# New drug targets for chronic cough - research you can literally sink your teeth into!

C.J. Jolley[1, 2*] & S.S. Birring[2, 3]

1. Centre of Human & Aerospace Physiological Sciences, School of Basic & Medical Biosciences, Faculty of Life Sciences & Medicine, King's College London, King's Health Partners, London, UK.
2. Department of Respiratory Medicine, King's College Hospital NHS Foundation Trust, King's Health Partners, London, UK.
3. Division of Asthma, Allergy & Lung Biology, School of Transplantation, Immunology, Infection & Inflammation Sciences, Faculty of Life Sciences & Medicine, King's College London, King's Health Partners, London, UK.

***Corresponding author:**

Dr Caroline Jolley

Centre of Human & Aerospace Physiological Sciences

King's College London

Shepherd's House, Rm 4.4

Guy's Campus

London SE1 1UL

UK

Email: caroline.jolley@kcl.ac.uk

**Take-home message:**

Human dental pulp stem cells & endocannabinoids- can new preclinical models translate to effective antitussive drugs?

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Cough is an essential neurally-mediated reflex that has evolved to protect the upper airways from obstruction, and to expel chemical and mechanical irritants. When heightened or persistent, however, cough presents a clinically-challenging source of considerable physical and psychological morbidity [1, 2]. In the US, cough continues to be the commonest single symptom for which patients seek a medical consultation [3]. Chronic cough, defined as cough continuing > 8 weeks, is also globally prevalent and accounts for 10% of respiratory referrals to secondary care [4].

The associated societal and healthcare costs of cough are huge. Acute cough contributes to approximately 34.0 million working days lost each year in the UK through minor illnesses [5], with more than £100 million pounds being spent on over-the-counter antitussive drugs each year [6]. Despite meticulous, and often lengthy, diagnostic protocols, chronic cough remains unexplained or refractory in 12 – 42% of cases [7], following which patients are frequently subjected to sequential trials of anti-tussives with limited clinical efficacy and/or undesirable side-effects [8]. A recent internet-based survey, conducted across 29 different European countries, found that most subjects with chronic cough responded that their cough medication had limited or no effectiveness (57% and 36%, respectively), with only 7% reporting that medications they had tried for their cough were effective [2]. This lack of effective treatment reflects our limited understanding of the mechanistic basis of cough. Indeed, it is increasingly recognised that in order to effectively treat cough syndromes, there is a need to look beyond the presence (or not) of underlying disease processes, and towards a better understanding of physiological control of the cough reflex itself.

This paradigm shift has heralded the recent description of the “cough hypersensitivity syndrome” (CHS) by a European Respiratory Society Taskforce. CHS is defined as a clinical entity characterised by cough as a major component, which is often triggered by low levels of thermal, mechanical, or chemical exposure [9]. The main mechanism of CHS has been suggested to be dysregulated sensory neural pathways and central processing in cough reflex regulation, as supported by a number of observations. Firstly, the symptom profile of CHS is similar to that of neuropathic disorders such as pain. CHS patients frequently report exaggerated coughing to known tussive stimuli, for example, strong odours and smoke (hypertussia) and to non-tussive stimuli such as talking and laughing (allotussia), and

abnormal sensations such as laryngeal paraesthesia (tickle) [10]. Secondly, neuropeptides, released from sensory nerves, can act as neurotransmitters and initiate local inflammatory responses and these are present in increased concentrations in the airways of patients with persistent cough [11, 12]. Central sensitisation to respiratory sensations, involving convergence of sensory bronchopulmonary C-fibres with low threshold A*δ*-fibres onto second order neurons in the brainstem, is suggested by animal models of bronchopulmonary C-fibre activation leading to an increase in A*δ*-mediated cough reflex sensitivity [13] [14]. Functional neuroimaging studies are beginning to provide insights into the neurobiology of chronic cough, including increased activation in the cortical and subcortical brain centres that integrate the intensity and location of the cough stimulus, and in regions previously implicated in voluntary cough suppression [15]. Centrally-acting neuromodulatory drugs such as gabapentin [16], amitriptyline [17] and morphine [18], and speech and physiotherapy interventions [19, 20], are also effective in some patients. Direct evidence for neural dysfunction is however lacking because, except for peripheral lung tissues, human neural tissues are very difficult to obtain. Thus, at present, CHS is still a conceptual entity.

The key regulator of CHS also remains elusive. Ion channels present on respiratory vagal afferent nerve termini can be activated by a wide variety of stimuli to elicit cough and other reflexes. The main family of ion channels implicated in the initiation of sensory reflexes are the transient receptor potential (TRP) channels, with most information pertinent to cough physiology having been gathered for transient receptor potential vanilloid (TRPV) 1, transient receptor potential ankyrin (TRPA) 1, TRPV4, and transient receptor potential melastatin (TRPM) 8 [21]. Capsaicin, the active ingredient of chilli pepper, binds TRPV1 receptors causing pain, burning sensation, cough and urge-to-cough, and is one of the most potent tussigens used in inhalation cough challenge tests [21]. TRPV1 is expressed by vagal afferent C- and A*δ*-nociceptive fibres innervating the airways [21] and TRPV1 receptor expression is increased in airway nerves of chronic cough patients [22]. However, despite efficacy being predicted in preclinical guinea pig and human *in vitro* vagal models [21], the potent TRPV1 receptor antagonist XEN-D0501 failed to significantly alter objective 24-hour cough frequency or subjective urge-to-cough in patients with refractory chronic cough in a recent clinical trial [23]. TRPA1 receptors are also present on vagal sensory afferents and bind a wide range of irritants (but not capsaicin) present in tussigenic environmental pollutants such as cigarette

smoke, as well as functioning as cold thermosensors [24]. However, despite the preclinical promise of TRPA1 receptors as effective anti-tussive targets [24], a TRPA1 antagonist did not show significant anti-tussive effects in humans (study completed in 2014 but unpublished) [25, 26]. Other examples where preclinical models of cough have failed to reliably translate to clinical efficacy are neurokinin (namely substance P) antagonists (reviewed in [27]), and the novel voltage-gated sodium channel blocker GSK2339345 [28].

These findings are, however, in stark comparison to those of a recent randomised, controlled clinical trial of an antagonist of the purinergic P2X3 receptor (AF219/MK7264), which caused a dramatic decrease in cough frequency in patients with refractory chronic cough [29]. P2X3 receptors are relatively specific for adenosine triphosphate (ATP), release of which is triggered by tissue inflammation and present in increased concentrations in the airways of chronic smokers and in COPD [30], after allergen challenge in asthmatics [31], and in fibrotic interstitial lung disease [32]. P2X3 and P2X2/3Rs are also present on the central projections of these neurons within the dorsal horn of the spinal cord and brainstem, where they are implicated in augmenting release of glutamate [33] and substance P [34], mediating central sensitisation at the first synapse. Animal studies implicate P2X3Rs in central sensitisation to pain including inflammatory hyperalgesia [35] and mechanical allodynia underlying bladder pain [36], and in arthritic and cancer pain [37] [38]. However, ATP administered to the bronchial tree does not cause a dramatic left-shift in cough reflex sensitivity [39]. Thus the P2X3 receptor may merely be a link in the chain of cough hypersensitivity rather than the primary mediator [25].

In this issue of the *European Respiratory Journal*, WORTLEY *et al.* [REF] report an elegant series of investigations in which they identify, for the first time, fatty acid amide hydrolase (FAAH) inhibition as a target for the development of novel, anti-tussive agents through modulation of the endocannabinoid system in preclinical guinea pig and human models. FAAH is an integral membrane protein found within the nervous system and is responsible for the hydrolysis of the endocannabinoid *N*-arachidonoyl ethanolamine (anandamide, AEA), and other related fatty acid amides (FAAs) such as palmitoylethanolamine (PEA), N-oleoylethanolamide (OEA) and linoleoyl ethanolamide (LEA). The recent identification of cannabinoid (CB1 and CB2) receptors and their endogenous lipid ligands has triggered an

exponential growth of studies exploring the endocannabinoid system and its regulatory functions in health and disease. Modulating the activity of the endocannabinoid system holds therapeutic promise in a wide range of disparate diseases and pathological conditions [40], notably including neuropathic pain [41]. The G-protein coupled receptors CB1 are the "brain receptors" for cannabinoids in the mammals, but are also present at much lower concentrations in a variety of peripheral tissues and cells. A second cannabinoid G-protein coupled receptor (GPCR), CB2, is primarily expressed peripherally in cells of the immune and hematopoietic systems but have also been identified in the brain, in nonparenchymal cells of the cirrhotic liver, in the endocrine pancreas, and in bone [40]. Activation of the CB2 receptor subtype has previously been shown to inhibit both guinea-pig and human airway sensory nerve activity and the cough reflex in guinea-pigs [42], modulation of sensory nerve activity being shown to be elicited both by the exogenous ligands capsaicin and hypertonic saline and by endogenous modulators such as PGE2 and low pH stimuli [43]. Although non-selective cannabinoids, such as anandamide, have been shown to suppress the cough reflex [44, 45], the associated (predominantly CB1-mediated) side effects such as sedation, cognitive dysfunction, tachycardia and psychotropic effects have hampered the use of such agonists for treatment purposes [46]. This suggested that the development of CB2 agonists, devoid of CB1-mediated central effects, could provide a new and safe antitussive treatment for chronic cough without these undesirable central side-effects.

Peripheral elevation of endocannabinoids provides an attractive alternative pharmacological strategy through which to indirectly target vagal afferent CB2 receptor activation. In a conscious guinea pig model, WORTLEY *et al.* [REF] demonstrate inhibition of citric acid (low pH) provoked cough in association with elevated FAAs, brought about by pharmacological inhibition of FAAH (FAAHi). This suggests FAAHi as a potential novel target for the pharmacotherapy of CHS. Then, in an isolated guinea-pig vagus nerve model, PEA is shown to cause a concentration related inhibition of both low pH- and capsaicin-induced depolarisation. WORTLEY *et al.* subsequently confirm this effect to be a CB2-, not CB1-, receptor-mediated mechanism, operating through activation of $Ca^{2+}$-activated $K^+$ ($SK_{Ca}$) channels. Remarkably, FAAHi-mediated inhibition of depolarisation is similarly demonstrated in a human vagal nerve preparation, again via a CB2-PP2A-$SK_{Ca}$ channel mechanism.

We await *in vivo* human studies of FAAH inhibition in chronic cough with the optimism that, counter to what might otherwise be predicted from previous experience [27], direct translation of physiological mechanism from rodent to man will translate to clinical efficacy. Lack of efficacy and safety are of course major causes of attrition in the pharmaceutical industry, and the former is likely to be a more significant contributor to attrition in therapeutic areas in which animal models of efficacy are unpredictive [47]. The parallels here between CHS and neuropathic pain are ominous [48], and highlight the need for robust preclinical human models; a problem because access to human vagal nerve preparations is challenging. A call to improve translation through a better understanding of, and control for, differences in human and animal preclinical and cellular models [27], together with the need to reduce, replace and refine the use of animals for scientific purposes, brings an urgent requirement for development of novel *in vitro* models of cough hypersensitivity based on human biology.

Could human sensory "peripheral neuronal equivalents" (PNEs), as introduced in this issue of the *Journal* by CLARKE *et al.*, meet this challenge? Human dental pulp stem cells (hDPSCs) are of neural crest origin and as such have a propensity to differentiate towards a neuronal phenotype [49]. CLARKE *et al.*, describe refinement of the hDPSC model to produce PNEs that have morphological, molecular and functional characteristics of sensory neurons. Moreover, these PNEs express functional TRPA1 and, intriguingly, exhibit TRPA1 channel hyper-responsiveness following stimulation with both nerve growth factor (NGF) and the viral mimetic Poly(I:C). Of course, TRPA1 channel *hyper-responsiveness* is physiologically distinct to neuronal *hypersensitivity*, and further work is required to study the latter phenomenon in the PNE model. Confirmation that a similar functional relationship exists between TRPA1 and live respiratory viruses to that demonstrated using the viral mimetic Poly(I:C) in the PNE model would also add relevance to the field of clinical respiratory medicine. An additional, albeit unavoidable, limitation of any *in vitro* peripheral sensory model of cough, human or otherwise, is that it is impossible to predict how the resultant pattern of afferent activity will be processed in central brain pathways. Most current clinically-effective drugs are centrally-acting [16, 18] and normalisation of cough frequency did not appear to be a prerequisite for clinical response to AF219/MK7264 [29]. This underlines the importance of understanding central pathways subserving cough perception in order to achieve antitussive effects.

These limitations should not, however, distract from the potential of the work of CLARKE *et al.* to provide a much-needed human *in vitro* model for the study of inflammatory TRP channel regulation and related CHS mechanisms. Such models should improve efficiency of translation to therapeutic development through larger-scale screening of pharmaceutical compounds on the basis of their functional interactions, improved drug dosage and frequency prediction before going to man, and a better understanding of interspecies differences. These could also prove helpful in improving the therapeutic ratio of drugs where the maximal clinical dose is limited by intolerable side-effect profiles [50]. The current level of interest in identifying antitussive targets and efficacy in preclinical models is reflected by the variety of alternate animal [51-54] and human cellular [55] options in development. These, together with identification of novel pharmacological targets [*ref* WORTLEY *et al.*], should provide opportunities to accelerate progress to positive first-in-man trials through a better understanding of the pathophysiology of human cough reflex hypersensitivity.

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