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Minireview

A Painful Trp Can Be a Bonding Experience

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The receptive field of the TRPA1 nociceptor is remarkably expansive when compared to other chemodetectors such as odorant receptors. The identification of a unique mechanism utilized by TRPA1 helps clarify how this protein can efficiently alert the cell to an array of reactive chemical agents, regardless of their structure.

The olfactory, gustatory, and trigeminal systems, known collectively as the "chemical senses," work in concert to detect chemicals in the environment. Indeed, these systems respond to many of the same compounds. However, the mechanisms employed by the various chemoreceptors within each system can be strikingly different, resulting in pronounced effects on receptor physiology and giving rise to experiences as disparate as the ephemeral perception of a lilac and the persistent tears from a sliced onion.

Odorant receptors often exhibit great sensitivity to nuances in the chemical structure of their ligands. Adding or subtracting a single carbon or shifting the location of a double bond can profoundly affect whether or not a compound is an effective agonist, as demonstrated by the markedly different responses to hexanal (Figure 1, 1) versus heptanal (Figure 1, 2) at rat OR-I7 (Araneda et al., 2000) and eugenol (Figure 1, 4) versus isoeugenol (Figure 1, 5) at mOR-EG (Kajiya et al., 2001). Thus, highly related chemicals typically populate the receptive field of a mammalian odorant receptor (Araneda et al., 2000; Gaillard et al., 2002; Spehr et al., 2003). In contrast, the range of compounds that activate the trigeminal nociceptor TRPA1 is staggeringly diverse—including plant-derived allylic mono-, di-, and trisulfides (Bautista et al., 2005; Macpherson et al., 2005), isothiocyanates (Bandell et al., 2004; Jordt et al., 2004), and α,β-unsaturated aldehydes (Bandell et al., 2004; Bautista et al., 2006). Despite being able to recognize a host of both aliphatic and aromatic compounds, TRPA1 simultaneously displays a surprising level of discrimination. For example, TRPA1 is readily activated by acrolein (a.k.a. *trans*-2-propenal [Figure 1, 8]) but is insensitive to the corresponding saturated aldehyde, propanal (Figure 1, 7; Bautista et al., 2006).

Two recent papers (Hinman et al., 2006; Macpherson et al., 2007) help reconcile this paradox by highlighting that the chemical *reactivity* and not structure per se is the critical feature of many TRPA1 agonists. These agonists, through a variety of functional groups, all possess an electrophilic carbon or sulfur that is subject to nucleophilic attack by the sulfur in cysteine side chains of TRPA1. The ability of benzyl isothiocyanate (Figure 1, 12; a strong electrophile) but not benzyl thiocyanate (Figure 1, 13; a weaker electrophile of nearly identical shape) to activate TRPA1 elegantly underscores this phenomenon (Hinman et al., 2006). Thus, while odorant receptors can readily distinguish between enantiomers of carvone (Figure 1, 25; Hamana et al., 2003), one would predict that electrophile stereoisomers differing only in their chirality or *cis/trans* arrangement should be equally effective at the TRPA1 nociceptor.

However, TRPA1's novel method of activation comes with a substantial tradeoff in terms of receptor kinetics. Odorant receptors such as mOR-EG recognize their ligands though transient, weak intermolecular interactions within a more traditional binding pocket (Katada et al., 2005). While they may have a more narrowly tuned receptive field, odorant receptors can respond repeatedly to multiple applications of an agonist with fair temporal fidelity. In contrast, the reaction with an electrophilic compound that underlies TRPA1's ability to detect such an expanded array of agents leaves a covalently linked adduct on the receptor. This modification of the protein persists for hours as monitored by reacting fluorescent tags with alkyne groups engineered into the electrophile (Macpherson et al., 2007). In consequence, the physiological response of TRPA1 to electrophiles is greatly prolonged as the compound cannot dissociate and the receptor remains activated. Experimentally, currents elicited by N-methyl maleimide (NMM; Figure 1, 14; Hinman et al., 2006) and cinnamaldehyde (Figure 1, 10; Bandell et al., 2004) last well beyond the stimulus duration, and the elevation in intracellular calcium in response to a cinnamaldehyde analog only gradually diminishes over the course of an hour (Macpherson et al., 2007). TRPA1 is thus greatly limited in its ability to respond to a closely timed second pulse of such electrophilic agonists.

While these covalent modifications pose unique challenges to signal termination within the cell, they can be exploited as a tool to directly identify which cysteines in TRPA1 are most reactive with a given electrophile. Macpherson et al. combined click chemistry (Speers et al., 2003) with protein degradation and mass spectrometry to empirically determine which amino acid residues react with an alkynated variant of the electrophile iodoacetamide (Figure 1, 15). Surprisingly, the critical cysteines reside within the intracellular N terminus of mouse TRPA1. Hinman et al. arrived at a similar conclusion for human

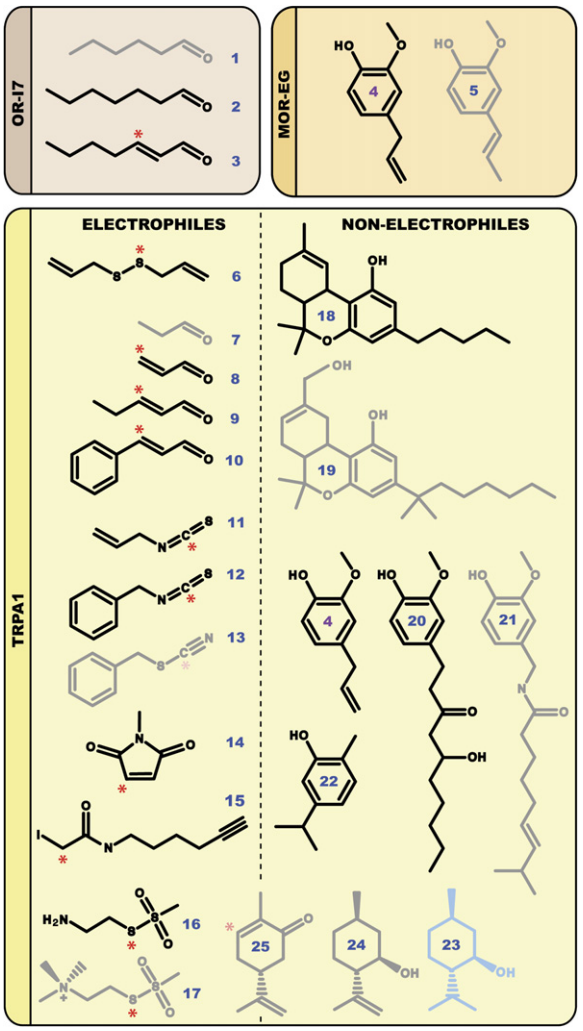

Figure 1. A Sampling of the Receptive Field of Two Odorant Receptors (Rat OR-I7 and Mouse mOR-EG) and the Nociceptor TRPA1

Key: black = active; gray = inactive, unknown inhibitory status; blue = inactive, demonstrated inhibitory; red asterisk = electrophilic atom. Chemical names: (1) hexanal; (2) heptanal; (3) *trans*-2-heptenal; (4) eugenol; (5) isoeugenol; (6) diallyl disulfide; (7) propanal; (8) acrolein/*trans*-2-propenal; (9) *trans*-2-pentenal; (10) *trans*-cinnamaldehyde; (11) allyl isothiocyanate/AITC; (12) benzyl isothiocyanate; (13) benzyl thiocyanate; (14) N-methyl maleimide/NMM; (15) iodoacetamide alkyne; (16) MTSEA; (17) MTSET; (18) Δ^9-tetrahydrocannabinol/THC; (19) HU-210; (20) gingerol; (21) capsaicin; (22) carvacrol; (23) L-menthol; (24) (−)-isopulegol; (25) L-carvone.

TRPA1 by systematic mutation of evolutionarily conserved cysteines.

An important consequence of an intracellular site of action is that the EC_{50} for such TRPA1 agonists, when assayed by whole-cell methods, reflects a combination of the electrophile strength and the compound's membrane permeability. Indeed, this effect was dramatically illustrated by comparison of the two highly similar electrophiles MTSEA (Figure 1, 16) and MTSET (Figure 1, 17). Both possess the same reactive thiosulfonate group and would likely elicit similar effects in excised inside-out patch recordings. However, the change from an amine to a charged trimethyl ammonium group renders MTSET membrane impermeable and wholly ineffective as an agonist in TRPA1-expressing oocytes (Hinman et al., 2006).

How the details of the detection mechanism shape the response profile of a chemoreceptor can be seen in a comparison between TRPA1 and the rat OR-I7 odorant receptor. The seven carbon long *trans*-2-alkenal (Figure 1, 3) is a robust OR-I7 agonist (Araneda et al., 2000), and TRPA1 is likewise potently activated by similar three (Figure 1, 8) and five (Figure 1, 9) carbon *trans*-2-alkenals (Bautista et al., 2006). However, OR-I7 and TRPA1 relay very different information about such aldehydes. OR-I7 demonstrates little regard for the electrophilic nature of the aldehyde (Figure 1, compare 2 versus 3), but it does exhibit a clear length preference for 7–11 carbon *trans*-2-alkenals (Araneda et al., 2000). This trend reflects steric constraints within a more traditional binding pocket (Hall et al., 2004), and it would result in a graph of EC_{50} versus carbon number with an abruptly appearing, bell-shaped distribution for panel members tested on OR-I7. For TRPA1, however, the critical feature of *trans*-2-alkenals is that they share the same reactive electrophilic group. Based on electrophilicity alone, all members of a panel of *trans*-2-alkenals, regardless of length, should activate TRPA1. But since increasing the alkyl tail length also gradually increases the hydrophobicity of the compound (enabling it to more readily enter the cell), one would thus expect an inverse linear relationship between EC_{50} and carbon number without evidence of cutoff for panel members tested at TRPA1. The response profiles of these two chemoreceptors thus reflect the specialized demands of their respective systems: discriminating between specific chemical shapes for olfaction and detecting reactive and potentially damaging chemicals for nociception.

An additional explanation for the unusually diverse receptive field of TRPA1 has also emerged through the study of mutant versions of TRPA1. Unlike odorant receptors, which have a single predicted odorant-binding pocket, TRPA1 appears to possess at least one other and possibly multiple other chemical detection sites. Mutating just one or a few particular cysteines to nonreactive serines or alanines was sufficient to render TRPA1 insensitive to most electrophiles (Hinman et al., 2006; Macpherson et al., 2007) regardless of their exact functional group. However, the mutant channels could still be activated by several nonelectrophilic compounds such as icilin (Macpherson et al., 2007). The currents elicited by these nonelectrophilic agonists are rapidly reversible (Jordt et al., 2004; Macpherson et al., 2007; Xu et al., 2006), suggesting that the alternate chemical detection site(s) more closely resembles a traditional binding pocket that only transiently interacts with appropriate ligands.

The nature of these noncovalently interacting site(s) within TRPA1 is still poorly understood. Δ^9-tetrahydrocannabinol (THC; Figure 1, 18) is a large heterocyclic plant-derived compound that can be detected even in TRPA1

cysteine mutants that no longer respond to electrophiles. A number of other, smaller plant-derived compounds such as eugenol (Figure 1, 4) and carvacrol (Figure 1, 22) can activate wild-type TRPA1 as well (Bandell et al., 2004). Although not yet tested in the TRPA1 cysteine mutants, these compounds are also likely detected by a secondary mechanism since the electrophile AITC (Figure 1, 11) can still elicit a moderate current following prior desensitizing pulses of carvacrol (Xu et al., 2006), similar to how AITC can trigger a large additional increase in intracellular calcium following prolonged prior application of THC (Jordt et al., 2004). These nonelectrophilic agonists happen to share a phenol core. However, this feature alone is insufficient to account for their effects, since it is also present in two compounds to which TRPA1 is insensitive: HU-210 (Figure 1, 19; Jordt et al., 2004) and capsaicin (Figure 1, 21; Macpherson et al., 2006). Interestingly, eugenol (Figure 1, 4) is also an agonist of the odorant receptor mOR-EG. In that system, an array of related small aromatic compounds has been used to probe the structural and functional requirements of both agonists and antagonists and how they relate to mOR-EG's binding pocket (Katada et al., 2005). Assaying TRPA1 with these compounds could prove a fruitful start to characterizing the apparently more traditional eugenol-binding site present in this nociceptor.

But while plants can trigger nociception by activating TRPA1 with both electrophilic and nonelectrophilic compounds, they also have provided at least a temporary balm in the form of menthol (Figure 1, 23). Menthol can inhibit both the current and elevated intracellular calcium induced by electrophiles at TRPA1 (Macpherson et al., 2006, 2007). However, the rapid reversibility of this inhibition shows that menthol merely masks the consequences of electrophile interactions with TRPA1; little if any change would be expected in the levels of covalently modified TRPA1 upon menthol treatment. In contrast, the cell permeable reducing agent dithiothreitol eliminates the adduct and leads to a sustainable decrease in intracellular calcium levels by breaking the covalent disulfide bond formed between the cysteine and electrophilic sulfur in compounds such as MTSEA (Figure 1, 16; Macpherson et al., 2007). Whether menthol can also inhibit the responses elicited by either THC-like or eugenol-like nonelectrophile agonists has not been determined. However, menthol's ability to also inhibit TRPA1's activation by cold (Macpherson et al., 2006) suggests that this compound may be an allosteric modulator with a more global effect on protein structure or gating.

A number of chemicals structurally related to menthol do not activate TRPA1 (Bandell et al., 2004), but whether they too have inhibitory effects has not yet been tested.

One particular compound, carvone (Figure 1, 25), will be particularly intriguing to revisit; although it shares several structural features with menthol (Figure 1, 23) and isopulegol (Figure 1, 24), it also possesses an electrophilic carbon. L-carvone was unable to activate TRPA1 when bath applied in a FLIPR assay (Bandell et al., 2004). However, whether any activation would surface following washout of the initial pulse and whether the D-enantiomer of carvone behaves any differently has not been reported. The methyl group on the α carbon of carvone does somewhat decrease its electrophilicity. Is carvone, like benzyl thiocyanate (Figure 1, 13), too poor an electrophile to substantially activate TRPA1? Or can carvone simultaneously inhibit its own induced current? Or is L-carvone sufficiently dissimilar to menthol that it would be unable to inhibit activation by even strong electrophiles? A careful dissection of the action of carvone could provide a fascinating glimpse of how TRPA1 integrates information from its multiple chemodetection mechanisms.

So, how does all this chemistry translate into a sensory experience? TRPA1 is a nonselective cation channel expressed in subsets of nociceptors of the dorsal root and trigeminal neurons. Presumably, the channel localizes to free nerve endings in the skin and mouth that, when depolarized, ultimately result in the sensation of pain. Thus, components of mustard (Figure 1, 11), cinnamon (Figure 1, 10), and garlic (Figure 1, 6), as well as environmental irritants such as those found in smoke (Figure 1, 8), elicit a common painful sensation by directly triggering TRPA1. However, natural compounds are not the only way to open the channel. TRPA1 is also critical for sensing inflammatory hyperalgesia (Bautista et al., 2006) and has a putative role in mechanosensation (Kwan et al., 2006). The channel is modulated by the activity of other receptors, including the TRPV1 (heat) and B2R (bradykinin) receptors (Bandell et al., 2004; Bautista et al., 2006), through intracellular signaling pathways. Intracellular Ca^{2+} sensitivity is conferred by an EF motif in the N terminus of TRPA1. While noxious cold was the first reported method of activating TRPA1 (<17°C; Story et al., 2003), it now appears that this effect is due to alterations in intracellular Ca^{2+} levels (Zurborg et al., 2007).

In sum, TRPA1 is a polymodal node that translates diverse hostilities into a singular experience. As we know the world through the filter of our sensory systems, we have no choice but to assume their fidelity. Natural compounds found in plants have particularly ingenious ways of "TRPing" these channels to create chemical illusions. We, in turn, have commandeered these compounds as instruments to improve our understanding of our sensory systems and, more importantly, our culinary experience.

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