Weakly acidic, but strongly irritating: TRPA1 and the activation of nociceptors by cytoplasmic acidification

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Our bodies and our surroundings contain an enormous array of chemicals. Although many of these chemicals are useful and even essential for survival, others are potentially harmful. A challenge all animals face is to sense which chemicals are useful and which are harmful. Considered in the context of feeding behavior, this challenge is highly asymmetric. Although animals need to recognize a sufficient set of useful chemicals to meet their dietary needs, such recognition needn't be comprehensive: if they fail to sense some potential sources of nutrition, they still have the opportunity to find others. On the other hand, consumption of just one toxin-laden meal can be fatal. Such potentially catastrophic outcomes would be expected to enforce a strong emphasis on the development of noxious chemical surveillance systems that are as comprehensive as possible. In this issue, Wang et al. significantly extend our understanding of how animals sense an important class of potentially harmful chemicals, weak acids that acidify the intracellular environment.

The comprehensive sensing of noxious chemicals is daunting given that damaging agents can come in virtually unlimited shapes and sizes. Many chemoreceptors sense chemicals through what could be termed a molecular recognition approach: highly specific, structure-dependent complementary binding interactions between ligand and receptor. Although this approach can endow chemosensory systems with remarkable discriminatory power, it imposes limitations on the spectrum of chemicals that can be recognized, even when a large family of chemoreceptors is deployed to help cover the chemical spectrum. An alternative strategy is what could be termed a "detection" approach. Such an approach relies not upon specific recognition of the chemical structures of various noxious chemicals, but rather on the detection of the harmful effects of these chemicals. For example, a chemoreceptor might respond to the reactivity of an entire class of noxious chemicals or to the damaging alteration in cellular physiology a class of noxious chemicals elicits. Such a detection strategy would sacrifice discriminatory power, but it would allow a cell to sense the presence of noxious chemicals of enormous structural diversity. It would also require a significantly smaller number of receptor molecules than the recognition approach.

Recent evidence indicates that the sensory neurons that respond to noxious chemical stimuli, chemical nociceptors, indeed use such detection approaches, at least for some classes of noxious chemicals. For example, nociceptors express multiple cation channels that respond to strong acids by detecting the drop in extracellular pH these chemicals cause (Caterina et al., 1997; Wemmie et al., 2006). In addition, recent work has demonstrated that a major class of reactive chemicals, strong electrophiles, is sensed by a detection approach. Reactive electrophiles (literally "electron lovers") include allyl isothiocyanate (the active ingredient of wasabi), cinnamaldehyde (found in cinnamon), and acrolein and formaldehyde (in cigarette smoke). Although structurally diverse, these chemical irritants all readily form covalent bonds with electron donors, which include molecules like protein and DNA. Such covalent modifications have a range of toxic effects, from enzyme inactivation to DNA mutation (Liebler, 2008). Rather than recognizing specific electrophiles based on their particular chemical structures, nociceptors detect reactive electrophiles based on their chemical reactivity (Hinman et al., 2006; Macpherson et al., 2007).

At the molecular level, the transient receptor potential A1 (TRPA1) cation channel is critical for the detection of structurally diverse electrophiles by nociceptors (Bautista et al., 2006; Kwan et al., 2006). TRPA1 detects electrophiles by serving as a target for electrophilic attack, with cysteine residues within TRPA1 acting as nucleophiles that form covalent bonds with noxious electrophiles (Hinman et al., 2006; Macpherson et al., 2007). Such covalent modification activates the TRPA1 channel, triggering nociceptor depolarization. In this way, chemical nociceptors are capable of detecting the presence of a broad spectrum of reactive electrophiles by sensing their fundamentally noxious property. Strikingly, this TRPA1-dependent mechanism for electrophile

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The Rockefeller University Press
J. Gen. Physiol. Vol. 137 No. 6 489–491
www.jgp.org/cgi/doi/10.1085/jgp.201110657

detection is at least 500 million years old, dating back to the common ancestor of vertebrates and invertebrates, and it has been highly conserved across animal evolution, with both fly and human TRPA1 channels relying on the same mechanism (Kang et al., 2010). Such conservation stands in stark contrast to chemoreceptors like canonical olfactory receptors that are highly divergent among animals, and it supports the notion that this TRPA1-dependent electrophile detection strategy is strongly beneficial for survival.

Of course, there are many other ways in which chemicals can damage cells in addition to extracellular acidification and electrophilic attack. What other kinds of noxious chemical insults do nociceptors detect based on their noxious properties? And what are the molecular sensors and detection mechanisms involved? The paper by Wang et al. (2011) in this issue begins to fill in some of the blanks. The authors demonstrate that weak acids constitute another class of noxious chemicals that nociceptors detect through a noxious chemical property, in this case their ability to acidify the cytoplasm. Such cytoplasmic acidification can have significant negative consequences on cell physiology, even triggering cell death (Matsuyama and Reed, 2000). Strikingly, the molecular sensor of intracellular acidification the authors identify is none other than TRPA1.

The present story grows out of a recently published investigation of the mechanism by which CO_2 activates nociceptors (Wang et al., 2010). As anyone drinking a fizzy soda can attest, carbonated beverages can elicit a sensation of stinging or pungency, a sensation that appears to be triggered by the ability of CO_2 to activate nociceptors from the trigeminal ganglia that innervate the oral and nasal cavity (Silver and Moulton, 1982; Wang et al., 2010). Wang et al. (2010) recently found that the subpopulation of trigeminal sensory neurons that respond to CO_2 also respond to the reactive electrophile cinnamaldehyde, implicating TRPA1-expressing neurons in the CO_2 response. Trigeminal neurons from TRPA1$^{-/-}$-mutant mice showed dramatic reductions in CO_2 responses, indicating that TRPA1 was critical for CO_2 responsiveness. Furthermore, when TRPA1 was expressed in heterologous cells, the channel could be activated by simply bubbling CO_2 through the media. Collectively, these data indicated that TRPA1 acts as a molecular sensor mediating nociceptor activation by CO_2.

The link to the current work came from examining how CO_2 exposure activates TRPA1. CO_2 freely diffuses across cellular membranes; once inside the cell, CO_2 can acidify the intracellular environment by reacting with water to yield bicarbonate and a proton. Consistent with intracellular acidification mediating TRPA1 activation by CO_2, acidifying the intracellular face of TRPA1-containing patches activated the channel. Meanwhile, buffering the intracellular pH of TRPA1-expresssing cells slowed their response to CO_2, suggesting that intracellular acidification was indeed a key step in TRPA1's response to CO_2 (Wang et al., 2010).

The ability of TRPA1 to respond to CO_2-mediated intracellular acidification raised the question of whether this mechanism might be used more generally to detect other chemicals that acidify the intracellular environment. In their current paper, Wang et al. (2011) examine this issue by focusing on a common class of chemical irritants that acidify the intracellular environment, weak acids like acetic acid (vinegar) and propionic acid (present in fermented foods like Swiss cheese). Unlike strong acids, which largely dissociate in solution, a significant fraction of weak acids remains in an undissociated neutral state that can readily diffuse across cell membranes. Thus, weak acids acidify both extracellular and intracellular environments. Although nociceptors were known to respond to a wide range of weak acids (Silver and Moulton, 1982; Bryant and Moore, 1995), whether the response was mediated by extracellular or intracellular acidification was unknown.

Wang et al. (2011) tested the ability of acids of varying strengths and hydrophobicity to activate trigeminal sensory neurons. Interestingly, although responses to a given acid increased with decreasing pH, the strongest responses were observed for weaker, more hydrophobic acids, suggesting that intracellular acidification was important for nociceptor activation. Reminiscent of their prior analysis of CO_2 detection, most trigeminal sensory neurons that responded to weak acids also responded to the electrophile cinnamaldehyde, whereas responses to weak acid were strongly reduced in TRPA1 knockout mice. Thus, TRPA1 is critical for nociceptor activation by weak acids as well as by CO_2 and reactive electrophiles.

At the molecular level, TRPA1 activation by weak acids proceeds via the same mechanism used to detect CO_2, intracellular acidification. In particular, the ability of a panel of weak acids to activate TRPA1 tightly correlated with their effects on intracellular pH rather than on extracellular pH. Furthermore, TRPA1 responded to intracellular acidification even when the external pH was held at 7.4. Collectively, these data make a convincing case that TRPA1 acts as a detector of intracellular acidification such as that triggered by exposure to weak acids.

Are similar or distinct mechanisms involved in the activation of TRPA1 by intracellular acidification and reactive electrophiles? The mechanisms appear distinct, as the authors found that an electrophile-insensitive mutant of TRPA1 was capable of responding to weak acids. The residues within TRPA1 important for responses to intracellular acidification remain to be determined.

Advances over the last several years have greatly expanded our understanding of how nociceptors are able to sense such a wide range of noxious chemicals. It is now clear that nociceptors express a relatively small set of ion channels that can detect the noxious properties of a broad range of tissue-damaging chemicals. Among the

surprises is how many different types of noxious chemical stimuli can be sensed by just one channel, TRPA1. And the list of noxious stimuli sensed by TRPA1 continues to expand; there is recent work indicating that TRPA1 also mediates nociceptor responses to heavy metals like zinc, cadmium, and copper (Hu et al., 2009; Gu and Lin, 2010). The sheer diversity of TRPA1 agonists makes understanding the mechanisms by which this channel is regulated a fascinating and important goal for the future. One also wonders how many other types of noxious chemical detection await discovery, and how many of these will be channeled through molecules like TRPA1.

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