Tiotropium modulates transient receptor potential V1 (TRPV1) in airway sensory nerves: A beneficial off-target effect?☆

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Background: Recent studies have suggested that the long-acting muscarinic receptor antagonist tiotropium, a drug widely prescribed for its bronchodilator activity in patients with chronic obstructive pulmonary disease and asthma, improves symptoms and attenuates cough in preclinical and clinical tussive agent challenge studies. The mechanism by which tiotropium modifies tussive responses is not clear, but an inhibition of vagal tone and a consequent reduction in mucus production from submucosal glands and bronchodilation have been proposed.

Objective: The aim of this study was to investigate whether tiotropium can directly modulate airway sensory nerve activity and thereby the cough reflex.

Methods: We used a conscious cough model in guinea pigs, isolated vagal sensory nerve and isolated airway neuron tissue– and cell-based assays, and *in vivo* single-fiber recording electrophysiologic techniques.

Results: Inhaled tiotropium blocked cough and single C-fiber firing in the guinea pig to the transient receptor potential (TRP) V1 agonist capsaicin, a clinically relevant tussive stimulant. Tiotropium and ipratropium, a structurally similar muscarinic antagonist, inhibited capsaicin responses in isolated guinea pig vagal tissue, but glycopyrrolate and atropine did not. Tiotropium failed to modulate other TRP channel–mediated responses. Complementary data were generated in airway-specific primary ganglion neurons, demonstrating that tiotropium inhibited capsaicin-induced, but not TRPA1-induced, calcium movement and voltage changes.

Conclusion: For the first time, we have shown that tiotropium inhibits neuronal TRPV1-mediated effects through a mechanism unrelated to its anticholinergic activity. We speculate that some of the clinical benefit associated with taking tiotropium (eg, in symptom control) could be explained through this proposed mechanism of action. (J Allergy Clin Immunol 2014;133:679-87.)

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

Inhaled muscarinic receptor antagonists are currently used as bronchodilators for the management of asthma and chronic obstructive pulmonary disease (COPD).[1] Their efficacy is believed to be based on the notion that they block increased vagal tone (through increased parasympathetic cholinergic contractile responses, acetylcholine release, and muscarinic receptor activation on airway smooth muscle), which is thought to be the major reversible component of airflow narrowing in patients with COPD.[2,3] Tiotropium was the first long-acting muscarinic receptor antagonist (LAMA), reaching the market in 2002.[4-6] Initially, tiotropium was prescribed for its bronchodilator effects in human subjects, but more recent evidence suggests that it might also be effective in improving patients' quality of life, reducing exacerbations, and increasing exercise capacity.[7] Tiotropium has also recently been shown to improve asthma symptoms and lung function in patients with inadequately controlled asthma.[8]

Preclinical and clinical studies have also suggested that tiotropium inhibits cough in tussive challenge models.[9,10] Dicipinigiatis et al[10] reported that tiotropium inhibits capsaicin (transient receptor potential [TRP] V1 agonist)–induced cough in patients with upper respiratory tract infections (URIs) in a prospective, randomized, double-blind, placebo-controlled clinical trial. Bouyssou et al[9] reported that tiotropium caused a dose-dependent inhibition of citric acid–induced cough in a guinea pig asthma model. An antitussive action of tiotropium in acute challenge models through its antimuscarinic activity is difficult to conceive. Various explanations have been proffered, including inhibition of vagal tone (and thereby mucus secretion and bronchodilation) elicited through blockade of muscarinic M_3 receptors on submucosal glands and airway smooth muscle, respectively. However, the mechanism behind the antitussive activity has never been fully elucidated.

The aim of this study was to investigate whether tiotropium can directly modulate airway sensory nerves and thereby tussive responses by using a range of techniques. We used an isolated vagus nerve preparation and calcium imaging of primary sensory jugular neurons to circumvent the potentially confounding

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Abbreviations used
$[Ca^{2+}]_i$: Intracellular calcium
COPD: Chronic obstructive pulmonary disease
DiI: DilC18(3)-1,1′-dioctacetyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate
DMSO: Dimethyl sulfoxide
ECS: Extracellular solution
K_{50}: 50 mmol/L potassium chloride extracellular solution
LAMA: Long-acting muscarinic receptor antagonist
MCh: Methacholine
Penh: Enhanced pause
PGE_2: Prostaglandin E_2
RTX: Resiniferatoxin
TRP: Transient receptor potential
URI: Upper respiratory tract infection

bronchodilator, anti-inflammatory, and antimucolytic properties of tiotropium, which are presumably associated with its antimuscarinic activity, and to avoid the pharmacokinetic and numerous other considerations that limit the interpretation of *in vivo* data. The ability to use human vagus nerve preparations also allowed us the opportunity to translate our findings to the clinical setting. An inhibitory activity on capsaicin-induced action potential firing *in vivo* confirmed an interaction of tiotropium with TRPV1 on airway-specific C-fibers.

In summary, our data suggest that tiotropium inhibits TRPV1 ion channel activity through a mechanism unrelated to its anticholinergic activity. This activity is not through a general inhibition of sensory nerve activity because TRPA1-mediated responses were not affected. In conclusion, we suggest that some of the clinical benefit associated with taking tiotropium could be explained through its inhibition of TRPV1 responses.

METHODS

Effect of tiotropium on capsaicin-induced cough

To establish an effective dosing regimen, we first performed a concentration response to inhaled tiotropium against methacholine (MCh)–induced bronchospasm (as estimated by changes in enhanced pause [Penh]). Conscious guinea pigs were exposed to either aerosolized vehicle (0.5% ethanol in saline) or tiotropium (3, 10, or 30 μg/mL; this equates to 6.35, 21.2, and 63.5 μmol/L solution) for 10 minutes and were challenged 50 minutes later with either saline or MCh (0.1 μg/mL). Changes in Penh were recorded for 5 minutes. From these data, doses of tiotropium were selected to be tested against capsaicin-induced cough, as previously described.[11-14] Briefly, after exposure to vehicle or tiotropium solution as above, cough was induced by exposing the guinea pigs to an aerosol of capsaicin (60 μmol/L) for 5 minutes. See additional methods in the Methods section in this article's Online Repository at www.jacionline.org.

Effect of tiotropium on isolated vagal sensory nerve tissue

Guinea pigs were culled with an overdose of pentobarbitone (200 mg/kg administered intraperitoneally). The 2 vagal trunks were carefully dissected free and placed in Krebs-Henseleit solution. The segments of vagus nerve were mounted in a grease-gap dual-recording chamber system, as previously described, and depolarization (as an indicator of sensory nerve activity) of the nerve was assessed.[11-14] Briefly, tissue was exposed to pre-established submaximal concentrations of the TRP agonist twice, treated with vehicle or test compound, and then rechallenged with the TRP agonist. After a wash step, the TRP agonist was reapplied. The effect of tiotropium was investigated on depolarization induced by a range of TRPV1 agonists, including capsaicin,[13,15] and against depolarization induced by the TRPA1 agonist acrolein (300 μmol/L)[12] and the TRPV4 agonist GSK1016790A (0.3 μmol/L).[16] Key experiments were repeated with human vagal tissue. Ethical approval to use recipient human lung/vagal tissue (transplant tissue) was obtained from the Royal Brompton & Harefield Trust (REC reference 09/H0708/72). See additional methods in the Methods section in this article's Online Repository.

Effect of tiotropium on airway-specific ganglion cells

Identification of airway-specific neurons was performed, as previously described.[17,18] Briefly, 14 days before the experiment, guinea pigs were dosed intranasally with the lipophilic retrograde tracer dye DilC18(3)-1,1′-dioctacetyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI). Guinea pigs were then killed, and the jugular ganglia were harvested to measure calcium movement and membrane voltage change, as described previously.[13] DiI-labeled neurons from jugular ganglia were then stained with both a ratiometric calcium-sensitive dye (Fura2-AM, 3 μmol/L) and a voltage-sensitive dye (Di-8-ANEPPS). The focus was on jugular ganglion cells because we have previously found these to be more responsive to capsaicin under normal conditions compared with airway nodose ganglion cells.[13] The responsiveness and viability of neurons were assessed by means of application of 50 mmol/L potassium chloride extracellular solution (K_{50}) at the start and end of recording. Intracellular calcium ($[Ca^{2+}]_i$) responses were recorded as the area under the curve, and membrane depolarization responses were recorded as the peak magnitude. All responses were normalized to the initial K_{50} application. See additional methods in the Methods section in this article's Online Repository.

Effect of tiotropium on capsaicin-driven TRPV1 FLIPR assays (performed with GenScript)

HEK293 cells were genetically modified to overexpress human TRPV1 and seeded in a 384-well, black-wall, clear-bottom plate at a density of 20,000 cells per well in 20 μL of medium. Cells were cultured for 18 hours before the day of the experiment and maintained at 37°C in 5% CO_2. Capsaicin concentration-response curves were performed to select a submaximal concentration in HEK293 cells overexpressing human TRPV1. Calcium-4, in conjunction with a Fluorescent Imaging Plate Reader, was used to record the signal. Assays were performed in duplicate. See additional methods in the Methods section in this article's Online Repository.

Effect of tiotropium on capsaicin-induced firing of single-fiber afferents and bronchospasm

Guinea pigs were anesthetized with urethane (1.5 g/kg) intraperitoneally. The trachea was cannulated, and bronchospasm was measured with an air-pressure transducer connected to a side arm of the tracheal cannula. Animals were paralyzed with vecuronium bromide, which was initially administered at an intravenous dose of 0.10 mg/kg and 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.[19] Briefly, after the vagus nerve was dissected clear of tissue, a single fiber was isolated and placed on the recording electrodes. After establishing it was an airway C-fiber, the animal was challenged with inhaled capsaicin after vehicle or inhaled test compound, and action potentials were recorded. See additional methods in the Methods section in this article's Online Repository.

Effect of tiotropium on isolated guinea pig tracheal contractions

Contractile responses to MCh or capsaicin were induced in isolated guinea pig trachea by using a system previously described.[20] Briefly, the trachea was placed in oxygenated Krebs-Henseleit solution at 37°C and exposed to