Theophylline inhibits the cough reflex through a novel mechanism of action☆

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Background: Theophylline has been used in the treatment of asthma and chronic obstructive pulmonary disease for more than 80 years. In addition to bronchodilator and anti-inflammatory activity, clinical studies have suggested that theophylline acts as an antitussive agent. Cough is the most frequent reason for consultation with a family doctor, and treatment options are limited. Determining how theophylline inhibits cough might lead to the development of optimized compounds.

Objective: We sought to investigate the inhibitory activity of theophylline on vagal sensory nerve activity and the cough reflex.

Methods: Using a range of techniques, we investigated the effect of theophylline on human and guinea pig vagal sensory nerve activity *in vitro* and on the cough reflex in guinea pig challenge models.

Results: Theophylline was antitussive in a guinea pig model, inhibited activation of single C-fiber afferents *in vivo* and depolarization of human and guinea pig vagus *in vitro*, and inhibited calcium influx in airway-specific neurons *in vitro*. A sequence of pharmacological studies on the isolated vagus and patch clamp and single-channel inside-out experiments showed that the effect of theophylline was due to an increase in the open probability of calcium-activated potassium channels. Finally, we demonstrated the antitussive activity of theophylline in a cigarette smoke exposure model that exhibited enhanced tussive responses to capsaicin.

Conclusion: Theophylline inhibits capsaicin-induced cough under both normal and "disease" conditions by decreasing the excitability of sensory nerves through activation of small- and intermediate-conductance calcium-activated potassium channels. These findings could lead to the development of optimized antitussive compounds with a reduced side effect potential. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Sensory nerves, vagus, cough, ion channels, capsaicin*

Theophylline has been used in the treatment of respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), for more than 80 years. Although in industrialized countries β-agonists and steroids are the preferred treatment, globally, theophylline remains a widely prescribed drug, particularly in patients with more severe disease, because it is cheap and readily available.[1] Although it has been primarily prescribed for its bronchodilator activity, there is a large body of evidence suggesting that it also possesses anti-inflammatory activity in both asthmatic patients and patients with COPD.[2-4] Furthermore, clinical studies have shown that adding theophylline to inhaled steroids in patients with mild-to-moderate asthma and to long-acting β-adrenoceptor agonists in patients with severe COPD provides additional clinical improvement. In addition to these properties, preclinical and clinical studies have suggested that both theophylline and another methylxanthine, theobromine, act as antitussive agents in several preclinical studies and in patients with a range of clinical conditions.[5,6] In children and adults with poorly controlled asthma, theophylline significantly improved symptom scores for cough and wheeze compared with placebo.[7] Theophylline has also been recommended for the treatment of cough in patients with COPD[8] and has been shown to be effective for treating angiotensin-converting enzyme inhibitor–related cough.[9]

Cough is a protective reflex and defense mechanism in healthy subjects.[10] However, cough is also the most common respiratory complaint for which medical attention is sought,[11] often presents as the first and most persistent symptom of many respiratory diseases, and can be idiopathic in nature.[12] Chronic persistent cough has a profoundly detrimental and debilitating effect on quality of life that can lead to social isolation and clinical depression.[13] Cough is a major problem that leads patients to use over-the-counter remedies as first-line treatments; United Kingdom sales were greater than £350 million in 2004, and US sales were greater than $2 billion.[14] However, a recent meta-analysis established that there was no good evidence for the effectiveness of such remedies.[15] Therefore the mechanisms underlying chronic cough and the identification of novel therapeutic targets for its treatment present a grossly neglected and unmet clinical need.

In this study we have demonstrated that theophylline inhibits sensory nerve activation and the cough reflex and uncovered a possible mechanism of action. This study highlights a previously unrecognized benefit of theophylline that could explain the

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Abbreviations used
BK_{ca} channel: Large-conductance calcium-activated potassium channel
$[Ca^{2+}]_i$: Intracellular calcium
COPD: Chronic obstructive pulmonary disease
DiI: DilC18(3)-1,1′-dioctacetyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate
ECS: Extracellular solution
IK channel: Intermediate-conductance calcium-activated potassium channel
PDE: Phosphodiesterase
PGE_2: Prostaglandin E_2
SK channel: Small-conductance calcium-activated potassium channel
TRP: Transient receptor potential

positive effects seen in patients with respiratory disease. Elucidating the mechanism of action could lead to the development of optimized compounds with a reduced side effect profile.

METHODS

For a more detailed discussion of the methods used in this study, please see the Methods section in this article's Online Repository at www.jacionline.org.

Animals

Male guinea pigs (Dunkin-Hartley, Harlan, United Kingdom) weighing 350 to 450 g (400-750 g for single-fiber *in vivo* studies) were housed in temperature-controlled (21°C) facilities with food and water *ad libitum* for at least 1 week before the experiment. All experiments were performed in accordance with the UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) Act of 1986.

Effect of theophylline on citric acid–and capsaicin-evoked cough in conscious guinea pigs

Conscious unrestrained guinea pigs were placed in individual plastic, transparent, whole-body plethysmograph chambers, and cough was detected, as previously described.[16,17]

Effect of theophylline on capsaicin-induced firing of single-fiber afferents and bronchospasm *in vivo*

Guinea pigs were anaesthetized with urethane (1.5 g/kg) intraperitoneally. The trachea was cannulated, and pressure was measured with an air pressure transducer connected to a side arm of the tracheal cannula. Animals were paralyzed with vecuronium bromide, initially administered at a dose of 0.10 mg/kg intravenously, followed every 20 minutes with 0.05 mg/kg administered intravenously to maintain paralysis. Firing of single-fiber afferents and bronchospasm was measured, as previously described.[18]

Effect of theophylline on depolarization of the vagus nerve preparation to various tussive agents

Guinea pigs were culled with an overdose of pentobarbitone (200 mg/kg administered intraperitoneally). The 2 vagus trunks were carefully dissected free and placed in Krebs–Henseleit solution. Segments of the vagus nerve were mounted in a "grease-gap" dual recording chamber system, as previously described.[19,20]

Primary culture of sensory neurons

Calcium imaging of primary sensory jugular ganglia was performed, as previously described.[21] Identification of airway sensory neurons was done by means of intratracheal administration of the retrograde neuronal tracer DilC18(3)-(1,1′-dioctacetyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate) (DiI; Invitrogen, Carlsbad, Calif) 2 weeks before collecting the ganglia and isolating the cells. Camptothecin was used to inhibit mitotic cell growth during the primary culture of neurons, and airway neuron staining with DiI was assessed with 531/40 nm and 593/40 nm excitation/emission filters (BS 565) before experimentation. Intracellular calcium $[Ca^{2+}]_i$ and membrane voltage changes were simultaneously optically measured in primary cultured dissociated jugular neurons (see the Methods section in this article's Online Repository). The effect of theophylline on small-conductance calcium-activated potassium channel (SK channel) and intermediate-conductance calcium-activated potassium channel (IK channel) currents and analysis of the direct effect of theophylline on calcium-activated potassium channels in the jugular ganglia were also investigated (see the Methods section in this article's Online Repository).

Cigarette smoke exposures

Guinea pigs were exposed to cigarette smoke from research cigarettes (3R4F, with filters removed; University of Kentucky, Lexington, Ky) for 1 hour twice daily, with 4 hours between each bidaily exposure period. Cigarette smoke exposure protocols are described in the Methods section in this article's Online Repository and were similar to those described by Eltom et al.[22]

Drugs and solutions

All compounds and drugs used are described in the Methods section in this article's Online Repository. The amount of vehicle (dimethyl sulfoxide) was limited to 0.1% to avoid possible side effects, and the effect of theophylline at each concentration was obtained in the presence of the same amount of vehicle each time. In all the experimental design paradigms, the effect of vehicle alone was assessed, and no significant effect observed.

RESULTS

Effect of theophylline in a conscious guinea pig cough model

Theophylline (1 hour before treatment) decreased the number of coughs induced by aerosolized capsaicin (60 μmol/L, n = 15; Fig 1, *A*) and citric acid (300 mmol/L, n = 12-15; Fig 1, *B*).

Effect of theophylline on C-fiber activation by capsaicin *in vivo*

Capsaicin, when nebulized into the lungs of anaesthetized guinea pigs, induced a burst of vagal C-fiber firing that was significantly reduced by theophylline (Fig 2, *A-C*). Theophylline administration *per se* did not modify the spontaneous firing observed in the vagal C-fibers (Fig 2, *C*). Capsaicin also induced a prolonged bronchoconstriction (which followed the C-fiber firing) that was almost entirely abolished in the presence of theophylline (Fig 2, *A*, *D*, and *E*). The possibility exists that some of the C-fiber firing could be induced indirectly after the bronchoconstrictor response elicited by capsaicin and that theophylline is merely acting as a bronchodilator. Aerosolized prostaglandin E_2 (PGE_2) was also used to induce bursts of C-fiber firing to address this concern. Action potential trains were obtained that were, on average, similar in total duration to capsaicin and were not associated with any bronchoconstriction (Fig 2, *F*, top panel). Theophylline significantly decreased PGE_2-induced firing (Fig 2, *F*, bottom panel).