Commentary

Interactions between Salt and Acid Stimuli: A Lesson in Gustation from Simultaneous Epithelial and Neural Recordings

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If for no other reason than the fact that eating and drinking gives us pleasure, it is of interest to understand the physiology of gustation. In this issue, Lyall et al. (2002a) have uncovered the key cellular mechanisms that occur in taste receptor cells (TRCs) when certain mixtures of chemicals (tastants) are applied to the anterior tongue. In much the same way as music produced by a quartet differs from that produced by each instrument, compared with the sensations produced by individual tastants, in mixtures they can produce an entirely different taste sensation, increase the intensity of one of the tastants, or, as shown in the work of Lyall et al. (2002a), suppress the response to one of the tastants.

One question frequently asked in gustatory circles is where the interaction between the different tastants first takes place. Is it at the periphery, in taste receptor cells and/or in higher CNS centers? Lyall and colleagues have shown that, in agreement with psychophysical studies, the neural responses to NaCl are reduced in the presence of acidic stimuli. Although previous nerve recordings have shown that neural responses to NaCl are inhibited at low pHs (Biedler, 1954; Ogawa, 1969), until this present study, the mechanisms underlying this effect have not been delineated. Lyall et al. (2002a) showed that these responses could be understood at the level of TRCs, where a decrease in intracellular pH reduces Na^+ influx through amiloride-sensitive epithelial sodium channels (ENaCs). This reduction in Na^+ influx results in a decreased neural response and ultimately in a decreased sensation to NaCl. One can appreciate the practicality of this result if one accidentally pours too much salt on a steak. The solution to reduce the salty taste is to add acid to the steak (I am not saying it will be palatable, just less salty). In this commentary, I will review some of the basic anatomy and physiology of the peripheral gustatory system and then show how it relates to measurements of the epithelial properties of the tongue, and simultaneous recordings from primary gustatory neurons.

Fig. 1 shows a taste bud embedded in a stratified epithelium. Taste buds are comprised of ~50 –100 neuroepithelial cells, the taste receptor cells that extend from the taste pore, which is in direct contact with the tastants in the mouth, to the basement membrane that separates the epithelium from the papillary layer. Tight junctions are located beneath the microvilli that project into the taste pore, which serve to make this a polarized epithelium (Holland et al., 1989). These tight junctions are the major barrier of the paracellular pathway. They are weakly cation selective and give rise to liquid junction potentials that change when the chemical (ionic) composition in the mouth changes, which alters the voltage across TRCs, and thus the neural responses (Elliott and Simon, 1990; Ye et al., 1993, 1994). The initial transduction events occur when chemicals interact with various types of receptors in the microvilli membrane. Presently, receptors have been identified for salts, acids (protons), amino acids, neuropeptides, and various sweet and bitter tasting compounds (Herness and Gilbertson, 1999; Nelson et al., 2001, 2002). At their basolateral membrane, TRCs also contain voltage-gated sodium, potassium, and calcium channels, a variety of ATPases, and ion exchangers that are necessary to maintain homeostasis. Individual rat TRCs are broadly tuned in that they respond to several of chemical stimuli (Gilbertson et al., 2001; Caicedo et al., 2002). TRCs in the anterior two-thirds of the tongue form synapses with broadly tuned primary gustatory neurons from the chorda tympani (CT) branch of the facial nerve (CNVII). The TRC-CT system has been shown to be important for tastant identification and discrimination (Spector, 2000). Chemical stimulation of TRCs in the back of the tongue evokes reflexive actions, such as gagging and swallowing (Spector, 2000).

Lyall et al. (2002a) have used three very different methods to show that acidic stimuli inhibit responses to NaCl in both TRCs and CT responses. In one set of experiments they measured whole nerve CT responses while simultaneously voltage-clamping an anterior section of rat lingual epithelium containing TRCs. As tastants such as salt (NaCl) and acid are applied alone or together to the tongue, the evoked CT responses can be used to infer processes that occur only in the TRCs. This approach is a "dream come true" for the

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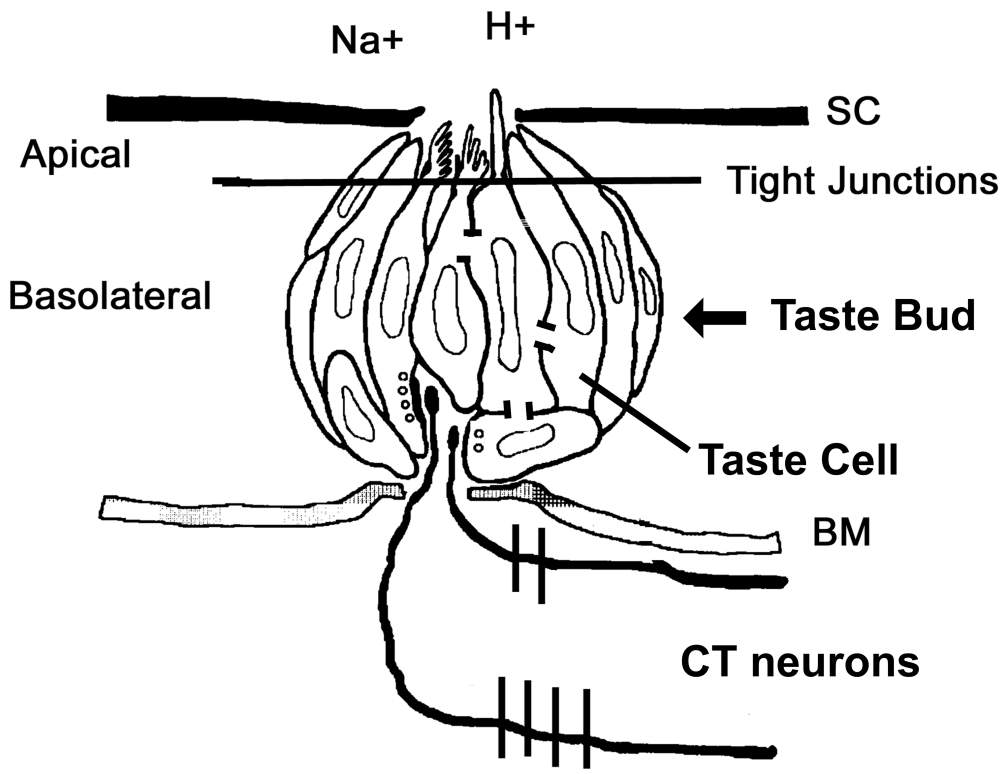

FIGURE 1. Diagram of a taste bud (arrow) that is embedded in stratified layers of epithelial cells (not depicted). The layer of tight junctions defines the apical and basolateral regions of the taste cells. Gap junctions couple clusters of taste cells. Coupled cells are indicated by short lines (see cell labeled "taste cell"). The stratum corneum (SC) of the epithelium opens to form a taste pore through which microvilli of taste cells protrude. Shown are a sodium ion and a proton about to enter the taste pore. Taste cells terminate at the basement membrane (BM), which separates the epithelium from the papillary layer. Two taste cells are shown to synapse with chorda tympani (CT) neurons.

many scientists who have worked with isolated epithelial preparations in Ussing chambers because the neural responses can be used to report the activity of a small and select population of cells in the epithelium. However, this elegant method only yields indirect information about events occurring in TRCs. To directly test their hypothesis regarding how acid stimuli inhibit CT responses to NaCl, they also measured changes in intracellular pH $(pH)_i$ and Na^+ $(Na^+)_i$ in an intact (polarized), but excised, piece of rat lingual epithelium (Lyall et al., 2001, 2002). To guide readers through this long and detailed article, especially those outside the taste field, I will briefly go through their methodology.

The imaging studies are straightforward. The lingual epithelium is removed enzymatically from the underlying papillary layer and is placed in a modified Ussing chamber in which the mucosal and serosal sides are separated. The TRCs are loaded from the serosal side with selected fluorescent dyes and measurements of pH_i and Na_i^+ are performed before and after changing the composition of the mucosal solutions.

The two other measurements are more complex, both in their execution and interpretation. The measurement of whole nerve CT responses involves placing the entire CT nerve on a wire and measuring the power (activity) in this nerve bundle. This response is then passed through an integrator (an RC circuit with a time constant selected to give a faithful representation of the CT response). In the continued presence of a stimulus, the CT response has a phasic (rapid) and tonic component, which reflects the adaptation to the stimulus. When four very different stimuli (NaCl, acid, sucrose, quinine), which represent four very distinct taste sensations, are placed on the anterior tongue at equal intensities the individual CT neurons vary in their responses (the rate of action potentials) to one of these stimuli (Frank et al., 1983). This paper concerns itself primarily with the type of CT neurons that respond best (in terms of more action potentials) to NaCl. The integrated CT responses are inhibited ~60% by the epithelial sodium channel blocker, amiloride (or its more potent analogue, benzamil). This inhibition represents the blockage of the "sodium best" neurons. The neurons that respond best to acid are also activated by NaCl and KCl, but are not inhibited by amiloride. CT responses to NaCl in the presence of other chemicals were measured with respect to the response evoked by 0.3 M NH_4Cl, because it gives a large and quite reproducible response to which those obtained to other stimuli can be compared.

Measurements of the electrical properties of an intact epithelial tissue usually require that it be placed in a chamber separating two solutions. With proper voltage-clamp circuitry, the open circuit potential (or zero current clamp potential, denoted as 0cc) can be measured. This transepithelial potential depends on the potential across the apical and basolateral membranes, transepithelial resistance (i.e., the resistance across the mucosal (Ra) and serosal (Rb) membranes), and the paracellular (Rp) or shunt resistance, which is in paral-

lel with the transepithelial resistance. In most actively transporting tissues, including lingual epithelia, the 0cc is positive (the serosal solution is positive with respect to the mucosal solution). The transepithelial current-voltage curve is then obtained by changing the voltage and measuring the corresponding current; the current when the transepithelial potential = 0 mV is the short circuit current. In many sodium-transporting epithelia, including rat tongue (DeSimone et al., 1984; Simon et al., 1988), the short-circuit is carried by Na^+ that enters the epithelial cells through amiloride-sensitive epithelial sodium channels (ENaCs) and leaves these cells through a ouabain-sensitive Na^+-K^+-ATPase (DeSimone et al., 1984; Simon et al., 1991). Chloride ions follow passively through the tight junctions. A major contribution to taste physiology made by DeSimone's laboratory was to develop the methodology to voltage-clamp an intact tongue in an anesthesitized rat. With this method, they could change the transepithelial potential from 0cc to potentials in either the depolarizing or hyperpolarizing direction. This is important because lingual epithelia consist of TRCs embedded in epithelial cells (see Fig. 1), so when the transepithelial voltage changes, whether by changing the applied voltage or by changing the composition of the mucosal solution, it will also change the voltage drops across apical and basolateral membranes. The changes in voltage in the taste cells, relative to the serosal solution, when the chemical composition on the mucosal surface is changed, are called the receptor potential (ΔVr). ΔVr also changes when ΔVt, the transepithelial potential measured with respect to the mucosal solution, is changed through the external electrodes: $\Delta Vr = -(1 - d)\Delta Vt$, where d is the ratio of mucosal resistance to the sum of the serosal and mucosal resistances. The key point is that the observed changes in the CT response are reflective of changes in ΔVr, which can be changed by altering the concentration of luminal tastants or by changing ΔVt.

Before highlighting the key experimental observations of their work, it is necessary to point out the "players" in the transduction process for Na^+ and H^+. Na^+ enters TRCs via two pathways: one is a "typical" channel of the ENaC family that is amiloride sensitive, Na^+ selective, and regulated by protons (Chalfant et al., 1999; Zeiske et al., 1999; Awayda et al., 2000); the other is a cetylpyridinium chloride (CPC)-sensitive, amiloride-insensitive pathway that is nonselective among several monovalent cations (DeSimone et al., 2001). The amiloride-sensitive channel is responsible for the characteristic taste of NaCl, as was demonstrated by showing that rats in the presence of amiloride cannot distinguish between NaCl and KCl (Spector, 2000). There are several possibilities that may account for the transduction pathways for protons that will lead to a sour taste sensation. These include two proton-gated cation-selective channels: hyperpolarization-activated channels (HCN; Stevens et al., 2001), and acid-sensitive ion channels (ASICs [Lin et al., 2002], which may also serve as mechanoreceptors [Mano and Driscoll, 1999]), and a proton-gated chloride channel (Miyamoto et al., 1998). There is also a poorly characterized amiloride-insensitive H^+-pathway on the apical membrane of TRCs. Finally, in the absence of Na^+, H^+ can enter the TRCs through the ENaCs (Gilbertson et al., 1993).

Acids come in two forms, strong and weak. Strong acids, like HCl, are completely dissociated at almost any pH. Weak acids, such as acetic acid, have a higher pKa (4.7) and can exist in two forms HA and A^- at reduced pH (say pH 3). The charged form is relatively impermeable to the membrane (unless it goes through a proton permeable channel), whereas the uncharged form will rapidly diffuse across the membrane (the larger the partition coefficient, the larger the permeability), dissociate in the cytoplasm, and reduce the intracellular pH. Lyall et al. (2002a) tested the effects of one strong acid (HCl) and two weak acids (acetic acid and CO_2); the latter rapidly hydrates and then dissociates, in the presence of carbonic anhydrase, into HCO_3^- and H^+.

Their first observation was that under open circuit conditions (the physiological condition), as the external pH (pH_o) increased from 2 to 10.3, the normalized CT response to 0.1 M NaCl increased linearly. Moreover, over this pH range the CT responses were voltage dependent, with the responses increasing at lumen-negative transepithelial potentials and decreasing at lumen-positive transepithelial potentials, indicating that the pathway involved in reducing the response is at the apical membrane of TRCs. Consistent with this observation is that throughout the pH_o range, the CT responses were markedly reduced (50–60%) by benzamil, indicating the involvement of the ENaC entry pathway in the CT response.

The question remained as to whether this inhibition arises as a consequence of extra- and/or intracellular pH changes. To address this question they kept pH_o at pH 6.1 and added acetic acid, at different concentrations, to the NaCl buffer. At this pH, CT responses decreased as the acetic acid concentration was increased. Perhaps more striking evidence that the inhibition of CT responses to NaCl arises from decreases in pH_i is that when CO_2/HCO_3 buffers with elevated pCO_2, but maintained at physiological pH, were added to the NaCl solution, the CT response decreased. Inhibiting carbonic anhydrase prevented this decrease.

More definitive evidence for the role of pH_i in decreasing CT responses to NaCl came from the imaging measurements of pH_i and Na_i^+ in individual TRCs from excised and polarized epithelia. Lyall et al.

(2002a) repeated the same experiments they did on the in vivo preparation, only now they measure the effects in individual TRCs. They showed that pH_i increases linearly over a pH_o range of 2–10.3. The CT responses also decreased linearly over this pH range and together these findings suggest that the acid-induced decrease in pH_i serves as a proximate stimulus for sour taste. Surprisingly, pH_i changed only ~0.3 pH units over this large change in pH_o. As with the CT experiments, addition of CO_2 decreased pH_i, and membrane-permeable inhibitors of carbonic anhydrase diminished this decrease. When the mucosal solution was kept at a constant pH, increasing the acetic acid concentration decreased pH_i in a concentration-dependent manner. Measurements of Na_i^+ showed that it decreased in the presence of amiloride, as would be expected if ENaCs were involved in Na^+ influx. Finally, Na_i^+ was decreased by lowering pH_o and increased by increasing pH_i (with NH_4Cl) in a manner consistent with the behavior of ENaCs in other cells (Zeiske et al., 1999). Thus, a very nice and consistent picture emerged regarding the interaction between NaCl and pH_i. One further observation reported in the paper has a bearing on the site of action of protons on ENaC responsible for their inhibition of apical sodium influx. The inhibitory action of acid on salt taste responses could be prevented by topical application of Zn or DEPC, suggesting that histidine residues on ENaC are the likely sites of H^+ modulation. What is missing to close the loop is to show that the increases in pH_i cause increases in Ca_i^{2+}, as Ca_i^{2+} is required for transmitter release from TRCs to CT neurons.

In summary, Lyall et al. (2002a) have provided the first good evidence for a peripheral mechanism that rationalizes why acid (sour taste), when mixed with NaCl (salty taste), reduces the intensity of the salty taste sensation. Their model, summarized in Fig. 22 of their paper, proposes that the interaction of acids with NaCl occurs at the level of TRCs. When protons enter the cytoplasm of TRCs, whether by diffusing through an apically located proton-permeable pathway (in rats), or by having the membrane-permeable form of a weak acid dissociate in the cytoplasm, Na^+ influx will be inhibited by protons binding to sites on amiloride-sensitive ENaCs. It is also possible, given the large pH gradient, that protons can diffuse through the tight junctions into extracellular space and activate proton-gated ion channels, such as HCNs or ASICs, on the serosal, resulting in depolarization of TRCs. However, these mechanisms may not generate a change in pH_i and are, therefore, unlikely to play a role in the acid–salt interaction. The recovery of pH_i occurs, in part, from the activation of Na^+-H^+ exchangers on the serosal side. The increase in Na_i^+ will also inhibit Na^+ influx through ENaCs until it is extruded from the TRC through Na^+-K^+-ATPases in basolateral membranes. The pH-induced inhibition of Na^+ influx means that TRCs will be depolarized less, which in turn will cause less neurotransmitter release, thus reducing the CT responses from "sodium-best" fibers and thereby resulting in a diminished salt sensation.

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