A permeability barrier surrounds taste buds in lingual epithelia

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Dando R, Pereira E, Kurian M, Barro-Soria R, Chaudhari N, Roper SD. A permeability barrier surrounds taste buds in lingual epithelia. Am J Physiol Cell Physiol 308: C21–C32, 2015. First published September 10, 2014; doi:10.1152/ajpcell.00157.2014.—Epithelial tissues are characterized by specialized cell-cell junctions, typically localized to the apical regions of cells. These junctions are formed by interacting membrane proteins and by cytoskeletal and extracellular matrix components. Within the lingual epithelium, tight junctions join the apical tips of the gustatory sensory cells in taste buds. These junctions constitute a selective barrier that limits penetration of chemosensory stimuli into taste buds (Michlig et al. J Comp Neurol 502: 1003–1011, 2007). We tested the ability of chemical compounds to permeate into sensory end organs in the lingual epithelium. Our findings reveal a robust barrier that surrounds the entire body of taste buds, not limited to the apical tight junctions. This barrier prevents penetration of many, but not all, compounds, whether they are applied topically, injected into the parenchyma of the tongue, or circulating in the blood supply, into taste buds. Enzymatic treatments indicate that this barrier likely includes glycosaminoglycans, as it was disrupted by chondroitinase but, less effectively, by proteases. The barrier surrounding taste buds could also be disrupted by brief treatment of lingual tissue samples with DMSO. Brief exposure of lingual slices to DMSO did not affect the ability of taste buds within the slice to respond to chemical stimulation. The existence of a highly impermeable barrier surrounding taste buds and methods to break through this barrier may be relevant to basic research and to clinical treatments of taste.

In a tissue-specific manner throughout mammalian epithelia (46). For instance, in mouse taste epithelia, claudins 4 and 8 are found in high abundance around the apical taste pore and claudin 6 is detected specifically inside the pore, consistent with the presence of tight junctions that prevent penetration of many taste compounds into the taste bud (32). The apical tight junctions not only prevent penetration of taste compounds into the taste bud, but they may also reduce access of topically applied drugs that might be used to modify the sensitivity of taste receptor cells.

However, there are other components to tissue barriers in addition to tight junctions. The epidermal barrier to water and solutes is also formed by a relatively impermeable extracellular matrix that is secreted into the spaces between cells in the stratum corneum (14, 44, 49). This raises the following question: Are there factors in addition to the zonula occludens of the taste pore that contribute to a barrier in gustatory end organs?

The present study focuses on tissue barriers of the taste bud and reveals that, in addition to apical tight junctions, there is a formidable barrier surrounding these sensory end organs, including their basolateral regions. This basolateral barrier prevents penetration of many compounds from the bloodstream into the taste bud and may represent a significant obstacle to the potential therapeutic use of systemic drugs in addition to topically applied agents. A number of studies, particularly in the fields of dermatology and drug delivery, have focused on reducing epithelial barriers (27, 28). Similarly, an important goal of our study was to identify the composition of the taste bud barrier and devise methods to reduce it (41).1

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the University of Miami Animal Care and Use Committee. C57BL/6J mice were killed by exposure to CO2 followed by cervical dislocation. In some of the experiments where dye was injected intravenously or directly into the tongue, deep anesthesia was achieved for 1–1.5 h with ketamine (120 mg/kg body wt) and xylazine (10 mg/kg body wt), with the animal monitored on a heating pad. The mouse was euthanized while still under deep anesthesia.

Dye permeation studies. Lingual slices (100–200 μm thick) containing circumvallate or fungiform papilla of mice were cut on a vibratome and incubated in Tyrode’s buffer [in mM: 130 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, 10 sodium pyruvate, and 5 NaHCO3, adjusted to pH 7.4 (318–323 mosM)] containing lipophilic or hydrophilic fluorescent dyes (Fig. 1). Incubation time and temperature varied according to the dye (Fig. 1). Adherence onto small pieces of nitrocellulose kept the slices flat for subsequent processing. Slices were then fixed in 4% paraformaldehyde, rinsed with PBS, cryoprotected in 30% sucrose for 30 min and cryosectioned

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1 This article is the topic of an Editorial Focus by Sami Damak (8a).
(20 μm thick), mounted in ProLong Gold mounting medium (Life Technologies), and photographed.

For in vivo experiments, dye was introduced via two routes. 1) In anesthetized mice, 50 μl of 5-chloromethylfluorescein diacetate (CMFDA, 25 μM) plus 70 μl of DMSO were injected with a 31-gauge hypodermic needle just below the lingual epithelium adjacent to the vallate papilla and allowed to diffuse into the lingual tissues surrounding taste buds for 10–30 min. After this procedure, lingual slices were prepared as described above. 2) In deeply anesthetized animals, dye was injected (typically, into a tail vein) for delivery via the systemic bloodstream (Fig. 1). Given the limited amount of CMFDA available, the dye was introduced directly into the heart, with the descending aorta clamped to restrict perfusion to the upper body.

Fig. 1. Fluorescent dyes used to assay the taste bud barrier. RT, room temperature.
Enzyme treatments. Lingual slices (100 μm, see above) of circumvallate papilla taste buds were treated with specific enzyme solutions or with their respective buffer solutions (Table 1) for 1 h at 37°C and washed twice with Tyrode’s solution for 10 min each. They were then transferred to 25 μM CellTracker Green CMFDA prepared in Tyrode’s solution and incubated at 37°C for 1 h and washed three times with Tyrode’s solution. Slices were then fixed with 4% paraformaldehyde, washed twice with PBS, counterstained with TO-PRO-3 (1:1,000 dilution) for 10 min, and washed with PBS. Slices were mounted with ProLong Gold mounting medium (Life Technologies) and then imaged with confocal microscopy (Olympus FV-1000) using Fluoview software.

Sheets of lingual epithelia were prepared by subepithelial injection of 100 μl of a protease cocktail containing Collagenase D (1 mg/ml) and Dispase II (2.5 mg/ml) into the region of the vallate papilla after the tongue was dissected from the animal. The tongue was aerated in Ca²⁺- and Mg²⁺-free Tyrode’s solution for 20 min; then an epithelial sheet containing vallate taste buds was carefully peeled free. Each epithelial sheet was bisected to yield two equal halves: one remained in Tyrode’s solution, and the other was reincubated in fresh enzyme cocktail for an additional 2 min and then washed with Tyrode’s solution. Both halves of the epithelial sheet were incubated in 25 μM CMFDA for 1 h and washed three times with Tyrode’s solution, fixed, washed once, and cryoprotected with 30% sucrose for 20 min. Tissues were embedded in optimal cutting temperature compound and cut at 25 μm, collected on glass slides, permeabilized (0.4% Triton X in PBS for 10 min), blocked (0.1% Triton X and 7% normal donkey serum in PBS for 30 min), and immunostained with goat anti-KCNQ1 (1:500 dilution; sc-10646, Santa Cruz Biotechnology) and donkey anti-goat-Alexa 594 (1:1,000 dilution; Invitrogen, Carlsbad, CA).

Microscopy. Tissue preparations were inspected and photographed using a Zeiss AxioPlan microscope and AxioCam camera or an Olympus Fluoview 1000 confocal microscope. Digital photomicrographs were collected with AxioVision 3.1 and Fluoview v2.0 software on the respective microscopes. Raw data micrographs (see Figs. 2–6) are presented without contrast enhancement, color balance.

Table 1. Enzymes used to permeabilize lingual epithelium

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Temperature</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase D + Dispase II</td>
<td><em>Clostridium histolyticum</em></td>
<td>RT and 37°C</td>
<td>2.5 mg/ml + 1 mg/ml in buffer, pH 7.2</td>
</tr>
<tr>
<td>Elastase</td>
<td><em>Bacillus polymyxa</em></td>
<td>RT and 37°C</td>
<td>0.25 mg/ml in buffer, pH 7.2</td>
</tr>
<tr>
<td>Chondroitinase ABC</td>
<td><em>Proteus vulgaris</em></td>
<td>37°C</td>
<td>2.5 U/ml in 0.1 M Tris-HCl and 0.1 M NaAcOH, pH 7.5</td>
</tr>
<tr>
<td>Heparitinase</td>
<td><em>Flavobacterium heparinum</em></td>
<td>37°C</td>
<td>0.1 U/ml in 0.1 NaAcOH, pH 7.0</td>
</tr>
</tbody>
</table>

Collagenase D and Dispase II were obtained from Roche, elastase from Worthington Biochemical, and chondroitinase ABC and heparitinase from Sigma Aldrich. RT, room temperature.

Fig. 2. Fluorescent dye permeation throughout the lingual epithelium reveals a barrier around taste buds. Freshly cut lingual slices (100–200 μm thick) from mouse vallate papilla were incubated in 5-chloromethylfluorescein diacetate (CMFDA, 25 μM) for 4 h at room temperature. A: schematic drawing of a lingual slice containing the vallate papilla. Green box shows approximate position of taste buds in B, B and C: CMFDA readily penetrated nontaste epithelium surrounding taste buds but, remarkably, was completely excluded from taste buds (dark, unstained regions, arrows). Connective tissue below taste buds was also stained with CMFDA, although staining is not as apparent due to the relatively low cellularity of that tissue compared with the overlying epithelium. D and E: CMFDA appeared to penetrate into and stain the cytosol of perigemmal cells immediately surrounding the taste buds (double arrows) but did not gain access into the taste bud (arrow). Cell nuclei in D and E are stained with TO-PRO-3 (red). Scale bars, 20 μm. [Drawing in A was modified with permission from Kim (24).]
Digital images were assembled using Adobe Photoshop 10.0.1 at 300 dpi without subsequent alterations that might distort the micrographs. Fluorescence intensity profiles for quantifying dye distribution in the epithelium were measured using ImageJ (v1.49b).

**Confocal Ca**^{2+} **imaging.** Taste cells were iontophotically loaded with Ca^{2+}-sensitive dye (2 mM Calcium Green dextran) via a 35-μm-tip-diameter borosilicate micropipette inserted into the trench of the circumvallate papilla, as described previously (7). Slices of vallate papilla were taken as described above, placed in a recording chamber, and attached to the coverslip base using Cell-Tak (BD Biosciences, San Jose, CA). Lingual slices were perfused at room temperature with Tyrode’s buffer containing elevated Ca^{2+} (8 mM) to improve the stability of the recordings and the signal-to-noise ratio. The experimental chamber was perfused with fresh Tyrode’s solution at a rate of 2 ml/min. We imaged cells confocally (Olympus Fluoview) to record Ca^{2+} transients in cells embedded within the slices (7, 9–11). High-K⁺ Tyrode’s solution for depolarizing taste cells contained 50 mM KCl in an equimolar substitution for NaCl to preserve physiological osmolarity.

![Fig. 3. The taste bud barrier is present in vivo. A highly penetrant fluorescent dye, CMFDA, was injected into tongue tissue surrounding taste buds subepithelially or into the circulatory system in deeply anesthetized mice. A: schematic drawing illustrating the site of CMFDA lingual injection in B and C. B and C: CMFDA (25 μM) was injected into the circumvallate papilla of a living mouse 1 h before tissue was harvested. Despite free diffusion of CMFDA throughout the nontaste epithelium, dye did not penetrate the taste buds (dark unstained regions in the epithelium, arrows). D and E: CMFDA (25 μM) injected directly into the vascular system uniformly penetrated the lingual epithelium, excluding taste buds (arrows). Connective tissue below the epithelium, as in Fig. 2, is sparsely cellular and, thus, does not take up and stain with CMFDA. Scale bars, 20 μm.](image.png)

![Fig. 4. DMSO disrupts the taste bud barrier, but agents affecting known tissue barriers are less effective. A and B: lingual slices were incubated for 1 h with Clostridium perfringens (C-CPE, 100 μg/ml), a claudin-binding protein used as a penetrant in other tissues, and then treated with CMFDA, as described in Fig. 2 legend. No improvement in dye permeation into taste buds was noted. C and D: verapamil, which blocks multidrug-resistant plasma membrane transporters and has been used to improve tissue penetration, only partially reduced the taste bud barrier. Tissue sections were incubated in verapamil (200 μM) for 20 min at room temperature and then in verapamil and CMFDA (25 μM) for 4.5 h. E and F: DMSO (75%) was applied to sections of taste tissue for 6 min, then tissue was incubated with CMFDA for 1 h (DMSO, in vitro). This treatment greatly enhanced CMFDA penetration into taste buds. G and H: DMSO (75%) and CMFDA (25 μM) were coinjected subepithelially into the tongue of a living animal, as described in Fig. 3, A–C. Dye access to taste buds was greatly improved, indicating that the barrier can be disrupted in vivo. Because CMFDA is taken up by cells and trapped in the cytosol, weak or no fluorescence in the connective tissue below the lingual epithelium (lamina propria) reflects the low cellularity of this tissue, not a barrier to dye penetration. Arrows indicate representative taste buds. Scale bars, 20 μm.](image.png)
**Ca^{2+} imaging data analysis.** Images were captured every 2 s, and responses are presented as changes in relative fluorescence: \[ \Delta F/F \] [i.e., \((F - F_0)/F_0\), where \(F_0\) is baseline fluorescence]. Baseline fluorescence was established in a period of 100 s before each stimulus was applied. Photobleaching of the sample was modeled typically with an exponentially declining baseline, and data were subsequently corrected, as fully described by Caicedo et al. (7). Only cells that maintained consistent responses for the duration of experimentation were included in our analysis.

We scored positive responses only if 1) peak \(\Delta F/F\) was at least twice baseline fluctuation, 2) responses could be elicited at least twice in the same cell by the same stimulus and were approximately of the same amplitude, and 3) responses were not observed in all dye-loaded cells in the field of view or in control areas external to the cells (i.e., signals were not generated by movement of the tissue or other stimulus artifact). Data were analyzed using Microsoft Excel and Prism V5 (Graphpad Software, La Jolla, CA).

**RESULTS**

**Taste bud barrier.** During the course of previous functional recordings from taste cells in lingual slices (7, 9–11), we observed that bath-applied fluorescein, used as a tracer to monitor perfusion, did not appear to penetrate into the body of taste buds. This led us to suspect that a barrier, distinct from that of apical tight junctions, surrounds the entirety of taste buds. On the basis of this observation, we designed assays to visualize this barrier and, further, applied those assays to test methods to reduce or eliminate the barrier.

Lingual slices of vallate papilla were incubated in fluorescent dyes of varying molecular weights and lipid solubility (Fig. 1). Despite marked differences in molecular properties, none of these dyes appreciably penetrated into taste buds that were embedded completely within the slice, even with prolonged incubation times. In marked contrast, all dyes produced intense staining of surrounding nonsensory epithelium and cells of connective tissues. Taste buds at the cut surfaces of lingual slices showed variable dye penetration, presumably because the taste buds were transected and any barrier was compromised. Such taste buds were excluded from further analysis. The dye that yielded the most reliable and consistent results and, hence, the dye we used for all remaining tests of the taste bud barrier was the low-molecular-weight, highly lipophilic fluorescent compound 5-chloromethylfluorescein diacetate (CMFDA). This compound readily penetrates cell membranes and enters the cytosol, where it is enzymatically converted into a membrane-impermeant fluorescent dye that remains trapped within the cytoplasm. Ironically, CMFDA is designed specifically to penetrate into cells and tissues and is used for long-term tracing of living cells, hence, its commercial name, CellTracker. Even with these properties, CMFDA failed to penetrate into and stain cells inside taste buds (Fig. 2). The failure of CMFDA to stain taste buds does not represent any inherent inability of taste cells to take up the dye. Indeed, in conditions where the taste bud barrier was disrupted, taste cells readily took up CMFDA (see below). Parenthetically, although Lucifer Yellow (LY) did not appear to diffuse into taste buds from the surrounding tissues, occasional individual taste cells were intensely stained with LY. This suggests that LY may be selectively taken up by certain taste cells, perhaps through ion channels in the apical, exposed tips of taste cells.

To identify the precise boundary of the taste bud barrier in greater detail, we carried out high-resolution confocal microscopy after treating lingual slices with CMFDA and staining cell nuclei with TO-PRO-3 (Fig. 2, D and E). We observed that the surrounding nonsensory epithelium, as well as the narrow layer of flattened perigemmal cells that form a tight sheath around taste buds cells (40), is intensely filled with CMFDA. This suggests that perigemmal cells do not play a major role in the taste bud barrier and that the principal barrier is formed by taste bud cells themselves.

![Fig. 5. Digestion of extracellular matrix reveals components of the taste bud barrier. Lingual slices were incubated with collagenase + dispase (A and B), elastase (C and D), heparitinase III (E and F), or chondroitinase ABC (G and H) for 1 h at 37°C (see Table 2) and then treated with CMFDA to test viability of the taste bud barrier. Only sections treated with chondroitinase ABC (G and H) showed significant penetration of CMFDA into the taste buds, indicating that the taste bud barrier was compromised. Arrows indicate representative taste buds. Scale bars, 20 μm.](http://ajpcell.physiology.org/)

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We next attempted to test the presence of a taste bud barrier under more physiologically relevant conditions, namely, by injecting CMFDA directly into the lingual tissue surrounding taste buds in a living animal (Fig. 3, A–C) or by perfusing CMFDA through the systemic circulation (Fig. 3, D and E). In both cases, the barrier remained prominent, with dye almost entirely excluded from taste buds, even though it had penetrated throughout surrounding nontaste epithelium. This would seem to imply that the taste bud represents a privileged environment that excludes access from the systemic circulation and from surrounding tissues.

A number of agents are known to disrupt epithelial barriers in other preparations: Clostridium perfringens enterotoxin (cCPE) (4, 30, 31) displays permeabilizing behavior and is known to bind to claudins, and verapamil (23, 48) blocks multidrug-resistant transporters used by cells to expel foreign matter. Clostridium perfringens enterotoxin treatment had no effect on the barrier (Fig. 4, A and B), and verapamil only partially reduced the taste bud barrier (Fig. 4, C and D). Negative results (data not shown) were obtained for EGTA and EDTA, which are routinely employed to permeabilize cells (13, 25), and sodium caprate, which is used clinically to open tight junctions (28, 39, 45). The fact that none of these standard methodologies proved particularly effective in our preparation would seem to suggest that the taste bud barrier is somewhat atypical in nature.

The aprotic solvent DMSO disrupts membranes through a number of postulated mechanisms, including the induction of water pores within the membrane (37, 38) and the introduction of structural defects in the bilayers through interactions with phospholipid acyl chains (52). When applied at sufficiently high concentrations (optimally, 75%), DMSO significantly improved CMFDA penetration into taste buds, whether applied to the slice of lingual tissue (Fig.

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**Fig. 6.** The taste bud barrier is partially disrupted in a peeled lingual epithelium. Mouse lingual epithelium was peeled from the tongue after subepithelial injection of an enzyme cocktail containing collagenase and dispase. Peeled epithelium was immediately incubated with CMFDA (A–C, “delaminated epithelium”) or reexposed to the enzyme cocktail for an additional 2 min and then incubated with CMFDA (D–F, “redigested epithelium”). Freshly peeled epithelia (A–C) showed taste buds with some taste cells loaded with CMFDA (green). In twice-digested peeled epithelium (D–F), dye was taken up by a greater proportion of taste bud cells. Sections shown in B and E were immunostained with the taste cell marker KCNQ1 (red) to identify location of taste buds. In individual taste buds isolated from peeled epithelium (G–J), the barrier appeared to be wholly destroyed; CMFDA penetrated all cells. G and I are differential interference contrast images of H and J, respectively. Scale bars, 20 μm (A–H) and 10 μm (I and J).
4, E and F) or injected into the lingual tissue directly (Fig. 4, G and H). In the latter case, the injection site was far enough from the taste buds to ensure that any dye permeation was not due to mechanical disruption.

**Enzymatic evaluation of the barrier.** Because the taste bud barrier appears to impede permeation from apical and basolateral aspects alike, we considered that extracellular matrix components surrounding taste cells might be responsible. To dissect the specific components that make up the taste bud barrier, we incubated 100-μm-thick lingual slices with enzymes specific for putative barrier constituents (Table 1).

Collagenase D readily breaks down collagen, whereas Dispase II more broadly targets fibronectin and some collagens and, thus, is favored for gentle tissue dissociations. This combination represents the most common protease mixture used in the isolation of taste buds. Yet, when applied to slices of circumvallate papilla, this enzyme cocktail resulted in little appreciable change in CMFDA penetration into taste buds (Fig. 5, A and B). Moreover, this cocktail resulted in extensive damage to the surrounding tissue, in particular, the subepithelial connective tissue and the deeper muscle layers of the lingual slice. The superficial epithelial layer was relatively well preserved, with CMFDA fluorescence in nontaste epithelial cells around and between the taste buds, but with little penetration inside the taste barrier. Elastase breaks down elastin fibers that form a mesh between cells of skin and other elastic epithelia. Elastase also is commonly used during the isolation of taste buds from a peeled lingual epithelium. As shown in Fig. 5, C and D, CMFDA failed to penetrate the taste bud barrier in elastase-treated slices.

Chondroitin and heparan sulfates, major glycosaminoglycan (GAG) side chains of epithelial proteoglycans, might be responsible for the taste bud barrier. Lingual slices treated with heparitinase, which depolymerizes heparan sulfate, did not promote significant penetration of the taste bud barrier (Fig. 5, E and F). However, significantly greater CMFDA penetration into the taste buds was allowed in lingual slices treated with chondroitinase ABC, which acts on isoforms of chondroitin sulfates, dermanatan sulfate, and, to some extent, hyaluronan than in control slices treated with buffer alone (Fig. 5, G and H). Disruption of the barrier was variable and not as complete as in DMSO-treated slices (Fig. 4, E–H). Taken together, the results suggest that the taste bud barrier relies, to some extent, on proteoglycans.

**Persistence of the taste bud barrier in peeled epithelium.** As a practical consideration, many laboratories, including our own, rely on protease isolation of taste buds from a peeled epithelium (8, 9, 22). Lingual epithelium containing taste buds is typically detached from underlying stroma with an initial collagenase digestion before it is peeled. Many investigators then secondarily treat the peeled epithelial section with a protease cocktail to permit removal of taste buds from the epithelium. Using the CMFDA dye penetration assay, we tested whether these procedures might disrupt the integrity of the barrier.

In peeled, freshly isolated epithelium, most of the taste buds contained areas devoid of CMFDA, indicating some persistence of a barrier (Fig. 6, A and C). Epithelia were subsequently immunostained with KCNQ1, a taste bud marker, to localize taste buds and verify their integrity (Fig. 6, B and E). In contrast, when the peeled epithelium was reexposed to collagenase plus dispase, there was greater penetration of CMFDA into taste buds (Fig. 6, D and F). Complete permeation was not observed until individual taste buds were removed from the lingual epithelium and exposed to CMFDA (Fig. 6, G–J).

Parenthetically, in Fig. 6, A–F, we noticed that, in taste buds where the barrier was partially compromised, some cells became fluorescent, while other regions in the same taste bud remained unstained. This finding is consistent with our interpretation that extracellular matrix components surrounding individual cells contribute to the taste bud barrier we describe here.

**Quantitative analysis of a taste bud barrier.** Figures 2–6 qualitatively assess the ability of a barrier to prevent CMFDA penetration into taste buds under normal conditions. Figures 2–6 also illustrate the partial disruption of this barrier by verapamil and its near-complete disruption by treatment with DMSO or chondroitinase. We quantified this taste bud barrier by measuring the distribution of fluorescent CMFDA dye in lingual epithelium under control conditions and after the treatments described in Figs. 2–6. We measured the fluorescence intensity profile of a line drawn across the widest part of a taste bud, including surrounding nontaste epithelium (Fig. 7). Fluorescence intensity for a distance 3–10 μm outside the border/edge of a taste bud (red lines in Fig. 7B) was averaged and defined as the extent of CMFDA penetration into nontaste epithelium surrounding the taste bud (perigemmal dye penetration). We took the average fluorescence of the intensity profile line plot 25–75% from either side of the taste bud (green line in Fig. 7B, i.e., the interior middle half of the line profile) as a measure of dye penetration into the taste bud (intragemmal dye). The ratio of intragemmal to extragemmal dye penetration was used as a measurement of the effectiveness of the barrier to CMFDA dye penetration for that taste bud.

**Fig. 7. Penetration of dye into lingual epithelium is quantified by measurement of fluorescence staining intensity.** A: micrograph of a taste bud in a lingual slice from mouse vallate papilla incubated in the fluorescent dye CMFDA. Line drawn across the taste bud (arrows) indicates position at which the fluorescence intensity profile was measured. Scale bar, 20 μm. B: fluorescence intensity profile along the line drawn in A. Red lines indicate average intensity at the boundaries of the taste bud (perigemmal fluorescence). Green line indicates average fluorescence intensity along the interior 50% of the line (intragemmal fluorescence). Dye penetration was quantified by the ratio of intragemmal (green line) to perigemmal (average of both red lines) fluorescence. In this example, this value is 34% (see text). y-Axis, fluorescence intensity [in arbitrary units (au)]; x-axis, distance along fluorescence intensity profile (arrows in A).
illustrated in Fig. 7, the ratio of intragemmal to extragemmal dye penetration was 34% (i.e., 19 arbitrary units of intragemmal fluorescence divided by the average of 53 and 62 arbitrary units of perigemmal fluorescence), suggesting a nearly threefold greater dye penetration into epithelium surrounding the taste bud than into the taste bud itself.

Individual taste buds were quantified in this manner and are presented as dot plots, superimposed with means ± 95% confidence intervals (Fig. 8). Each symbol in Fig. 8 represents data from one taste bud, as described for Fig. 7. As apparent from Figs. 2–5, the plots indicate that the taste bud barrier is somewhat reduced following treatment with verapamil and substantially disrupted following treatment with DMSO or chondroitinase.

Physiological assessment of the taste bud barrier. Next, we tested whether the barrier to dye penetration might also apply to physiologically relevant ions. Specifically, we perfused lingual slices with buffer containing elevated KCl (50 mM) to depolarize taste cells. Membrane depolarization opens voltage-gated Ca$^{2+}$ channels in a subset of taste cells; the resultant influx of Ca$^{2+}$ into those taste cells can be monitored with confocal Ca$^{2+}$ imaging (12). We tracked the equilibration of KCl buffer in the recording chamber by including the far-red fluorescent dye 7-hydroxy-9,9-dimethylacridine-2-one) (DDAO). Figure 9A shows data from three separate cells from taste buds from two different animals (dotted lines). The onset of KCl buffer equilibration in the bath (DDAO fluorescence) is shown as a single thick trace. There is a significant delay between KCl buffer perfusion (solid line, DDAO) and change in intracellular Ca$^{2+}$ concentration (dotted lines), indicating that K$^+$ did penetrate into taste buds, but only slowly. In contrast, when lingual slices were pretreated with 75% DMSO for 5 min (Fig. 9, thin solid lines), the change in intracellular Ca$^{2+}$ concentration was more rapid, almost matching the kinetics of solution exchange, and KCl-evoked Ca$^{2+}$ responses were significantly larger (Fig. 9, B and C). Importantly, not only was the taste bud barrier disrupted by DMSO, but this treatment did not compromise the physiological responses of taste bud cells.

Enzymatic component of the taste bud barrier. In addition to what appears to be some sort of barricade to the penetration of taste buds by dyes and ions, there exists a further aspect to the insular environment of taste buds. A host of active enzymes intrinsic to taste buds that catabolize transmitters are released during gustatory stimulation. For example, a potent ectonucleotidase, nucleoside triphosphate diphosphohydrolase 2 (NTPDase2), is expressed on the surface of certain cells within the taste bud. NTPDase2 catabolizes the taste transmitter ATP and its metabolite ADP (2, 11, 47). Acetylcholinesterase is also present in taste tissue (29), perhaps related to ACh released during taste stimulation (10) or to its trophic properties. When ATP or an equivalent concentration of its nonhydrolyzable analog ATPγS, an agonist with equal potency, was applied, a notably stronger response to the nonhydrolyzable form was observed (Fig. 10, A and B). This suggests the presence of an active enzymatic barrier within taste buds, consistent with the demonstrated role for NTPDase2 (47). Similarly, carbachol, a nonhydrolyzable analog of ACh, elicited significantly greater intracellular Ca$^{2+}$ mobilization than did ACh (Fig. 10, C and D). These results confirm that, in addition to a physical barrier...
that limits penetration of exogenous compounds, the taste bud also maintains an intrinsic enzymatic “barrier” to the diffusion of synaptically released taste transmitters throughout its core.

DISCUSSION

This report describes an unusual situation where sensory organs embedded in an epithelial sheet are protected from their immediate surroundings by a pronounced permeability barrier. Specifically, end organs of taste, i.e., taste buds, are surrounded by a selective and formidable barrier that maintains a privileged environment for gustatory sensory cells and separates them from the surrounding lingual epithelium. A general lingual epithelial surface barrier for nonelectrolytes, including fructose and sucrose, was characterized >40 years ago by Mistretta (34). The lingual surface barrier was later shown to consist of specialized intercellular junctional complexes, including at the apical pore of taste buds (1, 19, 26, 32). The present report extends and refines these earlier studies by showing that there is an additional barrier that isolates the entire body of taste buds. This perigemmal barrier prevents certain chemicals in the systemic circulation from reaching the underlying cell membrane. The mechanism by which GAGs act as a receptor for many GAGs, including hyaluronan, heparan, and chondroitin sulfate. The GAGs, located on the taste cell membranes or in the pericellular space, might provide a physical and/or physiological barrier that prevents molecules, including those that are generally cell-permeant, from reaching the underlying cell membrane. The mechanism by which GAGs do so is unknown but is unlikely to be charge-sensitive, since CMFDA and many other dyes we tested were neutral. The ineffectiveness of proteases in enhancing permeation suggests that the barrier is not merely proteinaceous in nature. Besides forming a permeability barrier, proteoglycans in the taste buds may have other functions, such as regulation of cell proliferation, migration, and differentiation, as in other tissues. Interestingly, Witt and

Fig. 9. Disruption of the taste barrier with DMSO promotes penetration of K\(^+\) into taste buds. Lingual slices through the vallate papilla were prepared for confocal Ca\(^{2+}\) imaging and placed in a recording chamber. Depolarization with bath-applied KCl (50 mM in Tyrode’s buffer) was used to open voltage-gated Ca\(^{2+}\) channels in taste cells, as previously described (12). Ca\(^{2+}\) influx into taste cells was measured with Calcium Green dextran and is shown as an increase in fluorescence over baseline (\(\Delta F/F_0\)). The far-red fluorescent dye 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) (DDAO) was added to the buffer containing elevated KCl to track stimulus perfusion in the recording chamber. A: KCl (50 mM) added to the bath (above traces) elicits Ca\(^{2+}\) influx in taste cells. For clarity, only the onset of perfusion with high-K\(^+\) buffer (tracked with DDAO, initial thick line) is shown. Lingual slices were incubated in Tyrode’s buffer (3 dotted traces) or in 75% DMSO (3 thin solid traces) for 5 min prior to recordings. DMSO treatment resulted in faster and larger responses to bath-applied KCl. B and C: summary of several experiments showing effect of DMSO pretreatment on amplitude and time to peak of KCl-evoked Ca\(^{2+}\) influx into taste cells. Values are means \(\pm\) SE; \(n = 29\) control cells and 26 DMSO-treated cells. *\(P = 0.0192, **P = 0.0001\) (by Student’s 2-tailed, unpaired t-test).
Kasper (50) showed that CD44 is involved in human embryonic taste bud development.

Brief treatment of isolated lingual tissue slices with DMSO disrupts the barrier. While 75% DMSO seems an unusually high concentration to apply to lingual slices, authors measuring permeability in other tissues have used DMSO at similarly high concentrations to enhance penetration (20). The permeability-enhancing properties of DMSO may be related to its ability to induce water pores in cell membranes or increase lipid fluidity (16, 17, 37, 38). However, the taste bud barrier seems to rely on extracellular matrix molecules, specifically GAG side chains of proteoglycans. How these might be altered by DMSO is unknown. Studies on keratin in hair concluded that DMSO replaces water and dehydrates the protein as it penetrates into the hair shaft (42). The ability of DMSO to substitute or remove water might be expected to alter the molecular configuration of extracellular matrix components and enhance penetration (43). Finally, the fact that verapamil appeared to allow limited penetration of dye into the taste bud (Fig. 4, C and D, and Fig. 8B) suggests that a multidrug-resistant transporter may also contribute to the taste bud barrier, although to a lesser extent. This is consistent with previous findings that treatment of lingual tissues with verapamil accelerated the accumulation of calcein acetyoxymethyl ester in taste bud cells (21).

Our studies have a number of implications. Topically applied or systemically injected pharmaceuticals may not readily cross the taste bud barrier. This may be important in designing effective taste modifiers or for explaining the lack of effect of many pharmacological agents on taste. Moreover, circulating hormones or peptides may have limited access to taste bud cells, and this might alter the interpretation of how and where hormones influence taste. In the laboratory, unless steps are taken to disrupt the taste bud barrier, we would expect that one might need to apply higher-than-expected concentrations of drugs onto taste tissues in situ to affect taste cell function. Lastly, our data raise the noteworthy possibility that peripheral sensory organs, at least in gustation, are afforded a selective, protected environment in which to transduce stimuli. This protected environment might be logical for chemosensory organs such as taste buds. It would be interesting to know whether other sensory endings, especially those that are encapsulated, might share this property.

NOTE ADDED IN PROOF

Holland et al. (Holland VF, Zampighi GA, and Simon SA, Tight junctions in taste buds: possible role in perception of intravascular gustatory stimuli, Chem Senses 16: 69–79, 1991) reported that ions and certain small molecules penetrated into taste buds in canine lingual epithelium. This could indicate there are species differences in the barrier. Moreover, as we had speculated in our report, Holland et al. (ibid) also suggested that intravascular taste might arise from chemical stimuli reaching receptors located on the apical, chemosensory tips of taste cells.

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AUTHOR CONTRIBUTIONS
R.D., E.P., M.K., N.C., and S.D.R. are responsible for conception and design of the research; R.D., E.P., M.K., R.B.-S., N.C., and S.D.R. performed the experiments; R.D., E.P., M.K., R.B.-S., N.C., and S.D.R. analyzed the data; R.D., M.K., N.C., and S.D.R. interpreted the results of the experiments; R.D., E.P., M.K., R.B.-S., N.C., and S.D.R. drafted the manuscript; R.D., E.P., M.K., N.C., and S.D.R. edited and revised the manuscript; R.D., E.P., M.K., R.B.-S., N.C., and S.D.R. approved the final version of the manuscript.

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