Online Data Supplement

TRPA1 Agonists Evoke Coughing in Guinea-Pig and Human Volunteers

Mark A. Birrell, Maria G. Belvisi,Megan Grace, Laura Sadofsky, Shoaib Faruqi, David J. Hele, Sarah A. Maher, Véronique Freund-Michel and Alyn H.Morice

METHODS AND MATERIALS

Functional characterization of cloned TRPA1 expressed in HEK293 cells.

The TRPA1 expressing HEK293 cells and method used to measure increases in intracellular calcium levels used in this study have been described previously (E1). Briefly, hTRPA1 (Accession number Y10601) was cloned from human lung fibroblasts, ligated into pcDNA3 and sequenced (MWG-Biotech, Ebersberg, Germany) before transfecting HEK293 cells, using the lipfectamine 2000 (Invitrogen) method, with the construct. Cells were maintained in geneticin containing media (DMEM 10% FCS, 1mM sodium pyruvate, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 250 ng ml^{-1} amphotericin B and 0.6 mg ml^{-1} geneticin) and three rounds of single cell cloning were carried out to select permanently expressing TRPA1-HEK cells.

Compounds and materials

In vitro vagus experiments: All Krebs salts were obtained from BDH (Dorset, UK), and Krebs Henseleit solution was made fresh on a daily basis. Cinnamaldehyde, Acrolein, Allyl isothiocyanate were dissolved in 100% DMSO and stocks stored (1M) until required when aliquots were then diluted down in Krebs solution for testing. All other agents were purchased from Sigma-Aldrich, Poole, Dorset, UK. *In vivo guinea pig experiments:* Acrolein was supplied in liquid form at 15M. A 1M stock was made in saline (0.9%) and diluted in saline to 100mM. HC-030031(30 mg/ml) was suspended in vehicle (0.5% methyl cellulose in sterile saline), dosing volume 10 ml/kg i.p. (1500 mg in 50ml). *Human cough challenge*: Cinnamaldehyde was obtained from Sigma-Aldrich, Poole, Dorset Citric Acid was provided by Sheffield Manufacturing Pharmacy and supplied by Formosa Laboratories Inc, Taiwan.

RESULTS

In vitro functional characterization of TRPA1 ligands on cloned human cells (HEK293).

The TRPA1 receptor was successfully cloned from primary human fibroblasts and permanently expressed in HEK293 cells. RT-PCR showed that HEK293 cells do not endogenously express TRPA1 mRNA (Figure E1, in the Online Data Supplement). A clear band for TRPA1 was observed for hTRPA1-HEK mRNA whereas no bands were amplified for hTRPV1-HEK or mock transfected HEK (pcDNA3-HEK). Actin was amplified to test cDNA viability. Actin was observed in all cDNA samples tested (Figure E1, in the Online Data Supplement).

Effect of TRPA1 ligands on the isolated guinea pig vagal nerve preparation.

Therefore, to demonstrate a functional role for the TRPA-1 channel in a native tissue, we demonstrated a concentration-related increase in the depolarization of the guinea pig isolated vagus (indicative of sensory nerve activation) with acrolein (Figure 2a). Furthermore, data obtained with cinnamaldehyde observed a similar pattern (Figure E2).

REFERENCES

E1. Sadofsky LR, Campi B, Trevisani M, Compton SJ, Morice AH. Transient receptor potential vanilloid-1-mediated calcium responses are inhibited by the alkylamine antihistamines, dexbrompheniramine and chlorpheniramine. *Exp Lung Res.* 2008; 34: 681-693.

FIGURE LEGENDS

Figure E1: RT-PCR for hTRPA1 mRNA or actin from hTRPA1-HEK, hTRPV1-HEK, pcDNA3-HEK or no cDNA in HEK293 cells. TRPA1mRNA was observed for hTRPA1-HEK mRNA whereas no bands were amplified for hTRPV1-HEK or mock transfected HEK (pcDNA3-HEK). β-actin was amplified to test cDNA viability and was observed in all cDNA samples tested.

Figure E2: Characterization of the depolarization (mV) responses elicited by the isolated guinea pig vagus nerve preparations in response to the TRPA1 agonist cinnamaldehyde. Results are expressed as the mean ± s. e. mean of 4-6 experiments. Results are expressed as the mean ± s. e. mean of 4-6 experiments.

Figure E1

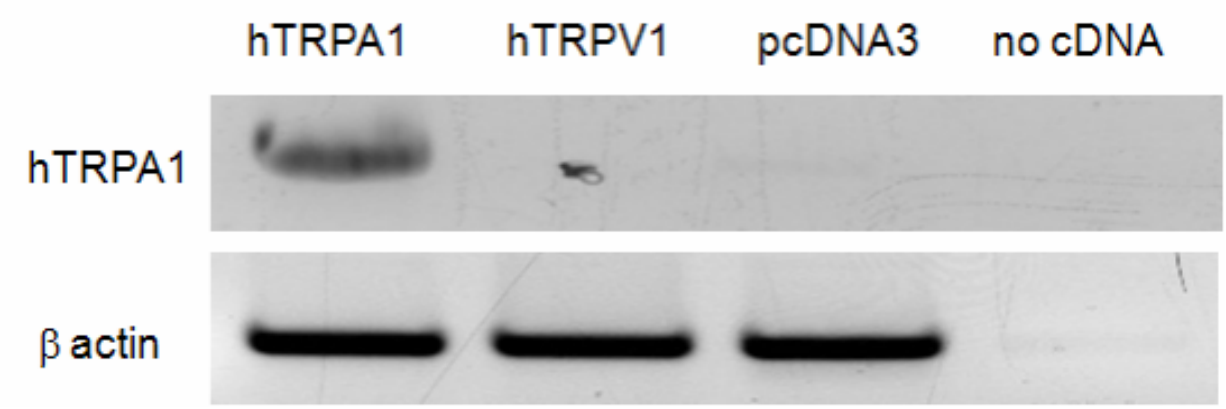

Figure E2