and also as a tool for the assessment of antitussive therapies. Since this time many different protocols have been published and these can vary greatly in terms of the nebuliser used, the tussive agent, single breath, single dose or dose response and even the method to count number of coughs required to attain a threshold. The ERS task Force on cough methodology [2] recommended that a definitive method for measuring cough sensitivity needs to be established to allow for comparison of the results from different groups. It was suggested that the standardisation of cough challenge would lead to a higher quality of research, better drug development and ultimately improve patient care.

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The tussive stimulus of citric acid has been used to demonstrate differences in cough response between the sexes [3,4] and has supported the efficacy of a number of cough medications including opiates [5] and diphenhydramine [6]. We have previously demonstrated that the most widely used antitussive, dextromethorphan, inhibited citric acid cough when given orally but not as inhalation [7]. The utility of citric acid in illustrating the pharmacokinetic and pharmacodynamic relationship of antitussives has also been demonstrated [8]. Thus citric acid is established as a tussive stimulus in cough challenge demonstrating both physiological alterations in cough reflex sensitivity as well as the pharmacological properties of antitussives.

Cough challenge methodology needs to be standardised to allow for comparison between studies, here we have compared citric acid challenge with two different methodologies and investigated the intra-day and inter-day variability to determine which provides the most reliable benchmark in clinical studies.

APPENDIX 4

Opiate Therapy in Chronic Cough

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Rationale: **Cough is the most common complaint for which medical attention is sought, and chronic cough can be both physically and mentally debilitating. There is currently no evidence supporting the use of antitussives in chronic treatment-resistant cough.**
Objectives: **We tested the hypothesis that morphine sulfate in the dose of 5 mg twice daily would bring about a reduction in cough frequency and severity in patients failing to respond to specific measures.**
Methods: **Patients recruited from the Hull Cough Clinic were enrolled into a randomized double-blind placebo-controlled study using 4 weeks of slow-release morphine sulfate and a corresponding period of matched placebo. An open-labeled extension of the core study allowed dose escalation to 10 mg twice daily. Cough was assessed using the Leicester Cough Questionnaire, daily symptom diary, and citric acid cough challenge.**
Results: **Twenty-seven patients completed the core study. A significant improvement of 3.2 points over baseline was noted on the Leicester Cough Questionnaire ($p < 0.01$). A rapid and highly significant reduction by 40% in daily cough scores was noted among patients on slow-release morphine sulfate ($p < 0.01$). Objective testing of the cough reflex using citric acid cough challenge tests did not show any significant changes. Eighteen patients continued into the extension study. Two-thirds of these patients opted to increase the morphine to 10 mg twice daily. At the end of 3 months, there was a similar improvement in cough between the 5- and 10-mg groups.**
Conclusion: **Morphine sulfate is an effective antitussive in intractable chronic cough at the doses of 5 to 10 mg twice daily.**

Keywords: chronic cough; antitussive; opiates; morphine sulfate

Cough is the most common complaint for which a medical consultation is made (1). Although acute cough is benign and self-limiting, chronic, persistent cough can have a devastating effect on the quality of life of sufferers (2, 3). The prevalence of chronic cough in the community has proven difficult to determine, but a recent questionnaire survey indicated that 7% of a representative population had coughing bouts at least weekly, sufficient to interfere with activities of daily living (4). Experience from specialist cough clinics suggests that a diagnosis (usually reflux disease or a variant of asthma) and a subsequent response to therapy is achievable in the majority of cases (5, 6). However, a significant minority remain undiagnosed or fail to respond to multiple therapeutic trials of antitussive therapy.

Opiates have long been advocated for the suppression of cough (7, 8). However, there are few trial data to support this recommendation. Although small, single-dose studies of codeine in chronic bronchitis have shown some benefit (9–11), a recent trial suggested an effect similar to that of placebo (12). The effect of opiates in intractable chronic cough has never been studied. Indeed, experience in acute cough and with cough challenge in normal volunteers suggests a lack of efficacy (13, 14). The aim of this study was to determine whether low-dose opiates in the form of slow-release morphine has a role in the management of patients with idiopathic cough or in those resistant to conventional treatments.

Some of these results have been previously reported in the form of an abstract (15)

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Opiates have been long advocated for the suppression of cough, but there are few trial data to support this recommendation.

What This Study Adds to the Field

Morphine sulfate is an effective agent for reducing the severity of chronic cough.

METHODS

Subjects were recruited from adult patients attending the Hull Cough Clinic who had a chronic, persistent cough of greater than 3 months' duration. Patients were assessed according to our previously published probability-based treatment algorithm (16), which excludes patients with significant lung disease. Those who failed to respond to trials of specific antitussive therapy were enrolled. Approval for the study was obtained from the Medicines and Healthcare Products Regulatory Agency (MHRA) (reference number MF 8000/13102) and Hull and East Riding Local Research and Ethics Committee and registered at www.controlled-trials.com (reference number ISRCTN 18474014). All patients were provided with written information prior to obtaining consent. The study was conducted in compliance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use–Good Clinical Practice (ICH-GCP) guidelines.

Because no previous studies of this type exist, a formal power calculation was impossible. Based on the observed effect of morphine in the clinic, the number of patients studied was considered to be sufficient to demonstrate any important effect. Patients were randomized into a double-blind placebo-controlled crossover study by using a computer-generated block randomization code. The patients were required to take 4 weeks of slow-release morphine sulfate (MST) 5 mg twice daily and 4 weeks of matched placebo (DHP Ltd, Monmouthshire, UK). No person involved in the analysis of the data had knowledge of the randomization. During the course of the trial, the patients were required to withhold any other cough remedies, including over-the-counter preparations.

The patients made three visits to the clinical trials unit at 4-week intervals. On each of these visits, they filled in the Leicester Cough Questionnaire (2), a validated and reproducible measure of the impact

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APPENDIX 5

Therapeutic Advances in Chronic Disease

Original Research

Is opiate action in cough due to sedation?

Ther Adv Chronic Dis

2014, Vol. 5(5) 200–205

DOI: 10.1177/2040622314543220

Rebecca S. Dickinson, Jaymin B. Morjaria, Caroline E. Wright and Alyn H. Morice

Abstract

Objectives: Opiates have been used for cough suppression for centuries. It is unclear whether this antitussive action is due to their known sedative effects. We aimed to assess correlation between cough suppression and opiate usage.

Methods: We performed a post hoc analysis of two published trials with three opioids. In study one, patients with chronic cough were treated with 4 weeks of modified release morphine sulphate (5 mg twice daily) or placebo in a double-blinded placebo-controlled fashion. Cough suppression was assessed subjectively by the Leicester Cough Questionnaire and objectively by citric acid aerosol (CAA) induced cough challenge. In study 2, normal volunteers were given single doses of placebo, codeine 30 mg or dextromethorphan 50 mg and cough suppression assessed using the CAA-induced cough challenge. Sedation was contemporaneously assessed by direct questioning.

Results: There were 14 episodes of patient-reported sedation; 2 with modified release morphine sulphate, 9 with codeine and 3 with dextromethorphan. There was no correlation between change in the Leicester Cough Questionnaire or the CAA-induced cough challenge and reported sedation.

Conclusion: This observational study suggests that sedation is unlikely to underlie the antitussive properties of these opioids. Eliciting the mechanism of these medications in cough may be a target for future tailored drug development.

Keywords: antitussive, codeine phosphate, cough therapies dextromethorphan, morphine sulphate (MST), sedation

Introduction

Chronic cough is a common problem which can have a significant negative impact on patient quality of life [French *et al.* 1998]. In a UK based primary care survey, 12% of subjects had a chronic cough with 7% feeling that it significantly impacted on their lives [Ford *et al.* 2006]. Acute cough sufferers regularly seek medical advice and purchase over-the-counter medications for cough relief at an estimated cost of £104 million per year [Morice *et al.* 2006] in the UK.

Opiates have had a long history of use as antitussives. Dextromethorphan (DEX), a synthetic opioid, is the most widely used over-the-counter cough suppressant which has been shown to be effective both in the clinical setting and in experimental cough challenge studies in normal volunteers [Mathys, 1983; Parvez *et al.* 1996]. Historically, codeine preparations have been widely used though their clinical efficacy is less certain [Mudge, 1778].

In chronic cough, opiates are recommended for cough suppression though there are major concerns with their use [Chung, 2005]. We have shown that low dose modified release morphine sulphate (MST, 5 mg twice daily) was associated with a significant reduction in subjective cough counts and improvement quality of life for patients with chronic cough [Morice *et al.* 2007].

The opiate mechanism of cough suppression is poorly understood. Opiates have been used as a cough remedy since the 18th century [Sanders, 2007]. One of the possible mechanisms could be through a sedative action. It is well recognized that opiates have sedative side effects [Forrest *et al.*, 1977; Bruera *et al.* 1989]. Hydrocodone, alfentanyl and fentanyl have been assessed as alternative sedative agents in fibreoptic bronchoscopy [Webb *et al.* 1989; Papagiannis and Smith 1994; Stolz *et al.* 2004] with subjective observer cough reduction as a secondary outcome.

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APPENDIX 6

ORIGINAL ARTICLE
COUGH

The effect of gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study

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Gefapixant reduces coughing in patients and blocks ATP- and distilled-water-induced cough, but not cough evoked by citric acid or capsaicin, suggesting a unique TRPV4/ATP pathway may underlie cough hypersensitivity seen in chronic refractory cough http://bit.ly/2Gcr9Lr

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ABSTRACT We evaluated the effect of gefapixant on cough reflex sensitivity to evoked tussive challenge.

In this phase 2, double-blind, two-period study, patients with chronic cough (CC) and healthy volunteers (HV) were randomised to single-dose gefapixant 100 mg or placebo in a crossover fashion. Sequential inhalational challenges with ATP, citric acid, capsaicin and distilled water were performed 1, 3 and 5 h after dosing. Mean concentrations evoking ≥2 coughs (C2) and ≥5 coughs (C5) post dose *versus* baseline were co-primary endpoints. Objective cough frequency (coughs·h^{-1}) over 24 h and a cough severity visual analogue scale (VAS) were assessed in CC patients. Adverse events were monitored.

24 CC patients and 12 HV were randomised (mean age 61 and 38 years, respectively). The cough challenge threshold increased for ATP by 4.7-fold (C2, p≤0.001) and 3.7-fold (C5, p=0.007) for gefapixant *versus* placebo in CC patients; in HV, C2 and C5 increased 2.4-fold (C2, p=0.113; C5, p=0.003). The distilled water C2 and C5 thresholds increased significantly (p<0.001) by a factor of 1.4 and 1.3, respectively, in CC patients. Gefapixant had no effect on capsaicin or citric acid challenge. Median cough frequency was reduced by 42% and the least squares mean cough severity VAS was 18.0 mm lower for gefapixant *versus* placebo in CC patients. Dysgeusia was the most frequent adverse event (75% of HV and 67% of CC patients).

ATP-evoked cough was significantly inhibited by gefapixant 100 mg, demonstrating peripheral target engagement. Cough count and severity were reduced in CC patients. Distilled water may also evoke cough through a purinergic pathway.

This article has supplementary material available from erj.ersjournals.com

This study is registered as a clinical trial (NCT02476890). Merck & Co., Inc.'s data sharing policy, including restrictions, is available at https://engagezone.merck.com/ds_documentation.php Requests for access to the clinical study data can be submitted through the EngageZone site or *via* email to dataaccess@merck.com

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