

## A possible role for TRPV4 receptors in asthma

Wolfgang Liedtke<sup>1,2</sup> and S. A. Simon<sup>2,3</sup>

Departments of <sup>1</sup>Neurology, <sup>2</sup>Neurobiology, and <sup>3</sup>Anesthesiology,  
Duke University Medical Center, Durham, North Carolina 27710

THE REPORT from Jia et al., the current article in focus (Ref. 15, see p. L272 in this issue), describes their findings in human bronchial smooth muscle cells in culture that can be stimulated with hypotonic solutions (15). This is an important finding with practical relevance for the understanding of the pathophysiology of bronchial hyperreactivity such as occurs in asthma. Asthma affects the populations of the United States and Europe (10, 22) and can become, in some patients, at times severe or even life threatening. Bronchial hyperresponsiveness is a hallmark of asthma. Asthma is accompanied by a denudation of the epithelial lining of the bronchi and bronchioli. This occurs within a pathophysiological framework described as “airway remodeling” (10, 22). In this condition, bronchial smooth muscles as well as nerve endings can thus become exposed directly to bronchial fluid that is itself hypotonic (see Fig. 1). The Jia et al. paper provides evidence that one member of the transient receptor potential (TRP) vanilloid subfamily (TRPV) family of the TRP channel superfamily of ion channels (8, 14), TRPV4 (26), that is found on airway smooth muscle cells, might function in the transduction of osmolarity/hypotonicity stimuli in airways.

The TRPV receptor subfamily consists of cation-selective, calcium-permeable ion channels that are widely expressed in both excitable and nonexcitable cells. Members of this family can be activated by diverse stimuli, including chemical irritants, protons, lipids, changes in cell volume possibly via membrane deformation, mechanical stimuli, and warm/noxious heat (1, 2, 4, 5, 14, 21, 25, 26). Despite recent progress, relatively little is understood regarding how members of the TRPV subfamily might respond to these stimuli *in vivo*.

Hypotonicity-activated ion channels permeable to calcium are ideal candidates to lead to contraction of bronchial smooth muscle cells. Of the TRPV subfamily of the TRP channel superfamily, TRPV4 [previously named VR-OAC, OTRPC4, TRP12, or VRL-2 (9, 18, 29, 32)] has been found by several groups to show activation in response to hypotonic stimulation in heterologous expression systems (18, 29, 32). We view the Jia et al. (15) paper as an important first step in elucidating the role of TRPV receptors in asthma. However, it is likely that it is part of a very complex story. To this point, the activation by hypotonicity and mechanical stimuli has been described recently for TRPV2 also (24) [previously named VRL-1 or GRC (6, 16)]. Because Jia et al. also detected the TRPV2 transcript in the bronchial smooth muscle cell line, its possible role also needs to be addressed. In this regard, in a recent study by Muraki et al. (24), vascular smooth muscle cells were found to express both TRPV4 and TRPV2, and their response to hypotonicity became greatly diminished after transfection with a TRPV2-specific antisense, which led to a reduction of TRPV2

protein expression. The investigations of Muraki et al. and Jia et al., when taken together, imply that we need to know about the expression of both TRPV2 and TRPV4 in smooth muscle cells in both bronchial airways and in vessels and other possible locations where smooth muscle surrounds a lumen. At the mRNA level, gene expression studies can be conducted at the transcriptome level in many species, and consequently, knowledge of the comprehensive expression profiles of TRP ion channels should be addressed in future studies. The approach by Muraki et al. to “knock down” protein expression of TRPV2 with an antisense strategy addresses the important issue of whether TRPV2 is necessary for the observed  $Ca^{2+}$  entry into the endothelial smooth muscle cells. Finally, it is important to elucidate the possible formation of TRPV2/TRPV4 multiprotein complexes in smooth muscle cells.

Although the “pharmacological” approach using 4 $\alpha$ -phorbol 12,13-didecanoate and ruthenium red aids in the identification of the receptors involved with airway hyperresponsiveness, the molecular approach of achieving a specifically targeted down-regulation of protein expression would yield less ambiguous results. This is because one transcript of a given *trpv* gene might respond to a certain ligand/activator, yet another one will not. On the other hand, a ligand/activator may stimulate two different channels derived from different genes. The former has been demonstrated for TRPV1 (7, 27) and the latter for anandamide and TRPV1/TRPV4 (28, 31, 34). In this context, a general question to be addressed is whether smooth muscle cells that line the lumina of various organs and that are endowed with responsiveness to osmotic stimuli (hypotonicity) and mechanical dilation are dependent on the same or a different molecular machinery to transduce the respective stimulus.

The physiological function of TRPV4 in (bronchial) smooth muscle cells *in vivo* can be approached by the use of *trpv4*<sup>-/-</sup> mice (19, 23, 30). These mice have been found to harbor abnormalities in their response to systemic osmotic and somatosensory mechanical stimuli. They have been investigated for their response to systemic osmotic stress and to external somatosensory stimuli. Thus lung function and smooth muscle cell function await exploration in these animals. In general, working with a general null genotype that is not lethal entails the possibility that other compensatory genes or pathways are upregulated in the null. This might be the case in *trpv4*<sup>-/-</sup> mice that show a relatively mild phenotype. To address more specifically the question of what TRPV4 does in live animals, tissue/cell-specific knockdowns will have to be generated. A smooth muscle/cell-specific *cre*<sup>+</sup> mouse has recently been reported by Xin et al. (33). In depth studies of gene-expression patterns (e.g., by gene arrays) can possibly lead to the isolation of bronchial smooth muscle cell-specific genes, which in turn will permit the generation of more specific *cre* mice. Transgenic expression of *cre* in mice can be accomplished not only under the control of tissue/cell-specific promoters, but also

Address for reprint requests and other correspondence: S. A. Simon, Depts. of Neurobiology and Anesthesiology, Duke Univ. Medical Center, Durham, NC 27710 (E-mail: sas@neuro.duke.edu).

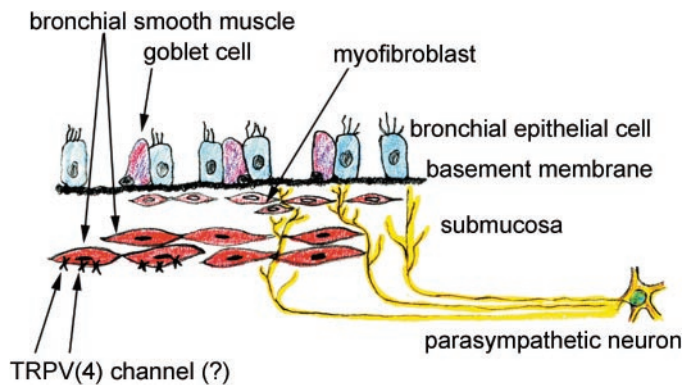


Fig. 1. Airway remodeling in asthma and a potential role for transient receptor potential vanilloid (TRPV4) on bronchial smooth muscle cells. Depicted is a schematic drawing of a bronchial epithelium in chronic asthma, a condition known to be associated with “airway remodeling.” The bronchial epithelium is changed from a regular single layer of bronchial epithelial cells with the occasional goblet cell, a mucin-producing cell, to a denuded layer with gaps, goblet cell hyperplasia, and rarefied bronchial epithelial cells, which have a tendency to transdifferentiate to squamous cell epithelial cells. The underlying basement membrane is thickened, and there is proliferation of myofibroblastoid cells between the basement membrane and the smooth muscle cells, which are hypertrophic and hyperplastic. A denuded, remodeled epithelial layer, as in chronic asthma or other chronic conditions associated with bronchial hyper-reactivity, would represent a pathological entryway for activators of TRPV4 channels possibly expressed on smooth muscle cells. This could also be true for TRPV2 channels. Free, unmyelinated nerve endings of the vagal nerve that innervate the edematous submucosa and that may also contain TRPV4 receptors are not depicted, for clarity. Also not shown are other pathological changes observed in asthma/airway remodeling such as excess luminal mucus, epithelial and luminal eosinophils, submucosal lymphoid cells and mast cells, mucous gland hypertrophy, and vasodilation of submucosal small vessels.

under the control of an inducible element, thus allowing temporal control (12). In principle, this permits the specific knock down of gene function in a specific organ/cell type in a live animal at a specific time point in the life of the animal. Given the physiological and clinical relevance of contraction of smooth muscle cells in vascular beds and bronchial airways, this appears to be a worthy long-term goal.

These considerations, in principle, also apply to the TRP melastatin (TRPM3) subfamily, which has been reported to be responsive to hypotonic stimuli when expressed in heterologous cellular systems, albeit with a slightly different response profile than TRPV4 and TRPV2 (most important being a more sluggish response kinetics) (13).

The biological role of TRPV4 in the lung is likely to be more complex than its role in bronchial smooth muscle. TRPV4 gene expression has been detected in tracheal epithelial cells, bronchial glandulae, and alveolar cells, particularly macrophages (gene expression was detected by either in situ hybridization or immunohistochemistry) (Ref. 9 and Liedtke, unpublished observations). If TRPV4 is present in sensory nerve endings in lung tissue, its activation could produce bronchoconstriction through an axon reflex.

In aggregate, the paper by Jia et al. (15) points toward the key relevance that an increased understanding of bronchial smooth muscle cell contractility will have for a rational targeting of new candidate drugs aiming at bronchial hyperresponsiveness. The osmotically/mechanically activated TRPV4 channel, which might function as a transduction channel for osmotic and mechanical stimuli (3, 11, 19, 20, 25), is now a

tentative new participant in this scenario. If confirmed, TRPV4 and possibly other TRP(V) channels will be targets for drug development for the treatment of bronchial hyperresponsiveness/asthma and maybe steroid-refractory asthma in particular. But we are all aware that asthma involves a diverse crowd of players, some of them from immunology, some of them from basic cell biology, and some, TRPV ion channels!

GRANTS

This work was supported by National Institutes of Health Grants ES-09844 (to S. A. Simon) and 7K08 MH-64702 (to W. Liedtke).

REFERENCES

1. Agopyan N, Bhatti T, Yu S, and Simon SA. Vanilloid receptor activation by 2- and 10-microm particles induces responses leading to apoptosis in human airway epithelial cells. *Toxicol Appl Pharmacol* 192: 21–35, 2003.
2. Agopyan N, Head J, Yu S, and Simon SA. TRPV1 receptors mediate particulate matter-induced apoptosis. *Am J Physiol Lung Cell Mol Physiol* 286: L563–L572, 2004.
3. Alessandri-Haber N, Yeh J, Boyd AE, Parada CA, Chen X, Reichling DB, and Levine JD. Hypotonicity induces TRPV4-mediated nociception in rat. *Neuron* 39: 497–511, 2003.
4. Caterina MJ and Julius D. Sense and specificity: a molecular identity for nociceptors. *Curr Opin Neurobiol* 9: 525–530, 1999.
5. Caterina MJ and Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci* 24: 487–517, 2001.
6. Caterina MJ, Rosen TA, Tominaga M, Brake AJ, and Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* 398: 436–441, 1999.
7. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, and Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389: 816–824, 1997.
8. Clapham DE, Runnels LW, and Strubing C. The TRP ion channel family. *Nat Rev Neurosci* 2: 387–396, 2001.
9. Delany NS, Hurle M, Facer P, Almadaf T, Plumpton C, Kinghorn I, See CG, Costigan M, Anand P, Woolf CJ, Crowther D, Sanseau P, and Tate SN. Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. *Physiol Genomics* 4: 165–174, 2001.
10. FitzGerald JM and Macklem P. Fatal asthma. *Annu Rev Med* 47: 161–168, 1996.
11. Gao X, Wu L, and O’Neil RG. Temperature-modulated diversity of TRPV4 channel gating: activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways. *J Biol Chem* 278: 27129–27137, 2003.
12. Grill MA, Bales MA, Fought AN, Rosburg KC, Munger SJ, and Antin PB. Tetracycline-inducible system for regulation of skeletal muscle-specific gene expression in transgenic mice. *Transgenic Res* 12: 33–43, 2003.
13. Grimm C, Kraft R, Sauerbruch S, Schultz G, and Harteneck C. Molecular and functional characterization of the melastatin-related cation channel TRPM3. *J Biol Chem* 278: 21493–21501, 2003.
14. Gunthorpe MJ, Benham CD, Randall A, and Davis JB. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci* 23: 183–191, 2002.
15. Jia Y, Wang X, Varty LA, Rizzo CA, Yang R, Correll CC, Phelps PT, Egan RW, and Hey JA. Functional TRPV4 channels are expressed in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 287: L272–L278, 2004.
16. Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, and Kojima I. Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat Cell Biol* 1: 165–170, 1999.
17. Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, and Heller S. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* 103: 525–535, 2000.
18. Liedtke W and Friedman JM. Abnormal osmotic regulation in *trpv4*<sup>-/-</sup> mice. *Proc Natl Acad Sci USA* 100: 13698–13703, 2003.
19. Liedtke W, Tobin DM, Bargmann CI, and Friedman JM. Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *C. elegans*. *Proc Natl Acad Sci USA* 100: 14531–14536, 2003.

21. Liu L, Zhu W, Zhang ZS, Yang T, Grant A, Oxford G, and Simon SA. Nicotine inhibits voltage-dependent sodium channels and sensitizes vanilloid receptors. *J Neurophysiol* 91: 1482–1491, 2004.
22. Maddox L and Schwartz DA. The pathophysiology of asthma. *Annu Rev Med* 53: 477–498, 2002.
23. Mizuno A, Matsumoto N, Imai M, and Suzuki M. Impaired osmotic sensation in mice lacking TRPV4. *Am J Physiol Cell Physiol* 285: C96–C101, 2003.
24. Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, and Imaizumi Y. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ Res* 93: 829–838, 2003.
25. Mutai H and Heller S. Vertebrate and invertebrate TRPV-like mechanoreceptors. *Cell Calcium* 33: 471–478, 2003.
26. Nilius B, Watanabe H, and Vriens J. The TRPV4 channel: structure-function relationship and promiscuous gating behaviour. *Pflügers Arch* 446: 298–303, 2003.
27. Schumacher MA, Moff I, Sudanagunta SP, and Levine JD. Molecular cloning of an N-terminal splice variant of the capsaicin receptor. Loss of N-terminal domain suggests functional divergence among capsaicin receptor subtypes. *J Biol Chem* 275: 2756–2762, 2000.
28. Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, Muir AI, Chambers JK, Randall AD, and Davis JB. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br J Pharmacol* 129: 227–230, 2000.
29. Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, and Plant TD. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat Cell Biol* 2: 695–702, 2000.
30. Suzuki M, Mizuno A, Kodaira K, and Imai M. Impaired pressure sensation in mice lacking TRPV4. *J Biol Chem* 278: 22664–22668, 2003.
31. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, and Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424: 434–438, 2003.
32. Wissenbach U, Bodding M, Freichel M, and Flockerzi V. Trp12, a novel Trp related protein from kidney. *FEBS Lett* 485: 127–134, 2000.
33. Xin HB, Deng KY, Rishniw M, Ji G, and Kotlikoff MI. Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. *Physiol Genomics* 10: 211–215, 2002.
34. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, and Hogestatt ED. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452–457, 1999.

