Commentary

The Salty and Burning Taste of Capsaicin

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The tongue is a kind of funny organ. From a physiological point of view, you can place solutions on the tongue that will be toxic to most any cell, and the tongue will give information, in a hopefully reversible manner, about the solution's chemical composition and whether it should be ingested. Remember the first time that you had a drink of vodka or scotch? It really burned. Do you know the reason for this? It turns out that neurons in the mouth, including the tongue, that carry nociceptive (tissue damaging) information contain receptors belonging to the transient receptor potential vanilloid (TRPV) family. The most investigated of these TRPV receptors is TRPV1. One reason TRPV1 is so extensively studied is that it is activated by capsaicin (CAP), the principle component in chili pepper that gives it its spicy or pungent taste (Caterina et al., 1997). It is also activated by acid and heat having a threshold temperature of 42°C. Previous studies on nociceptive neurons that innervate the face and mouth, as well as TRPV1-expressing HEK293 cells, showed that TRPV1 channels responded to ethanol in a concentration-dependent manner (Trevisani et al., 2002). Specifically, ethanol potentiated the response of TRPV1 to CAP and protons and lowered the threshold for heat activation of TRPV1 from 42°C to 34°C, which is near the temperature of the tongue. This provides a likely mechanistic explanation for the ethanol-induced sensory responses that occur at body temperature and for the sensitivity of inflamed tissues to ethanol, such as might be the case in esophagitis, neuralgia, or wounds (Hirota et al., 2003). For us gourmands, however, these data help rationalize why the pungent sensation of spicy food increases when we drink alcohol. Given the temperature dependence of TRPV1 receptors, the pungency should be reduced if one has a cold beer rather than some heated brandy.

For many years it has been known that ethanol also produces taste (as opposed to painful) responses (Hellekant, 1965; Sako and Yamamoto, 1999). However, the molecular and cellular mechanisms regarding the transduction mechanisms remain unknown. In a series of papers, Vijay Lyall and colleagues have identified the presence of a TRPV1 variant in taste receptor cells (Lyall et al., 2004, 2005a,b,c). From the above discussion, it should not be surprising that they found this receptor to be also sensitive to ethanol. They also found that this receptor has other important functions in gustatory physiology. Specifically, in the presence of salt, it is responsible for the amiloride-insensitive salt taste. They also found that the application of just ethanol causes a transient decrease in the volume of taste receptor cells and produces responses in taste cells and taste-sensitive neurons. Note that these results were obtained with a 20% vol/vol ethanol solution, which is 3.43 M. At the very minimum, this should serve as a warning to all of us who dissolve chemicals in hyperosmotic solutions containing ethanol (or DMSO) and add them to cells.

Below we present background information to lead you through these two papers by first reviewing some of the basic anatomy and physiology of the peripheral gustatory system and then showing how they relate to measurements of properties of taste cells from fungiform papillae and recordings from gustatory neurons from the chorda tympani branch of the facial nerve. We then review briefly the methodology and guide readers through these long and detailed articles.

Taste receptor cells (TRCs) in taste buds from fungiform papilla synapse with chorda tympani (CT) neurons (Finger and Simon, 2002). Taste buds are comprised of ∼50–100 taste receptor cells that extend from the taste pore, which is in direct contact with tastants placed in the mouth, to the basement membrane that separates the epithelium from the papillary layer. Taste cells comprise a simple epithelium that is embedded in a protective stratified epithelium. The tight junctions that are located beneath the microvilli (that project into the taste pore) serve to make this a polarized epithelium and protect the basolateral surface from the various solid and liquid foods that are placed in the mouth. Taste cells are frequently exposed to highly nonisotonic solutions (water, vodka); they can reversibly respond to the resultant volume flux changes because of the small surface area of taste cells that are exposed to the external solutions (Holland et al.,

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Abbreviations used in this paper: CAP, capsaicin; CT, chorda tympani; RTX, resiniferatoxin; TRC, taste receptor cell; TRPV, transient receptor potential vanilloid.

1989). Taste buds are surrounded by TRPV1 containing peptidergic nociceptors from the trigeminal nerve that are largely responsible for the burning sensation when we eat foods containing CAP.

The initial taste transduction events occur when chemicals interact with various types of receptors located on the apical ends of taste cells become activated. This receptor activation eventually results in an increase in intracellular Ca^{2+} that, in turn, increases the probability of transmitter release to activate chorda tympani neurons. Information from these neurons then is transmitted to the brain where it may be interpreted as taste and reward so that it can be ingested or rejected.

The two papers by Lyall et al. in this issue of the Journal concern themselves with two types of receptors, both of which are located on the apical membrane of taste cells that permit the entry of Na^+. The first is the amiloride-sensitive sodium channel (ENaC), which, when activated, is responsible for what we perceive as salty (NaCl). In the rat, the ion selectivity of these channels is $Na^+ >> K^+$. In many of the experiments of Lyall et al., ENaC was blocked by either amiloride or its more potent analogue, benzamil. The second sodium-permeable channel in taste cells is the TRPV1 variant. One major result of the present study is that this receptor can account for the amiloride/benzamil-insensitive salt responses. Rats can easily distinguish equi-intensive NaCl from KCl, but they cannot in the presence of amiloride (Spector, 2000). Indeed, recordings from the CT nerve have shown that upon the application of NaCl to the tongue, the CT responses also have amiloride-sensitive and -insensitive components (Elliott and Simon, 1990), thereby showing the presence of two distinct pathways.

Lyall et al. (2005a,b) used a variety of methods to show that ethanol induces changes in taste cell volume and increases the sensitivity of taste TRPV1 channels to ethanol in both TRCs and in CT responses. In one set of experiments using anesthesitized rats and in $TRPV1^{-/-}$ mice they measured whole nerve CT responses. As ethanol or NaCl (or some impermeant cation such as $NMDG^+$ or benzamil) is applied to the tongue alone or in combination, the evoked CT responses can be used to infer processes that occur only from the taste cells. This method, however, yields indirect information only about events occurring in TRCs. To directly test their hypothesis regarding the role of TRPV1 channels, they measured the changes in intracellular Na^+ in an intact (polarized), but excised, piece of rat lingual epithelium as well as in isolated TRCs.

In these imaging studies, the lingual epithelium is enzymatically removed from the underlying papillary layer and placed in a modified Ussing chamber. The mucosal and serosal sides thus can be independently perfused. The TRCs are loaded from the serosal side with fluorophore (usually with sodium green but also with the ratiometric dye SBFI), and measurements of Na_i^+ are performed before and after changing the composition of the solutions bathing the mucosal surface. Individual taste buds can be obtained from the excised epithelium by punching them out with a pipette. Changes in Na_i^+ were performed in the presence and absence of extracellular Na^+ and were used as a measure of changes in the taste cells' volume or changes in Na^+ influx in the presence and absence of benzamil. For changes in volume, in the absence of permeable cations, increases in the osmolality will result in an increase in the fluorescence intensity of the fluorophore.

The measurement of whole nerve CT responses involves placing the entire CT nerve on a wire and measuring (through an amplifier circuit) the power (activity) in this nerve bundle before and after a taste solution is flowed over the anterior region of the tongue. This response is then passed through an integrator, which is an RC circuit with a time constant selected to give a faithful representation of the CT response.

In their experiments, they first applied a control stimulus, such as artificial saliva, and then the taste stimulus which may include ethanol. Relative to the control, the evoked CT response has a phasic (rapid) and tonic (steady state) component. There have been many studies regarding the interpretation of the phasic and tonic components (e.g., Smith et al., 1978; DeSimone and Ferrell, 1985), and the ethanol modulation of CT responses have yielded a variety of responses. Lyall et al. (2005a) resolved this long standing puzzle by showing that the morphology of the response (relative magnitude of phasic and tonic response) depends on a variety of factors, which includes the flow rate, temperature, and ionic composition of the fluid.

At the usual concentrations of 0.1 M NaCl, the integrated CT responses (both phasic and tonic components) are inhibited ~60% by the ENaC blocker, amiloride or its more potent analogue, benzamil. This ENaC inhibition is thought to represent the blockage of the subset of neurons that among other tastants at comparable concentrations produce the greatest number of action potentials to NaCl. In the presence of benzamil, neurons that produce the greatest activity are those activated by Cl^- salts of H^+, NH_4^+, Na^+, K^+, and Ca^{2+}. Lyall et al. (2005b) suggest that, at least in part, these neurons are the ones that are activated by the TRPV1 receptors (but see below for acid stimulation).

Before proceeding, we review some characteristics of TRPV1 channels. In addition to being activated by CAP ($EC_{50} \sim 0.7 \mu M$), they are activated also by resiniferatoxin (RTX), which is ~10,000 times as potent as CAP. TRPV1 receptors are inhibited by ruthenium red, cap-

sazepine, and SB-366791. For the usual ionic conditions (high extracellular Na^+ and high intracellular K^+), TRPV1 receptors have a reversal potential near 0 mV, meaning they are not very selective between Na^+ and K^+. This last point is important since in gustatory physiology the extracellular ionic conditions can be distilled water, sugar, salt, and even ethanol solutions, which should alter the potential across the apical and basolateral membranes; the latter being related to the receptor potential. Moreover, the particular response will depend the temperature at which the experiments are performed. Experiments that used cells or excised tissues usually are performed at ∼20°C; one may not obtain the same results when solutions are placed on the tongue, which is likely to be at a higher temperature.

Lyall et al. (2005a,b) called their receptor a TRPV1 variant because it shares some similarities and differences with rTRPV1. Natural variants of TRPV1 that are insensitive to acid have already been identified (Lu et al., 2005). Although the taste TRPV1 variant (hereafter called TRPV1t) is activated by CAP, RTX, and heat, it differs from rTRPV1 in that it is not activated by acid, that it is active at 23°C, and it has a lower threshold temperature (38°C). Another difference between TRPV1t and TRPV1 that is relevant to this work is that, unlike TRPV1, the threshold temperature is not altered by ethanol. TRPV1 channels can be activated by voltage and heat and the probability of being in one of its many open or closed states depends on the activation energies between the various states whose magnitudes depend on voltage and temperature (Voets et al., 2004). It is not known how ethanol affects these temperature- and voltage-dependent rate constants.

Lyall et al. (2005a,b) demonstrated that the tonic phase of the CT response arises from the activation of TRPV1-type receptors because, in recordings from wild-type mice, the CT response to a lingual application of NaCl in the presence of benzamil exhibited both phasic and tonic components. In contrast, under identical conditions, the CT responses in TRPV1$^{-/-}$ mice exhibited only a phasic component. The origin of the phasic response to NaCl + benzamil in TRPV1$^{-/-}$ mice is unclear.

In recordings from the rat CT, Lyall et al. showed that ethanol will sensitize the effect of CAP or RTX in a dose-dependent manner, and that the tonic response could be blocked by using antagonists of TRPV1 receptors. They also showed that the ethanol-induced responses were enhanced at higher temperatures, but did not alter the threshold temperature.

Lyall et al. (2005a,b) also show that the CT phasic response due to ethanol in the absence of ions can be attenuated by preshrinking the taste cells with hypertonic mannitol, which provides further evidence that a transient decrease in taste cell volume is a precursor to the phasic neural response to ethanol.

In their studies on ethanol effects on taste receptor cells in fungiform papillae, Lyall et al. have previously shown that TRPV1 agonists (CAP, RTX, elevated temperature, and ATP) increase the apical membrane conductance and enhance the flux of Na^+, NH_4^+, and Ca^{2+} across the apical membrane of fungiform TRCs (Lyall et al., 2004, 2005c). Now they show that, in the presence of nonpermeable ions (NMDG$^+$), the addition of ethanol (plus benzamil) produces a dose-dependent decrease in taste cell volume whereas, in the presence of Na^+ and benzamil, ethanol produces a sustained response without an accompanying change in volume. Importantly, TRPV1 antagonists inhibit this response.

That is, using genetic and pharmacological interventions and in vitro and in vivo recordings, Lyall et al. showed that a TRPV1 variant, TRPV1t, is necessary to produce the tonic responses in taste cells and CT nerve fibers when ENaC receptors are blocked and when mineral salts are present. Among the very interesting properties of this channel (in addition to being acid insensitive), it is constitutively active at the resting membrane potential and at 23°C.

Regarding transduction mechanisms for salt taste, Lyall et al. proposes that Na^+ can enter cells through either ENaCs or TRPV1ts. Other ions such as K^+, NH_4^+, and Ca^{2+} can enter taste cells through TRPV1t receptors and perhaps through other pathways; protons must use a different pathway. Whatever pathway, the cation entry will tend to depolarize the cells. This will increase intracellular Ca^{2+}, through voltage-gated calcium channels including TRPM5 channels (Perez et al., 2003) or TRPV1t, which in turn will increase transmitters to be released from taste cells and activate CT fibers. Decreasing the TRC volume will presumably also increase intracellular Ca^{2+} because a CT response was evoked. Cell volume and ion homeostasis are regulated by the presence of various ATPases and cotransporters in the taste cells.

These papers are a great contribution to gustatory physiology because they identify the role of TRPV1t receptors for the amiloride-insensitive salt taste responses, but questions remain. What is the molecular identity of TRPV1t, by what mechanisms does ethanol modulate channel function, what other tastant receptors are present in TRCs that contain TRPV1t? The latter is important in the context of the labeled-line model of coding at the periphery (Mueller et al., 2005).

It is important to characterize the properties of TRPV1t in TRCs using patch clamp either in taste cells or after it is cloned, to determine how it differs from rTRPV1. In addition, to not being activated by acid and being active at 23°C, TRPV1t differs in another property from TRPV1 in that RTX seems to be readily re-

versible and does not induce desensitization. These characteristics reflect the absence of Ca^{2+} in the apical solution whose entry into the cell could activate intracellular pathways that promote receptor desensitization and tachyphylaxis (Koplas et al., 1997). How small hydrophobic molecules affect ion channels is a topic of great interest to readers of this journal (Suchyna et al., 2004). In the case of TRPV1 receptors, amphiphilic molecules like ethanol and nicotine (Liu et al., 2004) seem to sensitize the channel, but local anesthetics such as lidocaine and prilocaine reduce channel activity (Hirota et al., 2003). So we need to consider at least two, not mutually exclusive possibilities: is ethanol an agonist in the pharmacological sense that it interacts directly with TRPV1 channels in some hydrophobic binding pocket (which might rationalize the different sensitivities of rat and human TRPV1's to ethanol); and, given the high concentrations, does ethanol (and other relevant compounds) alter the membrane's material properties, which could also alter channel activity (Lundbaek and Andersen, 1994)?

From a physiological perspective, how are the TRPV1t's voltage- (and temperature-) dependent properties related to the physiology of the TRC, and what determines phasic and tonic responses when solutions of NaCl are applied? Specifically, we need to understand what part of the tonic response that arises as a consequence of adaptation of the ENaC channels and what part that is due to the Na^+ permeability across TRPV1t receptors. This information is relevant to studies where the sodium salts contain large anions that do not permeate the tight junctions and that eliminate virtually all amiloride-insensitive currents.

R E F E R E N C E S

Caterina, M.J., M.A. Schumacher, M. Tominaga, T.A. Rosen, J.D. Levine, and D. Julius. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 389:816–824.

DeSimone, J.A., and F. Ferrell. 1985. Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. *Am. J. Physiol.* 249:R52–R61.

Elliott, E.J., and S.A. Simon. 1990. The anion in salt taste: a possible role of tight junctions. *Brain Res.* 535:9–17.

Finger, T.E., and S.A. Simon. 2002. The cell biology of lingual epithelia. *In* The Neurobiology of Taste and Smell. T.E. Finger, W.L. Silver, and D. Restrepo, editors. Wiley-Liss, New York. 287–314.

Hellekant, G. 1965. Electrophysiological investigation of the gustatory effect of ethyl alcohol. I. The summated response of the chorda tympani in the cat. *Acta Physiol. Scand.* 64:398–406.

Hirota, K., D. Smart, and D.G. Lambert. 2003. The effects of local and intravenous anesthetics on recombinant rat VR1 vanilloid re-

ceptors. *Anesth. Analg.* 96:1656–1660.

Holland, V.F., G.A. Zampighi, and S.A. Simon. 1989. Morphology of fungiform papillae in canine lingual epithelium: location of intercellular junctions in the epithelium. *J. Comp. Neurol.* 279:13–27.

Koplas, P.A., R.L. Rosenberg, and G.S. Oxford. 1997. The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. *J. Neurosci.* 17:3525–3537.

Liu, L., W. Zhu, Z.S. Zhang, T. Yang, A. Grant, G. Oxford, and S.A. Simon. 2004. Nicotine inhibits voltage-dependent sodium channels and sensitizes vanilloid receptors. *J. Neurophysiol.* 91:1482–1491.

Lu, G., D. Henderson, L. Liu, P.H. Reinhart, and S.A. Simon. 2005. TRPV1b, a functional human vanilloid receptor splice variant. *Mol. Pharmacol.* 67:1119–1127.

Lundbaek, J.A., and O.S. Andersen. 1994. Lysophospholipids modulate channel function by altering the mechanical properties of lipid bilayers. *J. Gen. Physiol.* 104:645–673.

Lyall, V., G.L. Heck, A.K. Vinnikova, S. Ghosh, T.H. Phan, R.I. Alam, O.F. Russell, S.A. Malik, J.W. Bigbee, and J.A. DeSimone. 2004. The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. *J. Physiol.* 558:147–159.

Lyall, V., G. Heck, T.-H.T. Phan, S. Mummalaneni, S.A. Malik, A.K. Vinnikova, and J.A. DeSimone. 2005a. Ethanol modulates the VR-1 variant amiloride-insensitive salt taste receptor. I. Effect on TRC volume and Na^+ flux. *J. Gen. Physiol.* 125:569–585.

Lyall, V., G. Heck, T.-H.T. Phan, S. Mummalaneni, S.A. Malik, A.K. Vinnikova, and J.A. DeSimone. 2005b. Ethanol modulates the VR-1 variant amiloride-insensitive salt taste receptor. II. Effect on chorda tympani salt responses. *J. Gen. Physiol.* 125:587–600.

Lyall, V., G.L. Heck, A.K. Vinnikova, S. Ghosh, T.H. Phan, and J.A. DeSimone. 2005c. A novel vanilloid receptor-1 (VR-1) variant mammalian salt taste receptor. *Chem. Senses.* 30:i42–i43.

Mueller, K.L., M.A. Hoon, I. Erlenbach, J. Chandrashekar, C.S. Zuker, and N.J.P. Ryba. 2005. The receptors and coding logic for bitter taste. *Nature.* 434:225–229.

Perez, C.A., R.F. Margolskee, S.C. Kinnamon, and T. Ogura. 2003. Making sense with TRP channels: store-operated calcium entry and the ion channel Trpm5 in taste receptor cells. *Cell Calcium.* 33:541–549.

Sako, N., and T. Yamamoto. 1999. Electrophysiological and behavioral studies on taste effectiveness of alcohols in rats. *Am. J. Physiol.* 276:R388–R396.

Smith, D.V., S.L. Bealer, and R.L. van Buskirk. 1978. Adaptation and recovery of the rat chorda tympani response to NaCl. *Physiol. Behav.* 20:629–636.

Spector, A.C. 2000. Linking gustatory neurobiology to behavior in vertebrates. *Neurosci. Biobehav. Rev.* 24:391–416.

Suchyna, T.M., S.E. Tape, R.E. Koeppe II, O.S. Andersen, F. Sachs, and P.A. Gottlieb. 2004. Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. *Nature.* 430:235–240.

Trevisani, M., D. Smart, M.J. Gunthorpe, M. Tognetto, P. Barbry, B. Campi, S. Amadesi, J. Grey, J.C. Jerman, S.J. Brough, et al. 2002. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. *Nat. Neurosci.* 5:546–551.

Voets, T., G. Droogmans, U. Wissenbach, A. Janssens, V. Flockerzi, and B. Nilius. 2004. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature.* 430:748–754.