Inflammatory stimuli acutely modulate peripheral taste function

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Kumarhia D, He L, McCluskey LP. Inflammatory stimuli acutely modulate peripheral taste function. J Neurophysiol 115: 2964–2975, 2016. First published March 23, 2016; doi:10.1152/jn.01104.2015.—Inflammation-mediated changes in taste perception can affect health outcomes in patients, but little is known about the underlying mechanisms. In the present work, we hypothesized that proinflammatory cytokines directly modulate Na+ transport in taste buds. To test this, we measured acute changes in Na+ flux in polarized fungiform taste buds loaded with a Na+ indicator dye. IL-1β elicited an amiloride-sensitive increase in Na+ transport in taste buds. In contrast, TNF-α dramatically and reversibly decreased Na+ flux in polarized taste buds via amiloride-sensitive and amiloride-insensitive Na+ transport systems. The speed and partial amiloride sensitivity of these changes in Na+ flux indicate that IL-1β and TNF-α modulate epithelial Na+ channel (ENaC) function. A portion of the TNF-mediated decrease in Na+ flux is also blocked by the TRPV1 antagonist capsazepine, although TNF-α further reduced Na+ transport independently of both amiloride and capsazepine. We also assessed taste function in vivo in a model of infection and inflammation that elevates these and additional cytokines. In rats administered systemic lipopolysaccharide (LPS), CT responses to Na+ were significantly elevated between 1 and 2 h after LPS treatment. Low, normally preferred concentrations of NaCl and sodium acetate elicited high response magnitudes. Consistent with this outcome, codelivery of IL-1β and TNF-α enhanced Na+ flux in polarized taste buds. These results demonstrate that inflammation elicits shifts in Na+ taste function, which may limit salt consumption during illness.

tumor necrosis factor-α; interleukin-1β; neuroimmunology; epithelial sodium channel; taste bud

ILLNESS AND INFECTION can impact the taste system, which plays an important role in food selection and nutrition. Transient taste dysfunction is common after infections of the upper respiratory tract and oral cavity, resulting in reduced taste sensitivity or distorted taste perception (Feng et al. 2014; Henkin et al. 1975; Mann 2002; Spielman 1998). Gustatory impairment can, in turn, negatively affect nutrition, general health, and quality of life (Bromley and Doty 2003; Solomdal et al. 2012). Infections may alter taste function through oral or systemic inflammation, reduced blood flow to the oral cavity, and possibly taste bud injury, though it is also difficult to rule out the contribution of medication and other variables (Bromley and Doty 2003; Schiffman et al. 2000; Schiffman and Zervakis 2002). Fortunately, studies in animal models are providing insight into mechanisms underlying immune-mediated changes in taste function (Feng et al. 2015; Shi et al. 2011; Steen et al. 2010).

Tastants activate distinct channels and receptor pathways in taste receptor cells, resulting in the transmission of taste information to the brain via multiple gustatory afferent nerves. The chorda tympani (CT) nerve, a branch of cranial nerve VII, transmits neural activity from fungiform taste buds on the anterior tongue to the rostral nucleus of the solitary tract. This brain stem nucleus also receives taste inputs from the greater superficial petrosal nerve (VII) innervating taste buds on the palate, the glossopharyngeal nerve (IX) from posterior lingual taste buds, and the superior laryngeal nerve (X) from the larynx (Witt et al. 2003). This process ultimately results in the perception of salt, sweet, umami, bitter, and sour (Spector and Glendinning 2009), while rodent studies also implicate fat (Gilbertson et al. 1997) and carbohydrates (Scalfani and Mann 1987) as primary tastants.

Neural responses to the prototypical salt taste stimulus, Na+, are particularly sensitive to regulation by the immune system (Cavallin and McCluskey 2005; Guagliardo et al. 2009; Phillips and Hill 1996; Shi et al. 2011; Steen et al. 2010). Salt taste transduction occurs as Na+ ions pass through heterodimeric, apical epithelial Na+ channels (ENaCs) in taste receptor cells (Chaudhari and Roper 2010). Salt taste perception, and neural responses to Na+, can be partially suppressed by the ENaC antagonist amiloride (Chandrashekar et al. 2010; Eylam and Spector 2003; Heck et al. 1984; Lin et al. 1999; Shigemura et al. 2008). Amiloride-insensitive neurophysiological and behavioral responses to Na+ indicate that additional membrane receptors or ion channels transduce Na+ in taste receptor cells, although the identity of these channels remains equivocal (Chaudhari and Roper 2010; Liman et al. 2014).

Cytokines are among the many regulators of ENaC, which is expressed in Na+-transporting tissues such as kidney, lung, and colon in addition to taste epithelia. For example, TNF-α decreases amiloride-sensitive current and electrogenic Na+ transport in lung epithelial cells and colon, respectively (Amasheh et al. 2004; Barmeyer et al. 2004; Dagenais et al. 2004; Yamagata et al. 2009). Many cell types produce and secrete TNF-α, including macrophages and neutrophils, which infiltrate the tongue after nerve injury or inflammation and alter taste responses to Na+ (Bradley 2008; Cavallin and McCluskey 2005; McCluskey 2004; Steen et al. 2010). Taste receptor cells express TNF-α and its receptors (TNFR1 and TNFR2) and upregulate TNF-α gene expression after systemic treatment with the inflammatory stimulus lipopolysaccharide (LPS) (Cohn et al. 2010; Feng et al. 2012, 2015). TNF-α also slows taste cell proliferation and decreases behavioral and neural responses to bitter tastants (Cohn et al. 2010; Feng et al. 2015). Thus taste receptor cells are targets for paracrine

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(and perhaps autocrine) sources of TNF-α, particularly as proinflammatory cytokine levels escalate during inflammation and infection (Bradley 2008).

IL-1β is another widely expressed proinflammatory cytokine shown to modulate ENaC expression and function. In both middle ear and alveolar epithelial cells, IL-1β decreases ENaC expression and amiloride-sensitive current, likely contributing to the dysregulation of fluid absorption during infection and injury (Choi et al. 2007; Kim et al. 2009; Roux et al. 2005). There are several constitutive sources of IL-1β in the peripheral taste system, including taste receptor cells and nerve fibers (Shi et al. 2011; Steen et al. 2010). After CT nerve injury, IL-1β levels are increased by infiltrating macrophages and denervated taste buds (Shi et al. 2011). This cytokine has a beneficial effect on peripheral taste function after nerve injury: blocking IL-1β selectively inhibits taste response to Na⁺ in the neighboring, intact CT (Shi et al. 2011).

In the present work, we test the hypothesis that TNF-α and IL-1β modulate Na⁺ taste function by measuring relative changes in fluorescence in dye-loaded polarized taste buds embedded within the lingual epithelium. Thus we directly explore cytokine-taste interactions indicated by prior neurophysiological studies (McCluskey 2004; Phillips and Hill 1996; Shi et al. 2011; Steen et al. 2010). We demonstrate that these proinflammatory cytokines have rapid, opposing effects on Na⁺ flux in taste buds through ENaC and additional Na⁺ transport pathways. Moreover, systemic inflammation enhances CT nerve responses to Na⁺, which may alter salt consumption during illness.

MATERIALS AND METHODS

Animals. All animal procedures were approved by the Augusta University Institutional Animal Care and Use Committee. Specified pathogen-free adult female CD rats (150–200 g; Charles River, Raleigh, NC) were used, based on previous studies (Lyall et al. 2002; McCluskey 2004; Shi et al. 2011; Steen et al. 2010). Rats were housed in a barrier facility on a 12:12-h light-dark cycle and had free access to autoclaved food and water. Animals that received CT or sham sectioning were housed in cages with barrier tops in a standard facility for <24 h after recovery from surgery.

Na⁺ imaging solutions. All reagents were from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. For a typical experiment, Na⁺-free or control Ringer solution (Lyall et al. 2002) was delivered apically with or without Na⁺ transport antagonists. Apical amiloride (50 μM) was delivered to localize changes in Na⁺ flux to ENaC (Brand et al. 1985). Apical capsazepine dissolved in DMSO before dilution in control Ringer solution (final concentration of 25 μM; Tocris Bioscience, Bristol, UK) was applied to block Na⁺ transport via TRPV1 (Lyall et al. 2004b). We confirmed that DMSO in Ringer solution alone did not alter relative fluorescence, responses to Na⁺-free solutions, or amiloride sensitivity (n = 3 experiments). Cytokines were freshly diluted in control Ringer solution before each experiment and delivered basolaterally at the following concentrations, based on ENaC-modulating doses in other epithelia: IL-1β: 0.5 ng/ml and 5 ng/ml, TNF-α: 10 ng/ml, and TGF-β: 5.0 ng/ml (R&D Systems, Minneapolis, MN) (Dagenais et al. 2004; Frank et al. 2003; Roux et al. 2005).

Dye loading and measurement of relative Na⁺ flux in polarized fungiform taste buds. Rats were euthanized by intracardiac overdose followed by thoracotomy. Tongues were rapidly removed and stored in ice-cold Tyrode solution bubbled with O₂. The lingual epithelium was then isolated by collagenase treatment and manual dissection. A portion of the anterior lingual epithelium containing one to three polarized fungiform taste buds was mounted as previously described (Lyall et al. 2002). We used a modified Ussing chamber (RC-50; Warner Instruments, Hamden, CT) with a custom 1.5-mm aperture to stabilize the epithelium during imaging. In this approach, taste receptor cells retain their apical and basolateral polarity within the taste bud and lingual epithelium.

Polarized taste buds were loaded with CoroNa Green AM Na⁺ indicator (1 μg/ml; Invitrogen, Grand Island, NY) dissolved in Pluronic F-127 (50 μl; Invitrogen) for 1 h. CoroNa Green is compatible with amiloride, which emits strong autofluorescence when excited with UV light (Lyall et al. 2002). After dye loading, the lingual epithelium was perfused apically and basolaterally with control Ringer solution for 15 min at room temperature.

Polarized taste buds were imaged from the basolateral side with an Olympus BX51WI upright fluorescence microscope and a ×60/1.10 W objective (Fig. 1, A–C; Olympus, Center Valley, PA). Images were captured with a Hamamatsu ORCA-03G digital camera (Middlesex, NY). Changes in intracellular Na⁺ concentration were monitored by excitation of CoroNa Green at 490 nm with the emitted light imaged at 535 nm at 10-s intervals. Six to eight regions of interest (ROIs, diameter of ~2 μm) were drawn around optimally loaded taste receptor cells in each taste bud, with each ROI containing one taste cell. Fluorescence (F490) was measured while apical and basolateral solutions were delivered with a VC-8 valve controller (Warner Instruments; 1 ml/min). Metafluor 7.7 software (Molecular Devices, Sunnyvale, CA) was used to monitor imaging, subtract background fluorescence, and measure changes in F490.

In most experiments, F490 was normalized to levels measured in response to control Ringer solution (i.e., baseline Na⁺ flux). Decreased F490 in response to Na⁺-free Ringer solution was used to verify dye loading and epithelial health and assist in drawing ROIs in Na⁺-sensitive taste receptor cells. In experiments to test whether Na⁺ flux is deficient after contralateral CT sectioning, we defined baseline responses to Na⁺-free Ringer solution as 100%. Differential responses to apical and basolateral Ringer solution confirmed the preservation of taste bud polarity.

Na⁺ imaging analysis. The area under the curve and peak fluorescence (F490) values over 60 s during matching conditions were collapsed across experiments, represented as the mean ± SE. Each experiment ended with apical and basolateral stimulation with control Ringer solution. To control for bleaching, we required the F490 measured at the end of the experiment to be ≤15% of the F490 measured during stimulation with control Ringer solution at the beginning of the experiment.

One-way ANOVAs followed by Newman-Keuls posttests were used to compare changes in relative F490 during stimulation with cytokine and/or Na⁺ channel antagonists with baseline values. We used the same method to compare the relative F490 in taste buds from CT- and sham-sectioned animals. All data analyses were conducted with Prism 3.0 software (GraphPad, San Diego, CA). The level of significance was set at P ≤ 0.05.

CT sectioning. After unilateral CT nerve sectioning, neutrophils infiltrating both sides of the tongue inhibit Na⁺ responses in the neighboring, intact CT (Steen et al. 2010; Wall and McCluskey 2008). We hypothesized that these neurophysiological changes are mediated by reduced amiloride-sensitive Na⁺ transport in polarized taste buds from the uninjured side of the tongue. These experiments were also used to verify that changes are consistent with neurophysiological responses in vivo, as shown by Lyall and colleagues (Lyall et al. 2002).

Rats were anesthetized with ketamine (40 mg/kg ip)-xylazine (10 mg/kg ip), and the CT nerve was approached ventrally between the anterior belly of the digastric and masseter muscles as previously described (Hill and Phillips 1994; McCluskey 2004; Shi et al. 2011; Steen et al. 2010). The CT nerve was sectioned after its bifurcation from the lingual nerve or visualized but not sectioned in surgical control animals (“sham sectioned”). The wound was flooded with...
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Fig. 1. Amiloride-sensitive Na⁺ flux in polarized taste buds from anterior tongue. A: brightfield image of the basolateral side of a single fungiform papilla with taste bud in the center (circled). B: fluorescent image of a CoroNa Green-loaded taste bud from the basolateral side. Taste receptor cells fluoresce in the presence of 150 mM Na⁺. C: pseudocolored image of the same taste bud in B, showing high levels of fluorescence in taste receptor cells (red). Scale bar, 50 μm. D: % change in fluorescence (F490) relative to baseline stimulation with 150 mM Na⁺.

We measured the height of summated, integrated neural responses minus the baseline at 20 s after stimulus application. Ratios for all steady-state responses were calculated relative to response magnitudes elicited by 0.5 M NH₄Cl. Only series bracketed by responses to NH₄Cl that did not deviate by >10% were included in further analyses. We used two-way ANOVAs posttests with rat as the covariate to determine the effects of stimulus concentration and systemic treatment (i.e., LPS or PBS) on responses to NaCl, NaAc, MSG, and NaCl + amiloride (STATISTICA software 9.0, StatSoft, Tulsa, OK). Responses to single concentrations of sucrose, QHCl, and HCl were compared between treatment groups with Student’s t-tests. The level of significance was set at \( P \leq 0.05 \).

RESULTS

Na⁺ transport in polarized taste buds. We first assessed Na⁺ flux in polarized taste buds during application of control Ringer solution (150 mM Na⁺), Na⁺-free Ringer solution (0 mM Na⁺), and control Ringer solution with the ENaC blocker amiloride (50 μM). As shown in Fig. 1, removing Na⁺ from the solution bathing the apical side of the lingual epithelia typically decreased \( F_{490} \) by 10–15% in taste receptor cells. Applying Na⁺-free solutions to both sides caused fluorescence to decrease up to 40%. We refer to these changes in relative \( F_{490} \) as a change in Na⁺ flux or transport (Lyall et al. 2002). Apical delivery of the ENaC blocker amiloride with control Ringer solution inhibited Na⁺ transport by 15–20% in taste receptor cells. These changes in Na⁺ flux are consistent with results reported by Lyall and colleagues although larger in magnitude, perhaps because of the improved loading of CoroNa Green compared with Sodium Green dye (Lyall et al. 2002).

We then moved to assessing the effects of inflammatory stimuli on Na⁺ transport in polarized taste buds. After confirming intact neural activity, we then administered LPS (E. coli 0111:B4, 5 mg/kg; Sigma, St. Louis, MO) or PBS vehicle intraperitoneally \((n = 7\) each). We recorded neural responses to taste stimuli beginning 1 h after systemic injection and completed all recordings within 2 h, before TNF-α levels return to baseline (Le Contel et al. 1990; Ulich et al. 1991). Responses were recorded during stimulation of the anterior tongue with concentration series of NaCl (0.05–0.50 M), Na⁺ acetate (NaAc, 0.05–0.50 M), monosodium glutamate (MSG; 0.1 and 0.3 M), 1.0 M sucrose, 0.01 M quinine (QHCl), and 1.0 N HCl. Stimuli were applied in this order to maintain stable posttreatment timing between animals. Three milliliters of each tastant was applied to the anterior tongue by hand with a syringe, followed 25 s later by a rinse of >1 min with distilled water. CT responses to a concentration series of NaCl with 50 μM amiloride as the solvent and rinse were also recorded.

After completing surgical procedures, we confirmed that robust neural activity was present before injecting either LPS (E. coli 0111:B4, 5 mg/kg; Sigma, St. Louis, MO) (Cohn et al. 2010) or PBS vehicle intraperitoneally \((n = 7\) each). We recorded neural responses to taste stimuli beginning 1 h after systemic injection and completed all recordings within 2 h, before TNF-α levels return to baseline (Le Contel et al. 1990; Ulich et al. 1991).

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Neurophysiology. Body temperature was maintained between 36°C and 39°C with a water-circulating heating pad. Rats were anesthetized with pentobarbital (40 mg/kg ip) and tracheotomized, and the hypoglossal nerves were sectioned bilaterally to prevent tongue movement. The CT nerve was then approached by a lateral dissection of the head, as described previously (Shi et al. 2011; Steen et al. 2010). Multifiber neural activity was amplified (Grass Instruments, Warwick, RI) and integrated with a time constant of 1.5 s. The summated signal was monitored with PowerLab hardware and software (ADInstruments, Colorado Springs, CO). This measure of multifiber activity reflects the sum of single-fiber responses and is an appropriate measure for studying the function of associated taste receptor cells (Beidler 1953). After completing surgical procedures, we confirmed that robust neural activity was present before injecting either LPS (E. coli 0111:B4, 5 mg/kg; Sigma, St. Louis, MO) (Cohn et al. 2010) or PBS vehicle intraperitoneally \((n = 7\) each). We recorded neural responses to taste stimuli beginning 1 h after systemic injection and completed all recordings within 2 h, before TNF-α levels return to baseline (Le Contel et al. 1990; Ulich et al. 1991).

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The small fluctuations in $F_{490}$ under static conditions likely reflect the dynamic status of intracellular $\text{Na}^+$ concentration, and are apparent in other cell types including HEK 293 cells stably transfected with human VR-1 (Grant et al. 2002).

Our analyses of $\text{Na}^+$ flux focused on taste receptor cells. Epithelial cells within fungiform papillae but outside the taste bud were sometimes dye loaded as reported for the sodium indicator dyes SFBI and Sodium Green (Lyall et al. 2002). These fungiform epithelial cells were typically unresponsive or only weakly responsive to $\text{Na}^+$ concentration. In Fig. 1D, for example, the relative $F_{490}$ in nontaste cells hovered around the baseline throughout the experiment, deviating only by an average of ±1.02% (maximum deviation ±1.08%; $n = 5$ ROIs). Nontaste epithelial cells in the anterior tongue (Shigemura et al. 2008; Stewart et al. 1995) are reported to express low levels of ENaC (Huang and Stahler 2009; Kretz et al. 1999; Lin et al. 1999), which may explain these small, inconsistent changes in $\text{Na}^+$ flux.

Contralateral CT sectioning reduces $\text{Na}^+$ flux. In these experiments we determined whether changes in $\text{Na}^+$ flux mediated decreased CT responses immediately following neighboring nerve injury. As shown in Fig. 2, $\text{Na}^+$ flux was reduced in polarized taste buds from sectioned vs. sham-sectioned animals. Both the mean peak $F_{490}$ (Fig. 2B) [$F(3,12) = 5.06; P < 0.01$] and the mean area under the curve (Fig. 2C) [$F(5,20) = 14.24; P < 0.01$] were significantly lower on day 1 after contralateral CT sectioning. Importantly, apical amiloride inhibited relative fluorescence to similar levels, eliminating differences between surgical treatment groups. These results demonstrate that $\text{Na}^+$ flux in polarized taste buds reflects neurophysiological responses in vivo (Wall and McCluskey 2008). They also indicate that ENaC is the primary $\text{Na}^+$-sensing pathway affected by neighboring nerve injury.

![Fig. 2. Contralateral CT nerve sectioning reduces Na⁺ flux. A: representative change in $F_{490}$ in polarized taste buds at day 1 after sham sectioning or contralateral CT sectioning (CTX). Responses to 150 mM Na⁺ decreased in taste buds opposite the denervated sensory field, in parallel with neurophysiological changes. B: mean Na⁺ flux is significantly lower in taste buds from CTX vs. sham-sectioned ($n = 5$) rats ($n = 5$ each). Apical amiloride reduces Na⁺ transport to the same levels in taste buds from sham-sectioned and CTX animals, indicating that ENaC is the primary salt-sensing pathway affected by contralateral neural injury. C: mean area under the curve is significantly lower in taste buds from CTX rats compared with sham-sectioned rats. *$P < 0.05$.](http://jn.physiology.org/doi/10.1152/jn.01104.2015)
IL-1β rapidly enhances Na\(^+\) flux through ENaC. We next determined the effects of IL-1β on Na\(^+\) transport in polarized taste buds. The IL-1β concentrations delivered to taste buds are similar to (or lower than) those that modulate ENaC in other epithelia and approximate levels in plasma and lingual tissues during inflammation (Barmeyer et al. 2004; Choi et al. 2007; Derijk and Berkenbosch 1992; Roux et al. 2005; Shi et al. 2011). We refer to IL-1β doses as low (0.5 ng/ml) and high (5.0 ng/ml) for convenience.

Acute basolateral application of the low dose of IL-1β induced small (2–10%), oscillating increases above the 100% baseline elicited by control Ringer solution. This modest increase in relative fluorescence was consistent across experiments (n = 6). A higher dose of IL-1β caused more dramatic increases in Na\(^+\) transport, up to 25% above baseline (Fig. 3A). The mean peak change in Na\(^+\) flux over 60 s of IL-1β delivery was significantly different between groups [F(3,15) = 347.9, P < 0.0001]. Specifically, the mean relative F\(_{490}\) elicited by either dose of IL-1β was greater than vehicle (P < 0.001–0.05) (Fig. 3C). Apical amiloride, delivered concurrently with basolateral IL-1β, reduced relative F\(_{490}\) to the same levels (P < 0.05). The rapid elevation in Na\(^+\) flux was reversed when basolateral cytokine was replaced with Ringer solution and relative F\(_{490}\) returned to baseline. Fungiform epithelial cells, which express low levels of the IL-1 receptor (Shi et al. 2011), responded weakly to high doses of IL-1β. For the experiment shown in Fig. 3, for example, fungiform epithelial cells exhibited a peak elevation in F\(_{490}\) of 106% (n = 5 ROIs) compared with 116% in taste receptor cells.

We also analyzed the area under the curve induced by IL-1β relative to the initial baseline of 100% (Fig. 3C). The mean area under the curve was significantly different between conditions [F(3,15) = 373.0, P < 0.0001]. Posttests revealed that both low (P < 0.01) and high (P < 0.001) doses of IL-1β elicited a greater area under the curve vs. vehicle. Applying amiloride concomitantly with both doses of IL-1β reduced the area under the curve to similar levels (P > 0.05). These results demonstrate that IL-1β rapidly and reversibly increases Na\(^+\) flux in taste buds. The amiloride sensitivity of the effect indicates that IL-1β enhances ENaC function in taste buds.

TNF-α inhibits Na\(^+\) flux through multiple transport systems. We tested whether another prominent proinflammatory cytokine, TNF-α, also has direct effects on taste bud function (Fig. 4). In contrast to IL-1β, basolateral TNF-α (10 ng/ml) caused Na\(^+\) flux to fall by 34–40% (Fig. 4A). This is similar to the reduction in transport seen when Na\(^+\)-free Ringer solution is applied to both sides of the epithelium (i.e., 40%) (Fig. 1C). In other words, Na\(^+\) flux is almost completely inhibited. The
relative $F_{490}$ increased gradually over the next 5–6 min and returned to baseline after replacement with control Ringer solution. Thus the effect of TNF-α/H9251 on Na$^{+}$/H11001 flux in taste buds was dramatic, rapid, and reversible. Fungiform epithelial cells outside the taste bud were unresponsive to TNF-α./H9251.

To test which Na$^{+}$/H11001 transport pathways are sensitive to TNF-α, we applied specific channel antagonists to the apical side of the lingual epithelium. The TRPV1 blocker capsazepine (Cap; 25 μM) and amiloride (50 μM) reduces Na$^{+}$ flux, basolateral TNF-α further decreases relative $F_{490}$ levels. C: mean peak Na$^{+}$ flux is significantly reduced by application of each of the following: apical capsazepine, apical amiloride, or basolateral TNF-α. Na$^{+}$ flux is significantly reduced by the antagonists or TNF-α compared with control Ringer solution and compared among conditions. D: mean area under the curve during stimulation with control Ringer solution and apical capsazepine, apical amiloride, or basolateral TNF-α. Progressive decreases in relative $F_{490}$ were elicited by Na$^{+}$ channel antagonists and, most strikingly, by TNF-α. Symbols represent statistical differences; brackets above 150 mM Na$^{+}$ indicate significance vs. each of the other conditions ($**P < 0.01$, ***$P < 0.001$).

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To test which Na$^{+}$ transport pathways are sensitive to TNF-α, we applied specific channel antagonists to the apical side of the lingual epithelium. The TRPV1 blocker capsazepine reduced mean relative Na$^{+}$ flux by >12%, while application of the ENaC antagonist amiloride decreased mean Na$^{+}$ transport by >20% (Fig. 4C). Importantly, basolateral delivery of TNF-α either alone (Fig. 4, A–C) or concomitantly with apical capsazepine and amiloride (Fig. 4B) further reduced mean Na$^{+}$ flux by 10–15%. The overall effect of treatment was significant [$F(4,16) = 105.8$, $P < 0.0001$]. All treatments significantly reduced Na$^{+}$ flux compared with control Ringer solution ($P < 0.001$) and each other ($P < 0.001$). Analysis of the effect of TNF-α, capsazepine, and amiloride on the area under the curve yielded similar results (Fig. 4D). There was a significant overall effect of treatment [$F(3,19) = 67.67$, $P < 0.001$]. Furthermore, while each of the two antagonists significantly reduced the area under the curve, TNF-α caused a further drop in this measure ($P < 0.05-0.01$). Together, these results demonstrate that TNF-α inhibits Na$^{+}$ transport in taste buds via both ENaC and TRPV1. Moreover, the decrease in Na$^{+}$ flux elicited by TNF-α in the presence of both antagonists indicates that additional Na$^{+}$-sensing mechanisms are targeted in taste receptor cells.

$TGF-\beta$ does not affect Na$^{+}$ flux in polarized taste buds. Since both IL-1β and TNF-α altered Na$^{+}$ flux, albeit in opposite directions, we next tested the specificity of cytokine effects on taste bud function. While TGF-β reduced taste receptor cell proliferation in vitro (Nakamura et al. 2010), we are not aware of acute effects of TGF-β on taste receptor cell function, making it an attractive “negative control.” Basolateral TGF-β did not cause consistent, acute changes in Na$^{+}$ flux (not
shown). This result confirms that protein delivery and/or flow rate (i.e., 1 ml/min) alone do not impact F<sub>490</sub>.

Systemic LPS acutely increases neural responses to Na<sup>+</sup>. IL-1β enhances Na<sup>+</sup> flux in taste buds in vitro while TNF-α has the opposite effect, yet inflammation and infection typically elevate both of these proinflammatory cytokines in vivo (Dinarello 1991). While the preparation used for Na<sup>+</sup> imaging studies preserves taste receptor cell polarity in the lingual epithelium, it lacks some features of the intact peripheral taste system, including synaptic connections with innervating sensory nerve fibers. Therefore, we investigated whether an inflammatory stimulus acutely changes Na<sup>+</sup> taste function in anesthetized rats and, if so, the net effect on Na<sup>+</sup> responses. In vivo neural recordings are also useful in determining whether inflammation selectively alters taste responses to Na<sup>+</sup> stimuli or extends to other taste modalities. To test this, taste-elicited responses were recorded from the CT nerve within 2 h after intraperitoneal injection of either sterile PBS or LPS. LPS purified from Gram-negative bacteria is widely used to stimulate inflammation and elevates both IL-1β and TNF-α (Dinarello 1991).

As shown in Fig. 5 and Fig. 6, neural responses to NaCl were elevated in rats receiving LPS (n = 7) compared with PBS (n = 7). Responses to NaCl were sensitive to stimulus concentration, as expected [F(3,46) = 40.79, P < 0.0001]. Systemic treatment also had a significant main effect on neural responses to NaCl [F(1,46) = 49.32, P < 0.0001]. Posttests revealed that LPS significantly increased responses to each concentration of NaCl tested (P < 0.0001–0.05) (Fig. 6A). Amiloride inhibited responses to NaCl in both groups (Fig. 6A), with significant main effects of treatment [F(1,40) = 17.05, P < 0.0001], concentration [F(3,40) = 102.10, P < 0.0001], and their interaction [F(3,40) = 3.11, P < 0.05]. Responses to 0.25 M (P < 0.01) and 0.50 M NaCl (P < 0.001) were inhibited to a greater degree by the ENaC antagonist. There were also significant main effects of NaAc concentration [F(3,44) = 47.70, P < 0.0001] and treatment [F(1,44) = 26.90, P < 0.0001] similar to NaCl responses, the difference between LPS- and PBS-treated groups was significant at each concentration of NaAc tested (P < 0.01–0.05) (Fig. 6B).

The effects of LPS were generally limited to Na<sup>+</sup> stimuli. Neural responses to bitter (QHCl), sweet (sucrose), and acid (HCl) stimuli did not differ between treatment groups (Fig. 6D) (P > 0.05). There was a significant effect main effect of concentration [F(1,22) = 16.48, P < 0.001] and treatment [F(1,22) = 19.41, P < 0.001] on MSG responses at both concentrations (P < 0.01–0.05). The Na<sup>+</sup> component of this umami stimulus is likely responsible for this slight increase in LPS-treated animals. In summary, systemic inflammation significantly enhanced the amiloride-sensitive component of neural responses to NaCl.

Concurrent IL-1β and TNF-α elevate Na<sup>+</sup> flux in taste buds. The hypersensitive Na<sup>+</sup> responses observed in LPS-injected animals were somewhat surprising, since TNF-α decreased Na<sup>+</sup> flux drastically in polarized taste buds. In other words, we expected an inflammatory stimulus that increases both TNF-α and IL-1β to decrease neural responses to Na<sup>+</sup>. To provide insight into the relative sensitivity of taste buds to IL-1β and TNF-α, we delivered these cytokines simultaneously to the basolateral side of the lingual epithelium. As shown in Fig. 7, this treatment elevated Na<sup>+</sup> flux relative to baseline stimulation with control Ringer solution. However, the mean F<sub>490</sub> during stimulation with both cytokines (~7%) was less than the 20% increase elicited by IL-1β alone. Consistent with neurophysiological findings, this indicates that taste receptor cells are more responsive to IL-1β when both cytokines are present.

DISCUSSION

We tested whether proinflammatory cytokines directly modulate Na<sup>+</sup> transport, since this taste modality is particularly sensitive to inflammation and nerve injury (Phillips and Hill 1996; Shi et al. 2011; Steen et al. 2010). We show that TNF-α and IL-1β have rapid, direct effects on Na<sup>+</sup> flux in polarized taste buds. TNF-α essentially shuts down Na<sup>+</sup> flux to levels elicited by Na<sup>+</sup>-free conditions. In contrast, IL-1β enhances Na<sup>+</sup> flux, in line with its protective effect on neural responses to neighboring nerve injury in vivo (Shi et al. 2011). Treatments that elevate both cytokines concomitantly elevated Na<sup>+</sup> transport in vitro and neural responses to Na<sup>+</sup> in vivo. Thus, while IL-1β has less dramatic effects on Na<sup>+</sup> flux, this cytokine is a more effective modulator of taste bud function.

Cytokine modulation of ENaC activity: potential mechanisms. The changes in Na<sup>+</sup> flux and neural responses to NaCl are amiloride sensitive, indicating that ENaC in taste receptor cells is the common target for these inflammatory stimuli. In fact, neural responses to NaCl were more sensitive to amiloride after LPS treatment. The speed of the change in F<sub>490</sub> narrows potential mechanisms for cytokine interaction with ENaC. Compatible mechanisms include those that rapidly increase the open probability of ENaC or insert ENaC into the membrane from a ready pool of subapical channels (Kashlan and Kleyman 2012; Snyder 2002). For example, PIP<sub>2</sub> and PIP<sub>3</sub> bind different sites on ENaC to induce functional changes in seconds (Gam-
per and Shapiro 2007; Ma and Eaton 2005; Pochynyuk et al. 2007).

Many studies in nongustatory epithelia demonstrate decreased ENaC expression after exposure to cytokines for longer periods (i.e., 4–24 h) as a mechanism for altered amiloride-sensitive current (Choi et al. 2007; Dagenais et al. 2004; Roux et al. 2005; Yamagata et al. 2009). There are, however, reports that TNF-α can alter ENaC function quickly, as we observed.

In distal nephron cells, TNF-α inhibits ENaC activity within minutes via ceramide regulation of a protein kinase C-dependent pathway (Bao et al. 2007). The lectinlike domain of TNF-α can also become internalized and bind directly to the α-ENaC carboxy terminal in alveolar epithelial cells. This noncanonical action of TNF-α stabilizes the PIP2-MARCKS complex to increase ENaC open probability in minutes and promote fluid clearance in the lung during inflammation (Czikora et al. 2014).

**TNF-α inhibits multiple Na⁺-sensing pathways.** ENaC in taste receptor cells is one target of TNF-α, as hypothesized on the basis of its role in other epithelia. However, the TNF-elicited decrease in relative F490 is also sensitive to capsazepine, a synthetic analog of capsaicin and competitive antagonist of TRPV1 (Caterina et al. 1997). TNF-α rapidly enhances TTX-resistant currents in dorsal root ganglion cells, likely through TRPV1 (Jin and Gereau 2006). Capsazepine also activates δ ENaC (Yamamura et al. 2004), which is expressed in human taste buds (Huque et al. 2009; Stahler et al. 2008). In polarized taste buds, the observed effects of capsazepine on Na⁺ flux are likely mediated by TRPV1 since the δ ENaC gene appears to be absent or a pseudogene in rats and mice (Giraldez et al. 2012; Hubbard et al. 2007; Ji et al. 2012).

Lyall and colleagues report that a TRPV1 variant is responsible for the amiloride-insensitive portion of CT nerve responses to Na⁺ (Lyall et al. 2004b), yet other groups demon-

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**Fig. 6.** LPS treatment enhances neural responses to NaCl. A: mean ± SE CT nerve response ratios to an increasing concentration of NaCl. There were significant main effects of concentration and treatment, as described in the text. Posttests revealed significantly higher responses to 0.05–0.50 M NaCl after LPS injection vs. PBS injection (n = 7 each). The ENaC antagonist amiloride reduced responses to NaCl in both groups, with significantly greater amiloride sensitivity in the responses to 0.25 M and 0.50 M NaCl in LPS-treated animals. B: mean ± SE neural response ratios to an increasing concentration of Na⁺ acetate (NaAc). There were significant main effects of concentration and treatment, resulting from significantly higher responses to each concentration of NaAc after LPS injection. C: there were significant effects of stimulus concentration and treatment on CT responses to the umami stimulus, MSG. LPS-injected rats had significantly higher responses to both concentrations of MSG. D: responses to sucrose, bitter (QHCl), and sour (HCl) tastants did not differ significantly between treatment groups (P > 0.05). *P < 0.5, **P < 0.01, ***P < 0.001.
the activity of channels that normally allow Na⁺ responsive to IL-1β elicited by TNF-α. These solutions typically elevate multiple cytokines as modulators. During concurrent application of these proinflammatory cytokines, Na⁺/H⁺ exchanger (NHE)-1, located in the basolateral membrane of taste receptor cells (Lyall et al. 2004a; Vinnikova et al. 2004). Although amiloride nonspecifically blocks NHE-1, the exchanger was likely isolated from apical amiloride delivery to polarized taste buds in our study. Another putative target of TNF-α is the Na⁺/H⁺ exchanger (NHE)-1, located in the basolateral membrane of taste receptor (Gao et al. 2009; Szebenyi et al. 2010; Vandenbeuch et al. 2008).

TNF-α dramatically reduces Na⁺ transport, but inflammation and infection typically elevate multiple cytokines as modeled by systemic LPS injection. Thus the heightened Na⁺ sensitivity revealed by combined cytokine treatment in vitro and LPS in vivo may be the most relevant to taste changes during clinical illness and infection. Moreover, the effects of LPS treatment on Na⁺ responses were highly sensitive to amiloride, suggesting ENaC vulnerability to inflammation. The decrease in Na⁺ transport elicited by TNF-α alone, in contrast, was capsazepine and amiloride sensitive. Relevant to our experiments in which TNF-α was delivered alone, treatment with antibodies to block or neutralize TNF in patients with autoimmune disease (Willrich et al. 2015) might affect both ENaC-sensitive and -insensitive Na⁺ taste pathways. Future studies will be needed to identify the third pathway sensitive to modulation by TNF-α and explore acute and delayed mechanisms that regulate ENaC activity.

**Na⁺-responsive and cytokine-responsive taste receptor cells in fungiform taste buds.** Taste receptor cells are commonly classified as 1) type I, 2) type II or receptor cells, and 3) type III or presynaptic cells. The identity of Na⁺-sensing taste receptor cells is not well defined compared with type II cells that transduce complex stimuli (i.e., sweet, bitter, and umami) (Chaudhari and Roper 2010). Although our goal was to test cytokine responsiveness, we note that the majority of fungiform taste cells exhibited robust fluorescence in the presence of 150 mM Na⁺ (Fig. 1). Consistent with this observation, several groups report widespread expression of α, β, and γ ENaC subunits in fungiform taste buds and in the apical, basolateral, and cytoplasmic compartments of taste receptor cells (Kretz et al. 1999; Lin et al. 1999; Stewart et al. 1995). The proportion of taste receptor cells responding to Na⁺ and their amiloride sensitivity in patch-clamp studies are more variable, perhaps reflecting differences in species and/or taste bud dissociation methods (Doolin and Gilbertson 1996; Miyamoto et al. 1999; Vandenbeuch et al. 2008).

One exception to the widespread Na⁺ sensitivity we observed within polarized taste buds was the relatively lower fluorescence surrounding the taste pore. Taste cells are indeed present in the center region of the bud (Fig. 1A). We also evaluated the regional consistency of dye loading at the start of each experiment. Alternatively, taste cells at the center of the bud might be less responsive to proinflammatory cytokines and/or Na⁺. When viewed from the basal side, brightly fluorescing cell bodies (which we sampled more frequently) may also obscure slender cellular processes tapering toward the apex of the taste bud. Taste cells in the center of the bud appear to express ENaC subunits (Chandrashekar et al. 2010; Huang and Stahler 2009; Kretz et al. 1999; Lin et al. 1999). However, the spatial distribution of other Na⁺ channels and transporters within the taste bud is generally not well understood.

In our Na⁺ imaging experiments, we observed a previously described basolaterally driven Na⁺ flux (Vinnikova et al. 2004). As described above, NHE-1 is expressed in the basolateral portion of taste cells and could be responsible for the change in F₄₉₀ in taste cells when basolateral Na⁺ is removed (Lyall et al. 2004a). Basolateral ENaCs may also contribute to this flux, though there are conflicting reports as to the existence of functional ENaCs in that compartment (Mierso et al. 1996; Miyamoto et al. 2000; Yoshida et al. 2009).

**Peripheral taste system is responsive to immune status.** Our Na⁺ imaging and neurophysiology results demonstrate that inflammatory mediators cause rapid changes in Na⁺ taste sensing, before potential regulation by transcription and/or translation. What is the behavioral significance of acute taste modulation during early stages of infection or inflammation? LPS and codelivery of proinflammatory cytokines induce hypersensitive taste responses to Na⁺, which may convert behavioral preference for Na⁺ to aversion. Indeed, Na⁺-depleted mice consume less 0.3 M NaCl within 1–2 h after intraperitoneal LPS injection (Almeida et al. 2011). A blunted Na⁺ appetite may contribute to “sickness behavior” resulting from infection (or experimentally by LPS) (Dantzer et al. 2008). These well-described behavioral changes, which reduce activ-
ity, thirst, and feeding, are thought to be an adaptive response that promotes fever (Dantzer et al. 2008).

We demonstrate that taste buds are acutely sensitive to inflammatory factors. Chronic inflammation has distinct effects on peripheral taste function. For example, in the MPR/lpr mouse autoimmune model, which exhibits lingual inflammation, neural responses to bitter and saccharin stimuli are reduced, with decreased behavioral sensitivity to stimuli mediated by type II taste receptor cells (i.e., bitter, sweet, and umami) (Kim et al. 2012). Furthermore, neurophysiological and behavioral responses to bitter tastants are reduced in TNF-deficient mice (Feng et al. 2015). CT responses to NaCl were unchanged in these knockout mice, perhaps reflecting the complex interactions of multiple cytokines acting on distinct taste transduction pathways during inflammation (Fantuzzi 2003).

Conclusions. Infection and inflammation often induce changes in appetite and taste perception. We demonstrate that Na⁺ responses from polarized taste buds and the associated afferent nerve are acutely sensitive to inflammatory stimuli. The net effect of proinflammatory immune signals is to elevate Na⁺ input from taste buds to the CNS. Taste receptor cells may serve as early responders to inflammation, initiating changes in ingestive behavior as part of a homeostatic response to infection.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.K., L.H., and L.P.M. performed experiments; D.K. and L.P.M. analyzed data; D.K. and L.P.M. interpreted results of experiments; D.K. and L.P.M. prepared figures; D.K. and L.P.M. drafted manuscript; D.K. and L.P.M. edited data; D.K. and L.P.M. interpreted results of experiments; D.K. and L.P.M. edited and revised manuscript; D.K., L.H., and L.P.M. approved final version of manuscript; L.P.M. conception and design of research.

REFERENCES


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Huang X, Stabler JA. Effects of dietary Na⁺ deprivation on epithelial Na⁺ channel (ENaC), BDNF, and TrkB mRNA expression in the rat tongue. BMC Neurosci 10: 19, 2009.


Huang X, Stabler JA. Effects of dietary Na⁺ deprivation on epithelial Na⁺ channel (ENaC), BDNF, and TrkB mRNA expression in the rat tongue. BMC Neurosci 10: 19, 2009.


