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AIM AND SCOPES

Cell Membranes and Free Radical Research is a print and online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+ - K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

C- Interaction Between Oxidative Stress and Ion Channels

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

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Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

Functional expression of TRPA1 cation channels in vestibular type II hair cells of the guinea pig

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List of abbreviations

AITC	allylisothiocyanate
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
URB597	3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate
TRP	transient receptor potential
TRPA	subfamily "A" (as in ankyrin) of TRP channels

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Abstract

Although the expression of TRPA1 (also known as ANKTM1) proteins has been demonstrated in hair cells of the inner ear, the role of this Ca^{2+}-permeable cation channel is unclear because TRPA1 knock-out mice have normal transduction currents in hair cells and do not show hearing impairment or vestibular problems. To test whether TRPA1 expression leads to the formation of intact ion channels in the plasma membrane of vestibular type II hair cells in the guinea pig, we measured whole-cell currents before and after stimulation with the specific agonists of TRPA1, allylisothiocyanate (AITC, 200 – 400 µM) and 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597, 100 µM). AITC induced currents with the typical current-voltage relation of TRPA1, as found in heterologous expression models. Currents densities reached maxima 168 ± 22 pA/pF (n = 41) at a holding potential of +60 mV and -62 ± 16 pA/pF at -60 mV. Current kinetics were characterized by an initial increase in amplitude over about 60 s, a subsequent plateau, and a complete current decline after wash-out of the drug. Repeated stimulations were possible. In the presence of URB597, similar currents developed but showed rapid desensitization under ongoing stimulation. We conclude that there is functional expression of TRPA1 in vestibular hair cells, at a current density relatively small in comparison to voltage gated currents. Thus, TRPA1 currents may modulate the electrical responses of hair cells. This may be relevant as potential side effects of many drugs and substances known to be activators of the polymodal channels TRPA1.

Keywords

TRPA1, vestibular hair cells, patch-clamp

Introduction

The cation channel TRPA1 is widely expressed in sensory neurons and can be activated by a large number of different stimuli, including varios pungent agents, cold temperature, alkaline pH and possibly mechanical membrane deflection (Jordt et al., 2004; Bandell et al., 2004; Bautista et al., 2005; Niforatos et al., 2007; Zurborg et., 2007). Thereby, TRPA1 enables the integrative transduction of a wide range of noxious stimuli. Moreover, it may mediate cellular responses to oxidative stress and membrane damage (Macpherson et al., 2007). Since it is expressed in hair cells of the cochlea or the vestibulum, it has been speculated that TRPA1 may contribute to the function of the inner ear. It has even been speculated that it may have a role in the transduction process of hair cells that involves an electrical response to a mechanical stimulus, i.e. the deflection of the stereocilia (Corey et al. 2004; Nagata et al., 2005). Such speculations, however, have not been supported by experiments on mice in which the gene for TRPA1 had been deleted. These TRPA1 knock-out mice do not show any sign of hearing impairment, in spite of extensive investigation. There was no indication of gross vestibular disorders (Kwan et al., 2006), either, although more subtle tests of vestibular functions are not easily possible in mice and have not been performed. Moreover, the transduction current in utricular hair cells induced by deflection of hairs was virtually identical in wild-type and knock-out mice (Kwan et al., 2006). Taken together, the role of TRPA1 in the inner ear seems unclear so far and is not likely to be prominent.

Evidence for TRPA1 expression in hair cells has been mostly provided with methods including in situ hybridization and antibody labelling (Corey et al. 2004; Takumida et al., 2008). These methods provide evidence for the presence of TRPA1 proteins but do not demonstrate that they form functional ion channels. In the light of the results on knock-out mice, it may be taken into doubt that operational TRPA1 channels exist in sensory cells of the inner ear that would provide ion currents in response to the established stimuli of TRPA1.

Therefore, the aim of the present study was an investigation of functional expression of TRPA1 in vestibular hair cells. We set up an electrophysiological study to measure whole-cell currents which might have the characteristic properties of TRPA1, as elsewhere reported either in overexpression models (Patil et al., 2010) or in cells endogenously expressing the channel (Vilceanu et al., 2010). We report functional evidence for TRPA1 expression in vestibular type II hair cells of the guinea pig even though the current density is comparably small.

Material and methods

Preparation of vestibular type II hair cells

Vestibular hair cells were prepared from young adult albino guinea pigs (weight 280–400 g) as described in detail previously (Düwel et al., 2005). In short, utricles were excised under microscopic control and dissected mechanically as well as enzymatically with the aid of collagenase IV and protease II. The procedure yielded 10–15 single cells, with visible hair bundles identified as vestibular type-II hair cells according to the morphological criteria proposed by (Ricci et al. 1997). Cells were transferred with an Eppendorf pipette into a bath chamber and allowed to settle and adhere to glass coverslips coated with Cell-Tak (Collaborative Biomedical Products, Meylan, France). All experiments were carried out in accordance with the German law on the protection of animals and international ethical guidelines for animal experiments.

Measurements of currents and membrane potentials with the patch-clamp technique

Patch clamp recordings were performed in the conventional whole-cell mode (Hamill et al., 1981) using a HEKA EPC-9 patch-clamp amplifier and a HEKA Pulse software (HEKA Elektronik, Lambrecht/Pfalz, Germany). For details see Haasler et al., 2009.

Cells had an average membrane capacitance of 4.25±1.03 pF and a series resistance of 18.83±4.3 MΩ (n=41). Cells were continuously superfused at a rate of 2.0 ml/min; the height of the bath was controlled and kept constant as described (Duong Dinh et al., 2006). The standard holding potential was -60 mV. Voltage ramps (from –80 to +80 mV and back, over 0.1 s) were applied every 20 s. For analysis, currents are given as current densities, calculated by dividing the current amplitude by the cell capacitance. All experiments were carried out at room temperature (21–23°C).

Solutions

The intracellular (pipette) solution contained: glutamic acid, 145 mM; NaCl, 8 mM; $MgCl_2$, 2 mM; ethylene glycol-bis (β-amino-ethylether)-N,N,N',N'-tetraacetic acid (EGTA), 10 mM; ATP, 0.3 mM; HEPES, 10 mM; pH 7.25 (CsOH). The extracellular (superfusate) solution contained tetraethylammonium chloride (TEA-Cl), 136 mM; $MgCl_2$, 1.8 mM; $CaCl_2$, 1.8 mM; N-2-hydroxyethylpiperazine- N'-2-ethanesulfonic acid (HEPES), 10 mM; pH 7.25.

For the stimulation of TRPA1 currents, the superfusate was changed to a solution supplemented with either allyl isothiocyanate (AITC, 200-400 μM) or the fatty

acid amide hydrolase inhibitor 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597, 100 µM, Cayman Chemical, Ann Arbor, USA).

All chemicals were from Sigma (Deisenhofen, Germany) if not indicated otherwise.

Statistics

Statistical evaluation was performed with the SPSS 12 software (SPSS Inc., Chicago, IL, USA). The effects of current changes were tested for statistical significance with the Wilcoxon nonparametric test for paired data. An error probability of $p<0.05$ was considered significant. Data are given as mean ± standard deviation (SD).

Results

Vestibular type II hair cells were tested for currents through TRPA1 in the conventional whole-cell mode of the patch-clamp technique. Voltage-gated K^+ currents were avoided by the composition of the intracellular and extracellular solutions, whereas voltage gated Ca^{2+} currents were minuscule in these solutions and did not occur at either -60 or +60 mV. Base-line currents were recorded during repeated voltage ramps from -80 mV to +80 mV until a stable plateau had been developed after about 20 seconds. Then, TRPA1 was stimulated, either with AITC in two concentrations (200 and 400 µM) or the synthetic TRPA1 agonist URB597 (100µm), usually for 200 s.

A representative experiment (out of n = 29) is documented in Figure 1, showing the characteristic response to 200 µM AITC that induced inward and outward currents with the typical current-voltage response frequently reported for TRPA1 (Macpherson et al., 2005). In particular, there was a marked outward rectification of whole-cell currents. Currents developed quickly and reached a plateau within 40-60 s which was maintained until AITC was washed out again after 200 s (Figure 1). In two further experiments, a constant current level was kept over a stimulation time of 400 s before a wash-out brought the current amplitude back to baseline levels. Repeated stimulations separated by a recovery of a few minutes resulted in a response virtually identical with the first one.

At a higher concentration (400 µM), AITC induced larger current amplitudes in the outward as well as in the inward direction (Figure 2). The rectification was still present but less prominent than at the lower concentration of the agonist.

URB597 was comparably effective as AITC on TRPA1 with respect to the current amplitudes in the outward direction. However, the rectification was less pronounced, resulting in fairly large inward currents. Moreover, the kinetics of the currents showed a different behavior. A current decline was consistently observed after about 65 ±10 s. At the end of the stimulation which was usually applied for 200 s, a complete current desensitization had developed (Figure 3).

For each experiment, the ramp with the largest deviation from base-line values was taken as maximal response and used to calculate the average of all

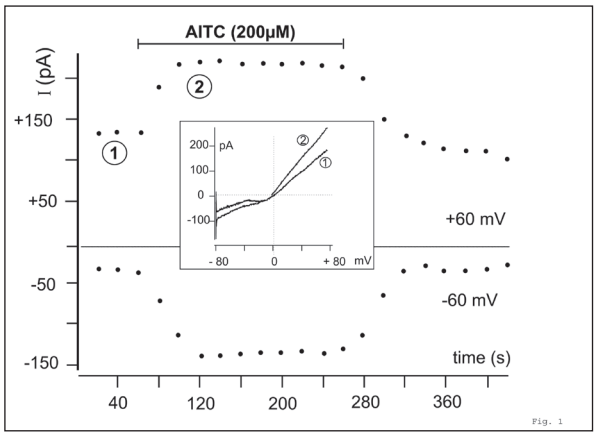

Figure 1. TRPA1 currents in vestibular type 2 hair cells under stimulation with AITC (200 µM, representative traces from one whole-cell patch-clamp experiments). Voltage ramps from -80 mV to +80 mV were regularly applied every 20 s. Current values are shown for -60 and +60 mV as determined from the ramps, before, during, and after superfusion of the cells with a solution containing 200 µM AITC. The holding potential between the ramps was -60 mV. The inset shows 2 ramps, obtained at time points 1 and 2 during the experiment.

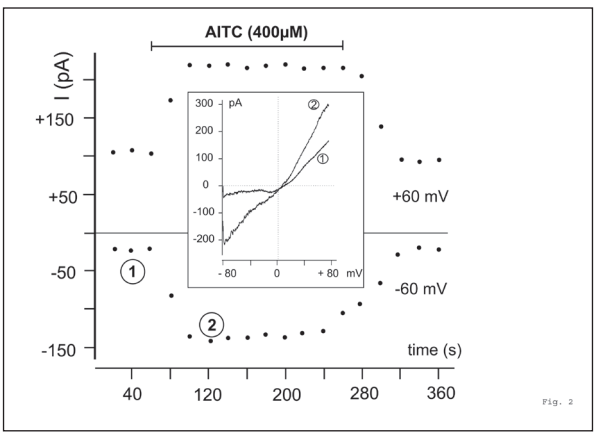

Figure 2. TRPA1 currents induced by AITC (400 µM). For details see legend of Figure 1. Note the larger inward current component in comparison to the experiment of Figure 1 with a lower concentration of the stimulus.

experiments, which is presented in Figure 4. All three stimuli induced significant ($p < 0.05$) currents at either holding potential but more prominently in the outward direction. In particular, the mean current densitiy in the presence of AITC (200 µM) was 112 ±8 pA/pF (± SD) at +60 mV but -22 ±5 pA/pF at -60 mV. The corresponding values in the presence of the higher concentration of AITC (400 µM) were 168 ±22 pA/pF (+60 mV) and -62 ±16 pA/pF (-60 mV), demonstrating that the effects of AITC were dependent on the agonist concentration. Currents with the least rectification were induced by URB597: 132 ±10 pA/pF (+60 mV) and -64 ±12 pA/pF (-60 mV).

Discussion

As main finding, we report evidence for functional expression of TRPA1 in vestibular type II hair cells because whole-cell currents characteristic for TRPA1 were stimulated by AITC and URB597 Thus, the reported absence of vestibular alterations in TRPA1 knock-out mice should not be attributed to the absence of functional TRPA1 channels the wild-type.

TRPA1 is the only channel known to respond to AITC, an important ingredient of garlic. This alone would indicate the currents observed in vestibular hair cells are attributable to TRPA1. Moreover, the currents induced by AITC were characteristic for TRPA1, as extensively studied in experiments using heterologous expression. In particular, they showed the typical outward rectification in the current-voltage-relation (Bautista et al., 2005). In addition, they were induced as well by URB597, again considered specific for TRPA1, although not as widely used (Niforatos et al., 2007). As a minor difference to previous results in overexpression models, no desensitization of the current took place under ongoing stimulation with AITC, whereas a moderate and slow desensitization was observed when the stimulation was performed with URB597. It is likely that the lack of desensitization is caused by the relatively low current density in hair cells because processes such as PIP2 depletion and Ca^{2+} influx are considered a factor of the desensitization of TRPA1 at high expression (Kim et al., 2008).

It has been demonstrated that knock-out mice display the same transduction current in hair cells as wild-type animals. Hence, any significant contribution of TRPA1 to the transduction process under normal conditions seems to be excluded (Kwan et al., 2006). On the other hand, TRPA1 is a channel with little constitutive activity and needs to be stimulated for biological effects, even though the spectrum

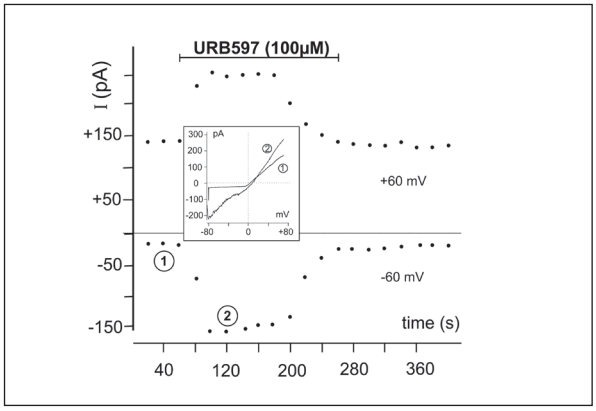

Figure 3. TRPA1 currents induced by the fatty acid amide hydrolase inhibitor 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597). For details see legend of Figure 1. Note the current decline starting already before the end of the superfusion with the stimulus.

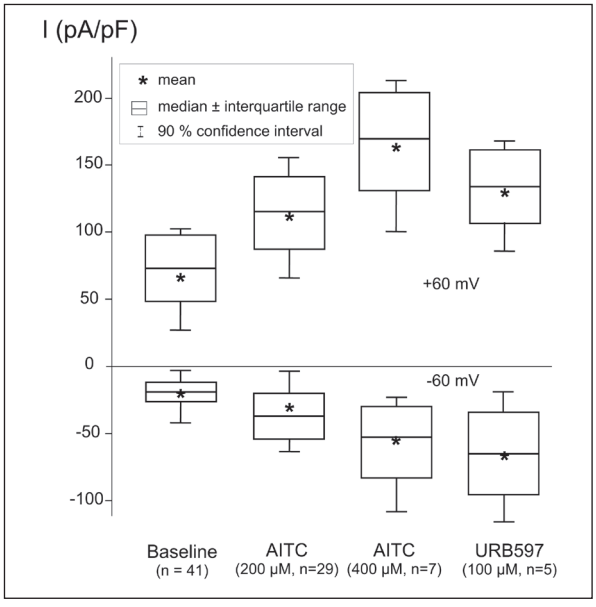

Figure 4. Summary of the effects of AITC and URB597 on inward and outward TRPA1 currents in vestibular hair cells. For each experiment, the maximal current density at -60 and +60 mV was determined at baseline as well as during stimulation and used for a calculation of the arithmetic mean (indicated by an asteric) and the median (horizontal line within each box), along with the the 90 % confidence interval (error bars) and the interquartile range (i.e. the range that contains the middle 50% of the data) shown as box.

of possible stimuli is rather wide. Therefore, the findings on knock-out mice do not rule out that TRPA1 may be used as a modifier of vestibular function when it is stimulated, either chemically such as by drugs or under pathological conditions.

The current density found in the present study under maximal stimulation and at -60 mV was about -80 pA/pF, whereas a current of 18 pA/pF was present already at baseline conditions. This baseline current may contain traces of TRPA1 but should mostly represent leak and non-specific currents through other cation channels; a stimulated TRPA1 activity is therefore in the range of 60 pA/pF. This value may be related to that of the transduction current; under similar conditions with respect to ion concentrations and transmembrane potential, a value of about 100 pA/pF has been reported in cochlear hair cells (Fettiplace, 2009). Therefore, a major change in vestibular transduction may take place when TRPA1 is maximally stimulated. However, an intermediate level of TRPA1 activation was mostly demonstrated by outward currents that occur at strongly positive holding potentials. Such potentials are unlikely to occur in situ and do not have direct physiological relevance. Inward currents at physiologically relevant membrane potentials were small in the presence of submaximal concentrations of AITC. Therefore, if situations exist when TRPA1 assumes a functional relevance, a requirement for a quite strong stimulation is expected.

As potential stimuli relevant in the inner ear in some conditions, various substances may be considered such as nicotine (Talavera et al., 2009) and propofol (Lee et al., 2008; Fischer et al.,2010). For both of these, an association with an increased incidence of vestibular vertigo has been implicated (Knox and McPherson, 1997; Lin and Young, 2001), although a direct link to TRPA1 is far from being established. Furthermore, TRPA1 may be a sensor for oxidative stress and reactive oxygen species (Hill and Schaefer, 2008; Bessac et al., 2008; Taylor-Clark et al., 2008). These findings may have major implications for the vestibular organ because oxidative stress is considered an important contributing pathological factor for vestibular diseases characterized by endolymphatic hydrops and attacks of vestibular vertigo (Labbé et al., 2005; Hayashi et al., 2010).

In conclusion, while TRPA1 does most likely not contribute to the function of the inner ear under normal conditions, especially not to the normal transduction process, the present study demonstrates the functional expression of the channel protein in vestibular hair cells and that currents may be induced by typical and specific stimuli of TRPA1. Therefore, a role of TRPA1 under some pathological conditions may be prevalent in the vestibulum. In particular, if a drug that potentially interferes with the multi-modal channel TRPA1 causes vestibular side effects, the action on TRPA1 may be considered as the molecular link.

REFERENCES

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ and Patapoutian A. 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41: 849-857.

Bautista DM, Movahed P, Hinman A, Axelsson H E Sterner O, Högestätt ED, Julius D, Jordt SE and Zygmunt P. M. 2005. Pungent products from garlic activate the sensory ion channel TRPA1. Proc. Natl. Acad. Sci. U.S.A., 102: 12248-12252.

Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, Jordt SE. 2008. TRPA1 is a major oxidant sensor in murine airway sensory neurons. J Clin Invest 118: 1899-1910.

Corey DP, García-Añoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfler BH, Duggan A, Géléoc GS, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, and Zhang DS. 2004. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432: 723-730.

Duong Dinh TA, Jüngling E, Strotmann KH, Westhofen M, Lückhoff A. 2006. Ultrasonic bath depth control and regulation in single cell recordings. Pflugers Arch 452: 784-788.

Düwel P, Haasler T, Jüngling E, Duong TA, Westhofen M, Lückhoff A. 2005. Effects of cinnarizine on calcium and pressure-dependent potassium currents in guinea pig vestibular hair cells. Naunyn Schmiedebergs Arch Pharmacol 371: 441-448.

Fettiplace R. 2009. Defining features of the hair cell mechanoelectrical transducer channel. Pflügers Arch 458: 1115-1123.

Fischer MJ, Leffler A, Niedermirtl F, Kistner K, Eberhardt M, Reeh PW, Nau C. 2010. The general anesthetic propofol excites nociceptors by activating TRPV1 and TRPA1 rather than GABAA receptors. J Biol Chem 285: 34781-34792.

Haasler T, Homann G, Duong Dinh TA, Jüngling E, Westhofen M, Lückhoff A. 2009.Pharmacological modulation of transmitter release by inhibition of pressure-dependent potassium currents in vestibular hair cells. Naunyn Schmiedebergs Arch Pharmacol 380: 531-538.

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. 1981. Improved patchclamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391: 85-100.

Hayashi K, Kobayashi R, Kitamura K, Goto F, Ogawa K, Matsumoto T. 2010. A novel model for prognosis of Meniere's disease using oxidative stress susceptibility of lymphoblastoid cell lines. Biosci Trends 4: 72-78.

Hill K, Schaefer M. 2008. Ultraviolet light and photosensitising agents activate TRPA1 via generation of oxidative stress. Cell Calcium 45: 155-164.

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED, Meng ID and Julius D. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427: 260-265.

Kim D, Cavanaugh EJ, Simkin D. 2008. Inhibition of transient receptor potential A1 channel by phosphatidylinositol-4,5-bisphosphate. Am J Physiol Cell Physiol 295: C92-99.

Knox GW, McPherson A. 1997. Menière's disease: differential diagnosis and treatment. Am Fam Physician 55: 1185-1190, 1193-1194.

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ and Corey DP. 2006. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50: 177-180.

Labbé D, Teranishi MA, Hess A, Bloch W, Michel O. 2005. Activation of caspase-3 is associated with oxidative stress in the hydropic guinea pig cochlea. Hear Res 202: 21-27.

Lee SP, Buber MT, Yang Q, Cerne R, Cortés RY, Sprous DG, Bryant RW. Br J Pharmacol. 2008. Thymol and related alkyl phenols activate the hTRPA1 channel. 153: 1739-1749.

Lin CY, Young YH. 2001. Effect of smoking on the treatment of vertigo. Otol Neurotol 22: 369-372.

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A. 2005. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15: 929-934.

Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP and Patapoutian A. 2007. An ion channel essential for sensing chemical damage. J Neurosci 17;27: 11412-11415.

Nagata K, Duggan A, Kumar G. and García-Añoveros J. 2005. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 25: 4052-4061.

Niforatos W, Zhang XF, Lake MR, Walter KA, Neelands T, Holzman TF, Scott VE, Faltynek CR, Moreland RB and Chen J. 2007. Activation of TRPA1 channels by the fatty acid amide hydrolase inhibitor 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597). Mol. Pharmacol 71: 1209-1216.

Patil MJ, Jeske NA, Akopian AN. 2010. Transient receptor potential V1 regulates activation and modulation of transient receptor potential A1 by Ca2+. Neuroscience 171: 1109-1119.

Ricci AJ, Cochran SL, Rennie KJ, Correia MJ. 1997. Vestibular type I and type II hair cells. 2: Morphometric comparisons of dissociated pigeon hair cells. J Vestib Res 7: 407-420.

Takumida M, Ishibashi T, Hamamoto T, Hirakawa K, and Anniko, M. 2008. Expression of transient receptor potential channel melastin (TRPM) 1-8 and TRPA1 (ankyrin) in mouse inner ear. Acta Otolaryngol. 1-11.

Taylor-Clark TE, McAlexander MA, Nassenstein C, Sheardown SA, Wilson S, Thornton J, Carr MJ, Undem BJ. 2008. Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal. J Physiol 586: 3447-3459.

Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JA, Damann N, Everaerts W, Benoit M, Janssens A, Vennekens R, Viana F, Nemery B, Nilius B, Voets T. 2009. Nicotine activates the chemosensory cation channel TRPA1. Nat. Neurosci 12: 1293-1299.

Vilceanu D, Stucky CL. 2010. TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons. PLoS One 5: 12177.

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA. 2007. Direct activation of the ion channel TRPA1 by Ca2+. Nat. Neurosci 10: 277-279.

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