The Pungency of Garlic: Activation of TRPA1 and TRPV1 in Response to Allicin

Lindsey J. Macpherson,1 Bernhard H. Geierstanger,2 Veena Viswanath,2 Michael Bandell,1,2 Samer R. Eid,1 SunWook Hwang,1,3 and Ardem Patapoutian1,2,*
1Department of Cell Biology
The Scripps Research Institute
La Jolla, California 92037
2Genomics Institute
Novartis Research Foundation
San Diego, California 92121
3College of Medicine
Korea University
Seoul 136-705
South Korea

Summary
Garlic’s pungent flavor has made it a popular ingredient in cuisines around the world and throughout history. Garlic’s health benefits have been elevated from folklore to clinical study [1–5]. Although there is some controversy as to the efficacy of garlic, garlic products are one of the most popular herbal supplements in the U.S. [6]. Chemically complex, garlic contains different assortments of sulfur compounds depending on whether the cloves are intact, crushed, cooked, or raw [7]. Raw garlic, when cut and placed on the tongue or lips, elicits painful burning and prickling sensations through unknown mechanisms. Here, we show that raw but not baked garlic activates TRPA1 and TRPV1, two temperature-activated ion channels that belong to the transient receptor potential (TRP) family [8–12]. These thermoTRPs are present in the pain-sensing neurons that innervate the mouth. We further show that allicin, an unstable component of fresh garlic, is the chemical responsible for TRPA1 and TRPV1 activation and is therefore likely to cause garlic’s pungency.

Results and Discussion

Raw But Not Baked Garlic Extracts Activate TRPA1 and TRPV1
Garlic’s most recognizable feature is its pungent odor and taste. This pungency is often credited to the activation of nociceptors in trigeminal ganglia [11, 12]. Despite its ubiquitous use, the identity of garlic’s active pungent components and their receptors are not known. Six members of the transient receptor potential (TRP) family of nonselective cation channels respond to a unique range of temperatures and are proposed to be involved in thermosensation [8–12]. In addition to temperature, many of the thermoTRPs can also respond to natural chemicals. TRPV1 is activated by noxious heat and by capsaicin, the pungent component of hot chili peppers [12–15]. TRPA1 is activated by noxious cold (but see [16]) and by pungent natural compounds present in cinnamon oil, mustard oil, and wintergreen oil [16–18]. Finally, TRPM8 is activated by innocuous cold temperatures and by menthol, the mint-derived cooling compound [19–21]. Together, these data suggest that thermoTRPs are an important component of chemesthesia, the somatosensory/trigeminal contribution to the sense of taste. We first tested whether crushed garlic could activate thermoTRPs expressed in Chinese hamster ovary (CHO) cells. Extracts of fresh garlic were able to activate TRPA1 and TRPV1, but not TRPM8, in calcium-imaging experiments (Figure 1A; data not shown). In this assay, garlic extract appears to be a better activator of TRPA1 than TRPV1. It is commonly known that baked garlic, although very flavorful, lacks pungency. We hypothesized that if TRPV1 and TRPA1 were indeed responding to the pungent components of garlic, then this activity would be eliminated by the use of extracts from baked garlic. Extracts from oven-roasted garlic (400°F, 60 min) were unable to activate either TRPA1 or TRPV1, consistent with the theory that these thermoTRPs are targets for garlic’s pungency (Figure 1A).

To quantify the calcium-imaging responses of the fresh garlic extracts, we used a fluorometric imaging plate reader (FLIPR) to perform dose-response curves on thermoTRP-expressing CHO cells. In one such experiment, the dilutions at half-maximal activation (EC50) for mTRPA1, rTRPV1, and hTRPA1 cells were calculated to be 1:8600, 1:3544, and 1:127 dilutions of garlic extractSeoul 136-705 respectively (Figure 1B). The EC50s from this experiment and two additional experiments are summarized below. In all three experiments, mTRPA1, closely followed by hTRPA1 and then rTRPV1, is the most sensitive to fresh garlic extract. Because calcium imaging and FLIPR are indirect assays of channel activity, the activities of fresh garlic extract on TRPA1 and TRPV1 were also assayed in electrophysiology experiments. Garlic extract at dilutions of 1:50 and 1:500 were able to activate both TRPA1 and TRPV1 channels expressed in Xenopus oocytes but not in control un.injected oocytes (Figure 1C; data not shown).

Activities of Garlic-Derived Sulfide Compounds
Intact cloves of garlic contain the compound alliin, which is converted into allicin by the enzyme allinase after the clove has been bruised, cut, or crushed [1, 6, 7]. Allicin is an unstable compound and is converted easily into a variety of more-stable sulfide compounds, including diallyl sulfide, diallyl disulfide, and diallyl trisulfide, over time or after heating [6, 7]. Although alliin is odorless, allicin and the sulfide compounds produced from alliin have characteristic garlic odors and tastes. It is unclear which (if any) of these compounds is responsible for the burning and prickling sensations produced in the mouth by fresh-cut garlic. In calcium-imaging experiments, diallyl sulfide, diallyl disulfide, and diallyl trisulfide were able to activate TRPA1 and TRPV1 (Figures 1D and 1E; data not shown). However, activation of these TRP-expressing CHO cells in re-
response to these sulfide compounds was slower and less intense than in response to garlic extracts (Figure 1D). Addition of allicin to TRPA1- and TRPV1-expressing CHO cells showed an immediate and strong calcium response, similar to the responses to garlic extract (Figures 1A and 2A). One hundred micromolar allicin was not able to activate other thermoTRPs (TRPV2 was tested by patch clamping of CHO cells, and TRPV3 and TRPV4 were tested by calcium imaging. One millimolar of 2-APB, 3 mM camphor, and 225 mM hypertonic solutions were used as positive controls for TRPV2, TRPV3, and TRPV4, respectively). Activation of TRPA1 and TRPV1 by allicin suggests that it might be the main pungent constituent of fresh garlic. Furthermore, as with fresh garlic extract, TRPA1 is more sensitive to allicin than TRPV1 is. Higher concentrations of garlic extract, diallyl disulfide, and allicin sometimes produced lower calcium-influx measurements, especially for TRPA1 (Figures 1A, 1D, and 2A). Dose responses assayed by FLIPR did not exhibit this phenomenon.

Dose-response curves for allicin on mTRPA1, hTRPA1, rTRPV1, and CHO were assayed by FLIPR. Three separate experiments were performed, and the EC50s from these experiments are tabulated below. One representative dose-response experiment is shown in Figure 2B. In this experiment, the EC50s calculated for mTRPA1, hTRPA1, and rTRPV1 are 1.32 μM, 1.91 μM, and 51.22 μM, respectively. In contrast to the potency of allicin, diallyl disulfide produced much higher EC50s for TRPA1 and TRPV1 (125 μM for mTRPA1, and responses were indistinguishable from background for rTRPV1; data not shown). Calcium-imaging experiments of TRPV1 and TRPA1 in response to alliin, the precursor of allicin, showed no activity in either channel for concentrations up to 500 μM (data not shown, four replicate experiments for each channel). In electrophysiological recording experiments, oocytes expressing TRPA1 and TRPV1 responded to allicin at a concentration of 10 μM (Figures 2C and 2D). One micromolar of allicin, on the other hand, activated TRPA1- but not TRPV1-expressing oocytes (data not shown), consistent with calcium-imaging experiments.

Although garlic extract and allicin are able to activate TRPV1, TRPA1 is much more sensitive to these treatments. We find that TRPA1 is at least ten times more sensitive to garlic and allicin than is TRPV1 assayed by calcium-imaging experiments of dorsal root ganglia (DRG) neurons (see below) and by FLIPR analysis of TRPA1 and TRPV1 channels stably expressed in CHO cells. When assayed in Xenopus oocyte electrophysiology experiments, differences among the two channels did not appear as varied. Activities of TRPA1 and TRPV1 in these experiments were approximately equivalent to those of garlic extract, although TRPA1 was

Figure 1. TRPA1 and TRPV1 Are Activated by Fresh Garlic Extract
(A) Responses of TRPV1 and TRPA1 to fresh and baked garlic extracts. Traces represent average fluorescent ratios of ~100 cells (A, D, E).
(B) Dose-response curve of fresh garlic extract on rTRPV1, mTRPA1, hTRPA1, and CHO cells assayed by FLIPR. Traces represent average fluorescence of four replicate wells. Error bars are 1 standard deviation (SD).
(C) Inward currents in representative TRPA1- and TRPV1-expressing Xenopus oocytes were evoked by fresh garlic extract at dilutions of 1:50 and 1:500. The black bar indicates addition of garlic extracts. Traces represent one of four replicate experiments.
(D and E) Comparison of responses of TRPA1 (D) and TRPV1 (E) to fresh garlic extract, diallyl disulfide, and diallyl sulfide.
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garlic extracts and allicin specifically activate TRPA1 and TRPV1 in native neurons, we performed calcium imaging of adult rat DRG neurons. We used capsaicin and cinnamaldehyde to mark TRPV1- and TRPA1-expressing neurons. We have previously shown that TRPA1 is expressed in a subset of TRPV1-positive neurons, and this is consistent with the capsaicin- and cinnamaldehyde-response profiles (cinnamaldehyde activates a subset of capsaicin-responsive neurons) [17, 18]. Addition of allicin or garlic extract to cultured rat DRG neurons activated a specific population of neurons. High concentrations of garlic extract or allicin (a dilution of 1:50 for garlic, and 100 μM allicin) activated the majority of capsaicin-sensitive DRG neurons (Figures 3B–3D). On the other hand, low concentrations of garlic extract and allicin (a dilution of 1:500 for garlic, and 10 μM allicin) activated only the cinnamaldehyde-sensitive neurons (a smaller subset of capsaicin-sensitive population) (Figures 3A and 3C–3D). Importantly, capsaicin-insensitive neurons never responded to garlic extract or allicin. These results agree with data from thermoTRP-expressing CHO cells to show that garlic specifically activates TRPA1 and, to a lesser extent, TRPV1. In addition, 100 μM allicin was also able to activate TRPV1- and TRPA1-expressing neurons of the trigeminal ganglia (21 out of 97 neurons, data not shown).

NMR Analysis of Garlic Extracts

If allicin is the main pungent component of garlic extracts, we would then expect it to be present in fresh but not baked garlic extracts. 1D 1H NMR spectrum of the aqueous extract of fresh garlic was compared to the spectrum of baked garlic extract. The spectra indicate that the extracts are complex yet similar mixtures. Identical resonance lines present in both fresh and baked garlic extracts indicate that the concentrations of many respective components are similar (Figure S1 in the Supplemental Data available with this article online). Despite their similarities, differences between the fresh and baked samples can be seen in the 5–6.1 ppm range (Figures 4A and 4B). These differences are mainly due to allicin, as indicated by a reference spectrum of pure allicin (Figure 4C). Resonance lines a–f are doublets arising from three of the four allyl protons of allicin (the fourth doublet, lines g and h, overlaps with a doublet of alliin in the fresh and baked extracts). Whereas resonance lines of allicin (Figure 4C, lines a–f) are very prominent in the spectrum of fresh garlic extract (Figure 4A), allicin is not detectable in the baked garlic extract (Figure 4B). The allyl resonances in the baked extract can be assigned to alliin (Figure 4B), lines g and h and resonances at 5.57 and 5.58 ppm). Small amounts of alliin are also detected in fresh garlic extract (Figure 4A). The absence of allicin but presence of alliin in baked garlic extracts is consistent with the biochemical pathway of allicin production [6, 7]. In baked garlic extracts, the enzyme alliinase is destroyed by baking and is unable to convert alliin into allicin. Small amounts of other diallyl sulfide species, mainly diallyl sulfide, can be seen around 5.2 and 5.85 ppm in both baked and fresh extracts. The amounts of diallyl sulfides were too small to be accurately quantified by nuclear magnetic activated by 1 μM allicin whereas TRPV1 was not. The differences in observed channel sensitivity to garlic extract and allicin may be due to the assay method, and more studies must be done to determine the physiological contribution of each channel to the sensation of garlic’s pungency.

Allicin and Garlic-Extract Activity in Cultured Rat DRG Neurons

Most of the thermoTRP channels (including TRPV1 and TRPA1) are expressed in the sensory neurons of the dorsal root ganglia (DRG) adjacent to the spinal column, as well as in the trigeminal ganglia in the head [8–12]. These neurons innervate all peripheral tissues, including the mouth and the tongue. To test whether...
garlic extracts before inactivation of alliinase. Reports cite diallyl sulfide compounds as comprising a significant percentage of garlic extract's chemical composition, but extraction method plays an important role in the production of these compounds [22, 23]. In our aqueous extracts, allicin was always the dominant allyl compound in the fresh extract whereas alliin was the most concentrated species in the extract of baked garlic. Resonance lines at around 2.2 and 2.5 ppm are also apparent in fresh but absent in baked garlic extract (Figure S1). However, the abundance of this unknown compound relative to allicin varies widely from sample preparation to sample preparation and does not correlate with activity of garlic, as allicin does (see below).

To further confirm the assignment of the allicin resonances, we added aliquots of pure allicin to the baked garlic sample. Figure 4D shows the baked garlic sample after the addition of allicin to a concentration of approximately 0.67 mM. The resonance lines of the allicin spiked into the baked garlic extract align perfectly with those in the spectrum of fresh garlic extract (and pure allicin) and are comparable in intensity. Peak integration (relative to TSP) of six resolved allicin-resonance lines, a–f, in the NMR sample of fresh garlic extract (Figure 4A) suggest a concentration of approximately 0.97 mM allicin, which corresponds to a concentration of 10.6 mM in the undiluted extract. These NMR experiments were repeated three times on three fresh preparations of garlic extracts, giving concentrations of allicin in fresh garlic extract at 10.6 mM, 10.4 mM, and 9.37 mM. These values compare well with the concentrations determined from FLIPR assays (Figure 4E).

The Concentration of Allicin in Garlic Extract Explains Its Activity on TRPV1 and TRPA1

Allicin’s activity on TRPA1 and TRPV1 is comparable to the activity of garlic extracts, and allicin is present in fresh but not baked garlic. Is there enough allicin in the garlic extract to account for all its activity? To answer this question, we compared the theoretical estimate of allicin concentration (assayed by FLIPR) that would account for the garlic-extract activity on thermoTRPs to the amount of allicin (calculated from the NMR studies) actually present in these same extracts. Three separate experiments were performed in this way. The EC50s for allicin and garlic extracts (in the form of a dilution factor) were determined by FLIPR for mTRPA1-, hTRPA1-, and rTRPV1-expressing CHO cells (Figure 4E). Multiplying the EC50 of allicin by the dilution factor of garlic extract at its EC50 for each cell type gives the concentration of allicin that would be expected to be present in the undiluted garlic extract on the basis of its activity (when one assumes that allicin accounts for all its activity). We then compared this activity-based calculation of allicin concentration to the concentration of allicin in garlic extract derived from NMR experiments performed on the same day (Figures 4E and 4F). If the concentration of allicin found by NMR was significantly lower than the concentration expected based on the activity of garlic extract, then we would conclude that there are other compounds within garlic that are activating these channels synergistically with allicin. However, because the concentration of allicin in garlic ex-
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tract matches well with its activity in TRPA1 and TRPV1 channels, allicin can account for the pungent activity of garlic. Although other diallyl sulfide species can activate these channels in vitro, their relatively high EC50s and low concentration in fresh garlic extracts indicate that these compounds play a minor role (if any) in TRPV1- and TRPA1-activation in vivo. Furthermore, the close correlation between garlic-extract potency and allicin content precludes any significant contribution from other garlic-derived compounds.

Conclusion

The pungency of garlic has most likely evolved as a defense mechanism to protect the bulb; many species, including European starlings, ticks, mosquitoes, and worms, are repelled by garlic [23–25]. Paradoxically, raw garlic is a popular food for humans. And although garlic has been enjoyed for millennia, the “burning” question remained: What is the biological mechanism through which garlic produces these sensations? Here, we show that fresh-cut garlic and allicin, one of its constituents, activate TRPA1 and TRPV1, two noxious thermoTRPs found in pain-sensing neurons that innervate the mouth and tongue. Activation by garlic and allicin is specific to neurons expressing these channels; no other populations of DRG or trigeminal ganglia neurons are activated by these stimuli. Among the chemical constituents of garlic extracts, allicin is by far the most potent activator of TRPA1 and TRPV1. Furthermore, the activity of allicin, given its concentration in garlic, is sufficient to explain all of garlic extract’s activity on these thermoTRPs. Finally, extracts of baked garlic (which differ from fresh extracts primarily in their lack of allicin) are unable to activate thermoTRPs. Allicin and other garlic components are expected to activate olfactory and gustatory neurons as well; however, the burning sensation that fresh garlic can cause must work through the trigeminal system. Therefore, we conclude that in garlic, allicin is the active ingredient that causes a burning sensation through activation of TRPA1 and TRPV1.

Supplemental Data

Supplemental Experimental Procedures and one figure can be found with this article online at: http://www.current-biology.com/cgi/content/full/15/10/929/DC1/.

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