THE EXPRESSION AND FUNCTIONAL ROLE OF AIRWAY EPITHELIAL TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS IN RESPONSES TO ENVIRONMENTAL CHEMICAL AND THERMAL IRRITANTS

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Exposure to chemical (e.g. cigarette smoke, air pollutants) and thermal (e.g. cold air) irritants is an important environmental health risk factor, especially in patients with respiratory diseases including asthma, chronic obstructive pulmonary disease (COPD) and chronic cough. Due to the development of hypersensitive airway reflexes even relatively innocuous stimuli such as aerosols, strong odours or changes in air temperature frequently provoke coughing and bronchospasm in such patients. In recent years, a novel superfamily of cellular receptors termed the Transient Receptor Potential (TRP) channels has been extensively characterised (Venkatachalam & Montell, 2007; Wu et al., 2010). These channels play key roles in sensing diverse environmental factors as well as in signal transduction in different cell types (Damann et al., 2008). There is also growing evidence for their involvement in various disease states, including respiratory diseases (Nilius et al., 2007). However, TRP receptors in the human respiratory epithelium remain poorly characterised.

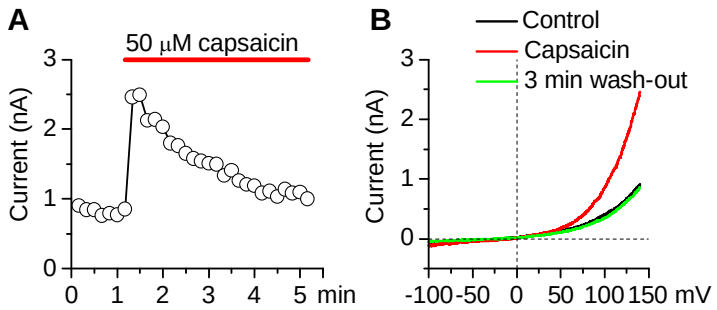

Fig. 1. Membrane current responses to TRPV1 agonist capsaicin application in PBECs. Time course at 140 mV (A) and current-voltage relationships (B) were measured by applying voltage ramps from -100 to 140 mV at 10 s interval.

We aimed to investigate the expression and functional roles of several TRPs, with primary focus on sensitive to temperature and pungent chemicals receptors, including TRPA1, TRPM8 and

TRPV1 nonselective calcium-permeable cation channels. TRP expression and function was examined in primary human nasal epithelial cells (NEC) obtained by nasal brushings, in primary human bronchial epithelial cells obtained by bronchial brushing from healthy volunteers (PBEC) and in cultured human bronchial epithelial cells (16HBE14o-). Molecular expression of TRP receptors was determined at an mRNA and protein level using quantitative real-time PCR (qRT-PCR), Western blots and immunocytochemistry. Functional expression was assessed based on the action of selective TRP agonists and antagonists with the use of patch-clamp recording techniques (whole-cell configuration) and laser confocal calcium imaging in Fluo-4 loaded cells.

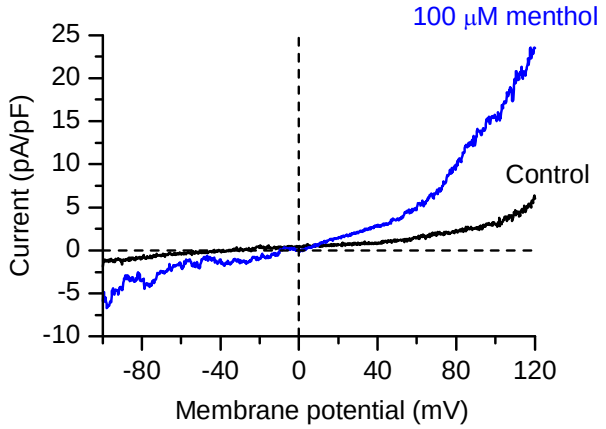

Fig. 2. Membrane current responses to menthol application (100 μM) in 16HBE14o- cells. Current-voltage relationships were measured by applying 500 ms voltage ramps from -100 to 120 mV in control and after TRPM8 agonist menthol application.

Gene transcripts for TRPA1, TRPM8 and TRPV1 were revealed in all cells by PCR and qRT-PCR. Protein expression for these channels was also detected in all cells, with the exception of TRPA1 in NEC.

TRPV1 is primary heat-sensitive receptor, which is also activated by a number of known respiratory irritants, in particular acidic gases and particulates contained in air pollution. Chemicals including capsaicin, the pungent extract from chilli pepper, cause burning sensation via activation of TRPV1 and readily induce cough in animals and humans. In contrast, TRPM8 and TRPA1 are primary detectors of cold, which also selectively respond to “cooling” compounds of plant origin such as menthol, eucalyptol and cinnamaldehyde. Confocal calcium imaging studies revealed a dose dependant activation of TRPM8 in HNEC in response to menthol application (EC_{50} ~ 60 μM) which was ablated in the presence of TRPM8 antagonist, 10 μM BCTC. TRPA1 functional expression was also revealed in PBEC by responses to cinnamaldehyde application (EC_{50} ~ 50 μM) which was ablated in the presence of TRPA1 antagonist, 30 μM HC030031. Patch-clamp experiments provided strong evidence for plasma membrane expression and function of these channels. Thus, application of specific TRP agonists, such as capsaicin (TRPV1 agonist, Fig. 1) and menthol (TRPM8 agonist, Fig. 2) produced robust membrane current responses with characteristic biophysical “TRP signatures” (current kinetics, outward rectification in current-voltage relationships) in bronchial epithelial cells.

These data show that TRPV1, TRPA1 and TRPM8 are functionally expressed in the lower and upper airways where they can play important roles in airway responses to chemical irritants, environmental pollutants and thermal stimuli. These channels are potentially important as they respond to noxious stimuli which typically trigger exacerbations of common respiratory diseases including asthma and chronic cough but further work is required to elucidate their involvement in respiratory diseases and validate them as putative novel drug targets.

References

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