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 nonsotonic 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.,

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

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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 anesthetized 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$^+$ 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$^+$ 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$_{50}$ ~ 0.7 μM), they are activated also by resiniferatoxin (RTX), which is ~10,000 times as potent as CAP. TRPV1 receptors are inhibited by ruthenium red, cap-
mannitol, which provides further evidence that a tran-
tenuated by preshrinking the taste cells with hypertonic
response due to ethanol in the absence of ions can be at-
not alter the threshold temperature.

Lyall et al. (2005a,b) demonstrated that the tonic
phases of the CT response arise 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 benzamid exhibited both pha-
sic and tonic components. In contrast, under identical
conditions, the CT responses in TRPV1<sup>−/−</sup> mice exhib-
ted only a phasic component. The origin of the phasic
response to NaCl + benzamid in TRPV1<sup>−/−</sup> 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 recep-
tors. They also showed that the ethanol-induced re-
sponses were enhanced at higher temperatures, but did
not alter the threshold temperature.

Lyall et al. (2005a,b) also show that the CT phasic re-
sponse due to ethanol in the absence of ions can be at-
tenuated by preshrinking the taste cells with hypertonic
mannitol, which provides further evidence that a tran-
sient 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 tem-
perature, and ATP) increase the apical membrane con-
ductance and enhance the flux of Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, and Ca<sup>2+</sup>
across the apical membrane of fungiform TRCs (Lyall
et al., 2004, 2005c). Now they show that, in the pres-
ence of nonpermeable ions (NMDG<sup>+</sup>), the addition of
ethanol (plus benzamil) produces a dose-dependent
decrease in taste cell volume whereas, in the presence
of Na<sup>+</sup> and benzamid, ethanol produces a sustained re-
response without an accompanying change in volume.
Importantly, TRPV1 antagonists inhibit this response.

That is, using genetic and pharmacological interven-
tions 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 in-
sensitive), it is constitutively active at the resting mem-
brane potential and at 23°C.

Regarding transduction mechanisms for salt taste, Ly-
all et al. proposes that Na<sup>+</sup> can enter cells through ei-
ther ENaCs or TRPV1t. Other ions such as K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>,
and Ca<sup>2+</sup> can enter taste cells through TRPV1t recep-
tors 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<sup>2+</sup>, through voltage-gated cal-
cium channels including TRPM5 channels (Perez et al.,
2003) or TRPV1t, which in turn will increase transmis-
ters to be released from taste cells and activate CT fi-
bers. Decreasing the TRC volume will presumably also
increase intracellular Ca<sup>2+</sup> 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 re-
sponses, but questions remain. What is the molecular
identity of TRPV1t, by what mechanisms does ethanol
modulate channel function, what other tasant recep-
tors are present in TRCs that contain TRPV1t? The lat-
ter 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 prop-
erty from TRPV1 in that RTX seems to be readily re-

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versatile and does not induce desensitization. These characteristics reflect the absence of $\text{Ca}^{2+}$ 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 (Lundback and Andersen, 1994)?

From a physiological perspective, how are the TRPV1’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 $\text{Na}^+$ permeability across TRPV1 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.

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