TRPA1: the species difference

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The recent *Journal of General Physiology* Perspectives (133:227–229, 245–262) deal with several key issues in TRPA1. The four informative papers (Bang and Hwang, 2009; Caspani and Heppenstall, 2009; Kwan and Corey, 2009; Latorre, 2009) provided a comprehensive review on the recent progress and remaining puzzles. Ample evidence suggests that the sensory function of TRPA1 is evolutionarily conserved. This notion is further supported by the fact that TRPA1 from several mammalian species (human, rat, and mouse) is activated by common ligands (a plethora of electrophylus) through a common mechanism (covalent modification). However, recent studies from us and others have revealed species-specific activation or blockade of TRPA1 by many ligands. Here, we wish to raise the awareness of the species difference issue, which has been largely overlooked thus far, but will have a profound impact on TRPA1 research and drug development.

The first case of species difference between TRPA1 orthologs was demonstrated by Klionsky et al. (2007). They reported the identification of several small molecules that potently block human TRPA1 (Fig. 1 A). However, when tested against rat TRPA1, these compounds are either inactive (AMG2504 and AMG7160) or demonstrate reverse pharmacology and function as activators of rat TRPA1 (AMG9090 and AMG5445) (Klionsky et al., 2007). From a high throughput screening, we identified dozens of structurally related, thioaminal-containing analogues that block human TRPA1 but activate rat TRPA1 (Chen et al., 2008) (e.g., CMP1-3; Fig. 1 A). Several lines of evidence indicate that these compounds interact with channel proteins through covalent modification. First, they are electrophilic compounds that are predicted to be reactive to nucleophilic cysteine and lysine residues. Second, when tested in La antigen-based ALARM NMR and ALARM MS studies, CMP1 modifies surface-exposed cysteines to form predicted adducts, confirming its chemical reactivity. Third, structural analogues with the reactive sulfur atom exhibit reactivity and effects on TRPA1, whereas close analogues without the reactive sulfur atom (e.g., CMP4 in Fig. 1 A) do not affect channel function. Finally, Cys-621 of human TRPA1 and Cys-622 of rat TRPA1, residues important for allyl isothiocyanate modification, were found to be important for CMP1-mediated effects. Therefore, thioaminals covalently

modify human and rat channels but produce opposite gating effects.

In addition to thioaminals, several nonreactive compounds also demonstrate species-specific effects. Menthol activates mouse TRPA1 at low micromolar concentrations and blocks at higher concentration. However, menthol exhibits only an agonist effect on human TRPA1 across a range of concentrations (Xiao et al., 2008). Furthermore, caffeine was shown to activate mouse TRPA1 but suppresses human TRPA1 activity (Nagatomo and Kubo, 2008). From high throughput screening and medicinal chemistry, we identified human TRPA1 antagonists from multiple structurally distinct chemical classes. When tested at rat TRPA1, only a small portion demonstrates consistent inhibitory effects, whereas a majority either has dramatic reduced potencies as antagonists (>10-fold) or functions as agonists of rat TRPA1. As more compounds are disclosed and evaluated in multiple species, we predict that the species difference for TRPA1 will likely be a prevalent issue.

What are the implications of the species difference? First, this has to be taken into consideration when studying the physiological and pathological function of TRPA1. TRPA1 has been proposed to be a molecular integrator of various endogenous stimuli (e.g., noxious cold, bradykinin, 4-hydroxynonenal, and 15-dPGJ$_2$) to mediate sensation, pain, and neurogenic inflammation (Bang and Hwang, 2009). This conclusion has been mostly derived from in vitro experiments using one ortholog and from in vivo experiments using rats or mice (Bautista et al., 2006; Kwan et al., 2006). It is tempting, of course, to extend these findings into human and other species. However, this practice may not have been adequately justified. One heavily disputed issue is the involvement of TRPA1 in noxious cold sensation (Bang and Hwang, 2009; Caspani and Heppenstall, 2009; Kwan and Corey, 2009; Latorre, 2009). Although activation of heterologoulsy expressed mouse TRPA1 by noxious cold has been well established (Story et al., 2003; Sawada et al., 2007; Karashima et al., 2009), human TRPA1 was not activated by noxious cold (Jordt et al., 2004). Whether this discrepancy is resulted from genuine species difference or from difference
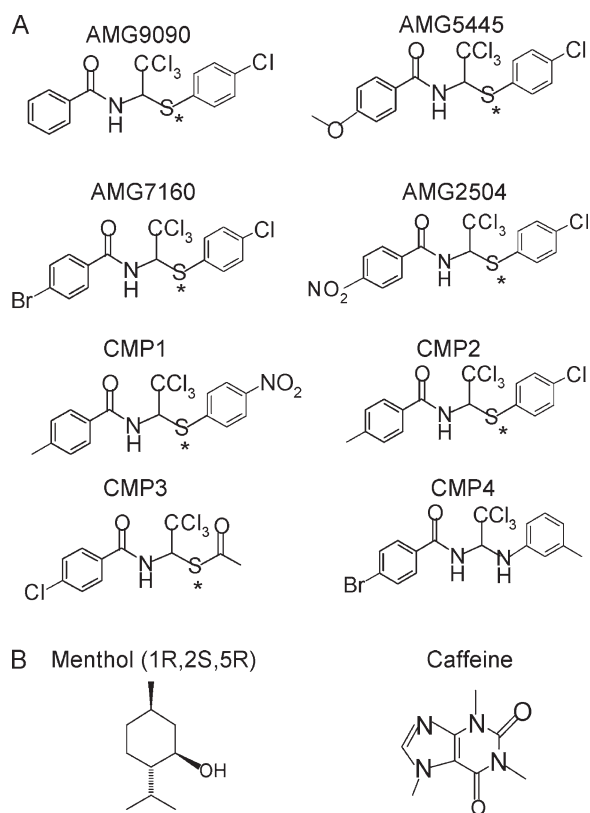
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Figure 1. Compounds with species-specific effects on TRPA1. (A) The electrophilic thioaminals (with a reactive sulfur atom labeled by an asterisk) block human TRPA1, but activate rat TRPA1 (AMG9090, AMG5445, CMP1, CMP2, and CMP3) or have almost no effects (AMG7160 and AMG2504) (Klionsky et al., 2007; Chen et al., 2008). CMP4, with a sulfur-to-nitrogen substitution, is not reactive and has no effects on human or rat channel. (B) Menthol is an activator of mouse TRPA1 at low concentrations and a blocker at high concentrations; but it only activates human TRPA1 (Xiao et al., 2008). Caffeine activates mouse TRPA1 but blocks human TRPA1 (Nagatomo and Kubo, 2008).

in experimental conditions has not been settled. Of note, dTRPA1 (from *Drosophila melanogaster*) is activated by heat, but not by cold or allyl isothiocyanate, indicating functional divergence during evolution (Viswanath et al., 2003). Another observation worth noting is the relatively low sequence homology among mammalian channels. For example, human and rodent TRPA1 are 79% identical, compared with 94% for TRPM8, 86% for TRPV1, 93% for TRPV3, and 95% for TRPV4, respectively. The high degree of sequence variation in TRPA1 may lead to different responses to endogenous ligands. In addition, the physiological function of TRPA1 can be shaped by metabolic mechanisms of ligands, temporal/spatial expression of the channel, signal transduction pathways, and animal physiology. Such parameters can vary between species and should be considered when extrapolating findings across species.

Second, the species difference presents significant practical challenges to drug development targeting TRPA1.

Currently, there is great interest in pursuing TRPA1 antagonists for pain and other indications. Testing in preclinical animal species, mandated by the FDA before any compound entry into human clinical trials, is an indispensable part of drug development. Animal models are not only used to assess the intended efficacy and discover new indications, but also to expose any on-target and off-target adverse side effects. Rats and mice are the most widely used species for pain research and compound assessment. Based on our experience and that of others, many antagonists of human TRPA1 have different effects on rat and mice channels. These compounds, albeit of potential utility for humans, must be excluded from the drug development process because assessing them in conventional rat/mouse models will not lead to useful information. One strategy to address this problem is to use higher species such as monkey and chimpanzee, which share high TRPA1 sequence homology with humans (97.1 and 99.7% identical, respectively). Therefore, monkey/chimpanzee may serve as faithful surrogate models for humans, but their practical usage is limited by the availability of pain models, low throughput, and huge expenses. Alternatively, transgenic animals can be generated for compound assessment. In any case, species difference is a serious challenge and should be overcome with creative technologies and heavy investment.

Finally, the cloud of species-specific difference does have a silver lining. Compounds with drastically species-specific effects (e.g., CMP1 and menthol) can be valuable tools to study channel biophysics and structure–function relationship. Through the characterization of rat and human TRPA1 chimeras, we identified residues in the upper portion of the S6 domains as critical determinants of the opposite gating by CMP1: Ala-946 and Met-949 of rat TRPA1 determine channel activation by CMP1, whereas equivalent residues of human TRPA1, Ser-943, and Ile-946 determine channel block (Chen et al., 2008). Through a similar approach, mouse-human chimeras revealed the pore domain (S5-S6) as critical to menthol-mediated inhibition. In addition, chimeras between dTRPA1 and mammalian channels led to the identification of several S5 residues as important to the menthol-mediated effects. Interestingly, some of the S5 and S6 residues were important for effects mediated by different ligands, including menthol, AP18, AMG5445, and CMP1 (Xiao et al., 2008). These residues may form a universal ligand-binding pocket. Alternatively, given the diverse structure and reactivity among these ligands, such residues may participate in channel gating instead of direct ligand binding. These and future findings using species difference will provide novel insights into how TRPA1 respond to stimuli.

REFERENCES

Bang, S., and S.W. Hwang. 2009. Polymodal ligand sensitivity of TRPA1 and its modes of interactions. *J. Gen. Physiol.* 133:257–262.

Bautista, D.M., S.E. Jordt, T. Nikai, P.R. Tsuruda, A.J. Read, J. Poblete, E.N. Yamoah, A.I. Basbaum, and D. Julius. 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell.* 124:1269–1282.

Caspani, O., and P.A. Heppenstall. 2009. TRPA1 and cold transduction: an unresolved issue? *J. Gen. Physiol.* 133:245–249.

Chen, J., X.F. Zhang, M.E. Kort, J.R. Huth, C. Sun, L.J. Miesbauer, S.C. Cassar, T. Neelands, V.E. Scott, R.B. Moreland, et al. 2008. Molecular determinants of species-specific activation or blockade of TRPA1 channels. *J. Neurosci.* 28:5063–5071.

Jordt, S.E., D.M. Bautista, H.H. Chuang, D.D. McKemy, P.M. Zygmunt, E.D. Hogestatt, I.D. Meng, and D. Julius. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature.* 427:260–265.

Karashima, Y., K. Talavera, W. Everaerts, A. Janssens, K.Y. Kwan, R. Vennekens, B. Nilius, and T. Voets. 2009. TRPA1 acts as a cold sensor in vitro and in vivo. *Proc. Natl. Acad. Sci. USA.* 106:1273–1278.

Klionsky, L., R. Tamir, B. Gao, W. Wang, D.C. Immke, N. Nishimura, and N.R. Gavva. 2007. Species-specific pharmacology of Trichloro-(sulfanyl)ethyl benzamides as transient receptor potential ankyrin 1 (TRPA1) antagonists. *Mol. Pain.* 3:39.

Kwan, K.Y., and D.P. Corey. 2009. Burning cold: involvement of TRPA1 in noxious cold sensation. *J. Gen. Physiol.* 133:251–256.

Kwan, K.Y., A.J. Allchorne, M.A. Vollrath, A.P. Christensen, D.S. Zhang, C.J. Woolf, and D.P. Corey. 2006. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron.* 50:277–289.

Latorre, R. 2009. Perspectives on TRP channel structure and the TRPA1 puzzle. *J. Gen. Physiol.* 133:227–229.

Nagatomo, K., and Y. Kubo. 2008. Caffeine activates mouse TRPA1 channels but suppresses human TRPA1 channels. *Proc. Natl. Acad. Sci. USA.* 105:17373–17378.

Sawada, Y., H. Hosokawa, A. Hori, K. Matsumura, and S. Kobayashi. 2007. Cold sensitivity of recombinant TRPA1 channels. *Brain Res.* 1160:39–46.

Story, G.M., A.M. Peier, A.J. Reeve, S.R. Eid, J. Mosbacher, T.R. Hricik, T.J. Earley, A.C. Hergarden, D.A. Andersson, S.W. Hwang, et al. 2003. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell.* 112:819–829.

Viswanath, V., G.M. Story, A.M. Peier, M.J. Petrus, V.M. Lee, S.W. Hwang, A. Patapoutian, and T. Jegla. 2003. Opposite thermosensor in fruitfly and mouse. *Nature.* 423:822–823.

Xiao, B., A.E. Dubin, B. Bursulaya, V. Viswanath, T.J. Jegla, and A. Patapoutian. 2008. Identification of transmembrane domain 5 as a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels. *J. Neurosci.* 28:9640–9651.