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T1 BACTERIA CAN TRIGGER AIRWAY SENSORY NERVES VIA THE ACTIVATION OF TLR2

MA Wortley, ED Dubuis, SJ Bonvini, MA Birrell, MG Belvisi. Imperial College, London, UK

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Introduction Excessive coughing is a key symptom of lower respiratory tract infections (LRTIs) especially when associated with exacerbations of respiratory diseases like asthma and COPD.^{1,2} As airway sensory nerve activation is required to trigger the cough response, we hypothesised that respiratory related bacteria could fire these nerves.

Methods *In vitro* tissue and neuron assay systems were employed, in conjunction with *in vivo* electrophysiology and a guinea pig cough model to investigate this hypothesis.

Results We show that a bacterial mimetic, LPS can activate guinea pig sensory nerve fibres *in vivo*. Furthermore, LPS, as well as heat-killed bacteria (*Haemophilus Influenzae* and *Streptococcus Pneumoniae*), activated isolated sensory nerve tissue (vagus) from guinea pigs (LPS 0.11 ± 0.04 mV; HI 0.14 ± 0.02 mV; SP 0.16 ± 0.05 mV, n=3) with similar Results in tissue obtained from human donor lungs and c57bl/6 mice. An investigation into the Toll-like receptor (TLR) involved revealed that it was TLR2, and not TLR4, with a TLR2 agonist (lipotechoic acid, LTA) causing activation of isolated vagus (0.14 ± 0.02 MTV), whereas a TLR4 agonist (ultra-pure LPS) did not (0.00 ± 0.00 mV). Furthermore LPS activated wild-type mouse vagus nerve (0.12 ± 0.01 mV) whereas it had little effect in TLR2-/- mouse vagus nerve (0.02 ± 0.00 mV). Indeed, using single cell RT-PCR, we found guinea pig airway-terminating neurons expressed TLR2 (3/69), but not TLR4 receptors (0/69). Further investigation showed that the TLR2 response at least partially required TRPA1 ion channels, with $55.25 \pm 3.51\%$ inhibition of LPS responses by the TRPA1 inhibitor J130.

Conclusion We have shown that bacteria can activate the sensory nerves associated with the cough response. We propose that this could be an endogenous defensive mechanism designed to expel bacterial infection, and/or could be a method harnessed by some infective agents to spread from carrier to new host. Furthermore, increased understanding of the mechanisms involved may lead to an insight into ways to modulate the excessive coughing, which plagues respiratory patients during exacerbation episodes.

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T2 OESTROGEN: AN ENDOGENOUS AGONIST FOR TRPM3 TRIGGERED SENSORY NERVE ACTIVATION IN THE AIRWAY?

SJ Bonvini, MA Wortley, JJ Adcock, E Dubuis, JA Bolaji, S D'Sa, J Ma, MA Birrell, MG Belvisi. Imperial College, London, UK

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Introduction In chronic lung diseases, activation of airway sensory nerves initiates respiratory reflexes including cough for which there is currently no safe and effective treatment. Ion

channels on sensory afferents can activate these reflexes and as such are attractive therapeutic targets. Using synthetic ligands we have shown that activation of TRPM3 can trigger human and guinea pig airway sensory nerves.¹ As TRPM3 is thought as a “steroid receptor” and women are consistently over represented in chronic cough clinics,² we hypothesised that oestrogen could be an endogenous agonist of TRPM3 mediated activation of airway sensory nerves.

Methods *Ex vivo* tissue and neuron assay systems were employed, in conjunction with *in vivo* electrophysiology and a guinea pig cough model to investigate this hypothesis.

Results *In vivo*, β -oestradiol caused firing of both C and A δ fibres and also elicited a cough response in conscious unrestrained guinea pigs. *Ex vivo*, β -Oestradiol caused a concentration dependant depolarisation of isolated guinea pig vagal nerves which was inhibited by the non-selective ER receptor antagonist ICI182780 ($92.7\% \pm 4.5\%$) and by the TRPM3 antagonist Isosakuranetin ($86.5\% \pm 6.8\%$). The ER receptor antagonist had no effect on the TRPM3 agonist (CIM0216) mediated depolarisation. Translational responses were obtained in human vagal tissue. Single cell PCR indicated that the TRPM3 ion channel and two oestrogen receptors GPER and ER α were expressed in airway specific nodose and jugular ganglia, and were co-expressed with TRPM3.

Conclusion These data show that the oestrogen can activate airway sensory nerves, and suggests that ER receptors may be activated upstream of TRPM3 activation. Further investigation is required, however this data may help to explain the higher number of females attending chronic cough clinics and suggests TRPM3 could be a novel therapeutic target for chronic cough.

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T3 INCREASED RESPIRATORY NEURAL DRIVE AND WORK OF BREATHING IN EXERCISE-INDUCED LARYNGEAL OBSTRUCTION

¹ES Walsted, ¹A Faisal, ²CJ Jolley, ¹LL Swanton, ¹MJ Pavitt, ³YM Luo, ⁴V Backer, ¹MI Polkey, ¹JH Hull. ¹Royal Brompton Hospital, London, UK; ²King's College London, London, UK; ³Guangzhou Medical College, Guangzhou, China; ⁴Bispebjerg University Hospital, Copenhagen, Denmark

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Background Exercise induced laryngeal obstruction (EILO), a clinical phenomenon in which the larynx closes inappropriately during physical activity, is a prevalent cause of exertional dyspnoea in young individuals and is often misdiagnosed as asthma. The physiological ventilatory impact of EILO and its relationship with dyspnoea has not been studied and we therefore sought to provide new insight by developing a methodology to facilitate synchronous evaluation of exercise related changes in laryngeal aperture on breathing pattern, pulmonary mechanics and respiratory neural drive.

Methods We prospectively evaluated six subjects with EILO and six healthy age- and gender-matched control subjects. Subjects underwent detailed physiological assessment and a symptom-limited incremental exercise test with simultaneous and synchronised laryngoscopic video, gastric-, oesophageal- and transdiaphragmatic pressures, diaphragm electromyography and respiratory airflow.