Self-Inhibition in Amiloride-sensitive Sodium Channels in Taste Receptor Cells

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ABSTRACT Electrophysiological recording techniques were used to study the Na⁺ dependence of currents through amiloride-sensitive sodium channels (ASSCs) in rat taste cells from the fungiform and vallate papillae. Perforated patch voltage clamp recordings were made from isolated fungiform and vallate taste receptor cells (TRCs) and Na⁺ transport was measured across lingual epithelia containing fungiform or vallate taste buds in a modified Ussing chamber. In isolated fungiform TRCs that contain Na⁺ currents sensitive to the diuretic amiloride, Na⁺ ions inhibit their own influx through ASSCs, a process known as sodium self-inhibition. Due to the interaction between self-inhibition and the driving force for Na⁺ entry, self-inhibition is most evident in whole-cell recordings at Na⁺ concentrations from 50 to 75 mM. In amiloride-sensitive cells, the Na permeability is significantly higher in extracellular solutions containing 35 mM Na⁺ than in 70 or 140 mM Na⁺. Compared with the block by amiloride, the development of self-inhibition is slow, taking up to 15 s to become maximally inhibited. Approximately one third of fungiform TRCs and all vallate TRCs lack functional ASSCs. These amiloride-insensitive TRCs show no signs of self-inhibition, tying this phenomenon to the presence of ASSCs. The sulfhydryl reagent, p-hydroxymercuribenzoate (p-HMB; 200 μM), reversibly removed self-inhibition from amiloride-sensitive Na⁺ currents, apparently by modifying cysteine residues in the ASSC. Na⁺ currents in amiloride-insensitive TRCs were unaffected by p-HMB. In sodium transport studies in fungiform taste bud-containing lingual epithelia, ~40% of the change in short-circuit current (Iₛ) after addition of 500 mM NaCl to the mucosal chamber is amiloride sensitive (0.5 mM). p-HMB significantly enhanced mucosal NaCl-induced changes in these epithelia at mucosal Na⁺ concentrations of 50 and above. In contrast, the vallate-containing epithelium, which are insensitive to amiloride, showed no enhancement of Iₛ during p-HMB treatment. These findings suggest that sodium self-inhibition is present in ASSCs in taste receptor cells where it may play a crucial role in performance of salt-sensitive pathways in taste tissue during sodium stimulation. This phenomenon may be important in the process of TRC adaptation, in the conservation of cellular resources during chronic sodium exposure, or in the gustatory response to water.

KEY WORDS: rat • patch clamp • salt • gustation

INTRODUCTION

The transduction of Na⁺ salts occurs in large part by means of an influx of Na⁺ ions through amiloride-sensitive sodium channels (ASSCs) localized on the apical membranes of taste receptor cells (TRCs). The influx of Na⁺ directly depolarizes TRCs, leading to the generation of action potentials and eventual release of neurotransmitter onto gustatory afferents (Gilbertson and Kinnamon, 1996; Kinnamon and Margolskee, 1996; Lindemann, 1996). Recent experiments in fungiform taste buds have revealed that Na⁺ transport into TRCs is saturable, highly selective, regulated by hormones, and inhibitable by amiloride and a number of its analogs (for review see Avenet, 1992; Gilbertson, 1993; Gilbertson and Kinnamon, 1996). Thus, ASSCs in TRCs apparently respond to the same regulatory cues that govern their function in other transporting epithelia such as the kidney, colon, and lung (Lindemann, 1984; Garty and Benos, 1988; Benos et al., 1996; Garty and Palmer, 1997).

An additional well-known feature of Na⁺-transporting epithelia, like that found in the kidney and colon, is that Na⁺ transport shows apparent saturation with increasing mucosal concentrations of Na⁺ (Lindemann, 1984; Van Driessche and Zeiske, 1985; Kroll et al., 1991). Several theories, which are not mutually exclusive, have been proposed to explain this decrease in Na⁺ permeability with increasing extracellular Na⁺ concentrations. One explanation attributes the inverse relationship between extracellular Na⁺ concentration and permeability to a direct interaction of extracellular Na⁺ ions with the ASSC, a process known as sodium self-inhibition (Fuchs et al., 1977). This process involves Na⁺ binding to a site on the extracellular face of the ASSC, within seconds causing a decrease in Na⁺ permeability. On the other hand, the feedback inhibition hypothesis proposes that saturation is due to a di-
rect or indirect inhibition of ASSCs by changes in intracellular Na\(^+\) (Ling and Eaton, 1989; Komwatana et al., 1996a, 1996b). Feedback inhibition involves a rise in intracellular Ca induced by increasing intracellular Na\(^+\) concentrations and a subsequent activation of G proteins (Komwatana et al., 1996b), of protein kinase C (Garty and Benos, 1988), and, presumably, phosphorylation. Unlike Na\(^+\) self-inhibition, the feedback inhibition mechanism may develop much more slowly, taking several minutes or more to see moderate changes in Na\(^+\) permeability (Lindemann, 1984; Ling and Eaton, 1989). A third potential cause of apparent Na\(^+\) saturation may be due to the decrease in driving force for Na\(^+\) ions resulting from a significant increase in intracellular Na\(^+\) concentration. The presence of this change in driving force would be apparent as a gradual shift in the reversal potential for Na\(^+\) current over time as Na\(^+\) concentrations built up inside the cell. Finally, saturation may be due to the actual saturation of the binding sites for Na\(^+\) within the pore of the ASSC itself. This would be predicted to be the most rapid of the potential mechanisms underlying saturation, typically in milliseconds (Hille, 1992), and thus would depend predominately on the time course of solution change.

We have used perforated patch and whole-cell patch clamp recording on rat isolated TRCs and transepithelial current recording on isolated lingual epithelia to determine the ability of Na\(^+\) ions to inhibit their own permeation through ASSCs. We have found that taste receptor cells that possess ASSCs show apparent sodium self-inhibition behavior and speculate this phenomenon may play important roles in the adaptation of taste receptor cells to Na\(^+\) salts, the conservation of cellular resources, and in the gustatory response to water. Clearly, evidence of sodium self-inhibition in taste cells has implications not only for the transduction of sodium salts, but also for the regulation of salt intake.

**METHODS**

**Tissue Preparation and Solutions**

Lingual epithelia and taste buds from the fungiform and vallate papillae were isolated from 2–4-mo-old male Sprague Dawley rats using methods previously described (Gilbertson, 1995; Doolin and Gilbertson, 1996). Briefly, tongues were removed posterior to the vallate papilla and immersed in ice cold Tyrode solution. For isolation of the epithelium used in epithelial transport experiments, tongues were injected beneath the lingual epithelia with 2–3 ml of Tyrode containing 3 mg/ml dispase (Type II; Boehringer Mannheim, Indianapolis, IN) and 1 mg/ml trypsin inhibitor (Type I-S; Sigma Chemical Co., St. Louis, MO). Injected tongues were incubated for 30 min in Tyrode solution, which was bubbled continuously with O\(_2\). For isolation of fungiform or vallate taste buds from the lingual epithelium, tongues were injected with ~2 ml of a mixture of 0.3 mg/ml collagenase A (Boehringer Mannheim Biochemicals), 4 mg/ml dispase, and 1 mg/ml trypsin inhibitor in Tyrode. These tongues were incubated in oxygenated Ca/Mg-free Tyrode for 30–35 min. After their specific incubation periods, the lingual epithelia containing the fungiform and vallate taste buds were removed from the underlying muscle layer and placed in a 35-mm petri dish containing either Tyrode (epithelial transport studies) or Ca/Mg-free Tyrode (patch clamp studies). For the isolation of individual taste buds, the lingual epithelia were subsequently pinned out serosal side up in a Sylgard-lined petri dish containing Ca/Mg-free Tyrode. Individual taste buds from the fungiform and vallate papillae were removed by gentle suction by a fire-polished pipette (~200 µm diameter) and plated into the recording chamber coated with Cell-Tak™ (Collaborative Biomedical Products, Bedford, MA) tissue adhesive. Taste buds isolated in this manner were viable and retained their distinctive morphology (Gilbertson, 1995; Doolin and Gilbertson, 1996) for ~6 h or more.

**Patch clamp solutions.** Extracellular saline (Tyrode) contained (mM): 140 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, 10 glucose, and 10 Na\(^+\) pyruvate. The pH was adjusted to 7.40 with NaOH. The only change made to prepare Ca/Mg-free Tyrode was to substitute 2 mM BAPTA (Molecular Probes, Inc., Eugene, OR) for the CaCl\(_2\) and MgCl\(_2\). Solutions in which NaCl concentration was altered were prepared by an equimolar substitution of N-methyl-D-glucamine chloride for NaCl. No other ions were altered in the reduced Na\(^+\) solutions. Amiloride and phloroxygenicuribenzoate (p-HMB), when used, were added to Tyrode or one of the reduced Na\(^+\) solutions.

Intracellular (pipette) solution used for perforated patch recording was prepared by first dissolving the pore-forming antibiotic nystatin (50 mg/ml) in DMSO. This solution was made daily. The nystatin solution was diluted to a final concentration of 250 µg/ml in a solution containing (mM): 55 KCl, 10 NaCl, 75 K\(_2\)SO\(_4\), 8 MgCl\(_2\), and 10 HEPES, pH adjusted to 7.20 with Tris-OH. Pluronic (Molecular Probes, Inc.) was added to the perforated patch solution to a final concentration of 0.2% (wt/vol) and the solution was sonicated and vortexed. Pipette tips were filled with nystatin-free solution and backfilled with nystatin-containing perforated patch solution. Once made, the perforated patch solution containing nystatin was used for up to 1.5 h.

In experiments where it was necessary to introduce compounds (e.g., GDP-β-S, p-HMB) intracellularly while patch clamp recording, the conventional whole-cell configuration was used. In this case, the pipette solution contained (mM): 140 KCl, 1 CaCl\(_2\), 2 MgCl\(_2\), 10 HEPES, 11 EGTA, 5 Na\(_2\)ATP, and 0.4 GTP. The pH was adjusted to 7.20 with KOH and the free Ca\(^{2+}\) was ~10^-8 M.

In several experiments, voltage-activated Na\(^+\) and K\(^+\) channels were inhibited. Tetrodotoxin (0.5 mM) was added to the extracellular solutions to inhibit voltage-activated Na\(^+\) channels. K\(^+\) channels were inhibited by addition of 10 mM tetrathylammonium bromide (10 mM) to the extracellular solutions and replacement of Cs\(^+\) for K\(^+\) in the perforated patch solution. Other ions were kept constant. These pharmacological manipulations eliminated most of the voltage-activated currents in the isolated TRCs and, as a result, a significant proportion of the remaining current was carried by Na\(^+\) ions through ASSCs.

**Epithelial transport solutions.** Modified Krebs-Heinsleit (KH) buffer contained (mM): 118 NaCl, 25 NaHCO\(_3\), 1.3 NaH\(_2\)PO\(_4\), 6 KCl, 2 CaCl\(_2\), 1.2 MgSO\(_4\), and 5.6 glucose. KH buffer was bubbled with 95% O\(_2\)/5% CO\(_2\) continuously, which produced a stable pH of 7.4. Mucosal solutions consisted of solutions varying in NaCl concentrations from 10 to 500 mM dissolved in distilled water.

**Patch-Clamp Recording**

Individual taste receptor cells maintained in isolated fungiform and vallate taste buds were recorded from using the perforated patch configuration (Horn and Marty, 1988; Korn et al., 1991) or
the conventional whole-cell variation of the patch clamp technique (Hamill et al., 1981). Patch pipettes were made from microhematocrit tubes (Scientific Products, McGaw Park, IL) pulled on a Flaming/Brown-type micropipette puller (P-97; Sutter Instrument Co., Novato, CA) and fire-polished on a microforge (MF-9; Narishige Ltd., Tokyo, Japan) to a resistance of 4–8 M\(\Omega\) when filled with intracellular solution. Seals resistances were typically in the tens of G\(\Omega\). In the perforated patch configuration, mean input resistance was 5.87 ± 1.02 (SD) G\(\Omega\), mean resting potential was −52.3 ± 8.9 mV, and mean cell capacitance was 10.2 ± 1.7 pF (\(n = 48\)). Similar values were obtained using standard whole-cell recording. Series resistance and capacitance were compensated before recording.

Whole-cell membrane currents were recorded in voltage-clamp mode by a high-impedance patch clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA) interfaced to a computer (486/33 MHz) by an A-to-D board (Digidata 1200A; Axon Instruments). Command potentials were delivered and currents recorded by computer-driven software (pCLAMP 6.0.3; Axon Instruments). Steady state and voltage-activated currents were recorded at a sampling rate of 10 kHz. For analysis and presentation, data were low-pass filtered at 2 kHz. Once the perforated patch configuration was achieved (typically 3–15 min after seal formation), or the whole-cell configuration was established, compensation for series resistance and cell capacitance was made. No records were leak subtracted in the present study. Solutions were applied by gravity and controlled manually by an 8-to-1 solenoid valve assembly (General Valve, Fairfield, NJ). The volume of the recording chamber was ~0.25 ml and flow rates were ~4 ml/min, allowing complete solution change in <4 s.

**Determination of Sodium Permeability**

To estimate the Na\(^+\) permeability (\(P_{Na}\)) of ASSCs under different extracellular Na\(^+\) concentrations (35, 70, and 140 mM Na) in tetradotoxin-containing extracellular solution and Cs and tetraethylammonium bromide containing intracellular solution (see above), each set of currents was measured at −100 mV for a given Na\(^+\) concentration and this value was used to calculate \(P_{Na}\) with the Goldman equation in the following form (Kroll et al. 1991):

\[
I = (VP_{Na}AF^2/RT) \cdot \frac{N_{Na_{out}} - (N_{Na_{in}} \cdot e^{VF/RT})}{1 - e^{VF/RT}},
\]

where \(I\) is the current measured at potential \(V = -100\) mV, \(P_{Na}\) is the Na\(^+\) permeability in centimeters per second, \(N_{Na_{out}}\) and \(N_{Na_{in}}\) are the extracellular and intracellular Na\(^+\) concentrations, respectively, \(A\) is the membrane surface area of the TRC determined from the measured whole-cell capacitance and assuming a specific capacitance of 1 \(\mu F/cm^2\), \(F\) is the Faraday constant, \(R\) is the gas constant, and \(T\) is the absolute temperature. \(N_{Na_{in}}\) was assumed to be 10 mM since all pipette solutions contained this concentration of Na\(^+\) and measurements were made at equilibrium. This equation has been used previously under conditions similar to those in the present study to estimate the permeability of ASSCs (Kroll et al., 1991). For purposes of the present study, the permeabilities of other ions through ASSCs were assumed to be zero since solutions contained no other cations known to be significantly permeant through ASSCs (Garty and Benos, 1988). The resulting \(P_{Na}\) in each solution was averaged and analyzed by one-way ANOVA for significant effects of \(N_{Na_{in}}\) on \(P_{Na}\) and subjected to Tukey's HSD post-hoc test at the 0.05 significance level.

**Transepithelial Current Recording**

Isolated lingual epithelia containing either the vallate or fungiform papillae were mounted in a bipartitioned Ussing chamber (World Precision Instruments, Inc., Sarasota, FL), each side of which had a volume of 0.75 ml and an opening with an area of 0.126 cm\(^2\). Epithelia were supported by a nylon mesh filter covering the opening to the serosal chamber that prevented damage to the tissue. The chamber was assembled and both chambers were filled with KH buffer and allowed to equilibrate for 30 min. Solutions were maintained at 34°C in a thermal bath and were applied by gravity flow to the two chambers through individual miniature solenoid valves (The Lee Co., Westbrook, CT) and 8:1 tubing connectors (Small Parts, Miami Lakes, FL). This arrangement allowed solution change without disturbance of the Ussing chamber and eliminated the possibility of introducing bubbles. Solutions were perfused continually through the mucosal and serosal chambers for the duration of the experiment at a rate of ~5 ml/min (complete change in <10 s). In the present study, keeping the solutions continually perfusing produced more reproducible results than when solution flow was stopped (our unpublished observations). Using this approach, epithelia were recorded for as long as 10 h without noticeable decrement. Once equilibrated, the open circuit potential difference (PD) for the fungiform-containing epithelia was 6.5 ± 1.2 (SD) mV, which was calculated by Ohm's Law from the measured \(I_{sc}\) in symmetrical KH buffer of 4.8 ± 0.7 \(\mu A/cm^2\) and the transepithelial resistance of 1.577 ± 185 \(\Omega cm^2\) (\(n = 19\) epithelia). In contrast, vallate-containing epithelia had a significantly higher transepithelial resistance (2.073 ± 141.2 \(\Omega cm^2\); \(n = 22\) epithelia) than the fungiform-containing epithelia. This resistance, coupled with a measured \(I_{sc}\) of 3.6 ± 0.8 \(\mu A/cm^2\), gave a calculated PD for the vallate-containing epithelia of 7.5 ± 1.8 mV with KH buffer on both sides of the epithelium.

Transepithelial currents were recorded by a dual voltage clamp (DVC-1000; WPI, New Haven, CT) connected to the Ussing chamber by Ag/AgCl electrodes. Each side of the chamber had a current and voltage electrode in series with 0.9% NaCl agar bridges. Fluid resistance was compensated before the introduction of an epithelium with symmetrical KH buffer. Short-circuit current was recorded on a two-channel PCM recorder (200; A.R. Vetter and Co., Inc., Rebersburg, PA) at a frequency of 44.1 kHz and displayed on a strip chart recorder (RS200; Gould Inc., Valley View, OH). Transepithelial resistance was monitored by −20-mV pulses delivered by a stimulator (S-900; Dagan Corp., Minneapolis, MN) connected to the voltage clamp. All experiments were conducted with the tissue voltage clamped to 0 mV. By convention (Mierson et al., 1996), positive \(I_{sc}\) indicates the net movement of cations from the apical to the basolateral side of the tissue.

**RESULTS**

**Patch clamp experiments.** To determine if there was evidence of sodium self-inhibition in isolated rat TRCs, we characterized the Na dependence of currents through ASSCs using whole-cell (perforated) patch clamp recording techniques. Approximately two thirds of mammalian fungiform TRCs have ASSCs (Gilbertson et al., 1993; Doolin and Gilbertson, 1996), whose activity is indicated by a reduction in a standing inward Na\(^+\) current at −80 mV (Fig. 1). In TRCs exhibiting ASSC activity, we have perfused salines varying in extracellular Na\(^+\) concentrations while recording steady state currents at −80 mV. In 13 of 14 cells that were able to be recorded long enough to test the full complement of solutions, the resting inward Na\(^+\) current was larger in
35 mM Na\(^+\) than 70 mM Na\(^+\), consistent with there being an inhibitory effect of extracellular Na\(^+\) ions (Fig. 1). In most cases, increases in Na\(^+\) concentration from 70 to 140 mM apparently overcame the self-inhibition due to the further increase in driving force for inward Na\(^+\) movement.

The time course for the development of self-inhibition was slow compared with the block of ASSCs by amiloride (e.g., Fig. 1). As illustrated in Fig. 2A, changing from 35 to 70 mM Na\(^+\) extracellularly caused a time-dependent decrease in conductance. This decrease in conductance reached a maximum \(\approx 15\) s after complete solution change in the recording chamber. After accounting for the inherent delay in our perfusion system (\(\approx 4\) s), we estimated the time constant for the development of self-inhibition for the decrease in current resulting from switching from 35 to 70 mM Na\(^+\) extracellularly (Fig. 2B). Our results show that the time constant is \(\approx 10\) s (Fig. 2B; \(n = 10\) cells), similar to values reported in ASSCs in other systems (Li and Lindemann, 1983; Kroll et al., 1991).

By varying only the extracellular Na\(^+\) concentration in our perfusate, we determined the concentration dependence for Na\(^+\) currents through ASSCs. A subset of fungiform TRCs showed extreme levels of self-inhibition, as illustrated in Fig. 3A. After blocking the voltage-activated Na\(^+\) and K\(^+\) channels pharmacologically and by ion substitution (see METHODS), current-voltage relations could be generated by applying ramps of voltage from \(-110\) to \(+50\) mV. Under these conditions, most of the current was through ASSCs (compare Fig. 1). Though a few TRCs showed the type of extreme self-inhibition seen in Fig. 3A, in the majority of TRCs the magnitude of the currents in 140 mM Na\(^+\) were greater than in 35 mM Na\(^+\). Fig. 3B shows the relation-
Figure 3. Na dependence of currents through amiloride-sensitive Na\(^+\) channels. (A) Instantaneous current–voltage relationship elicited by voltage ramps in varying extracellular Na\(^+\) concentrations. This TRC showed the most extreme case of sodium self-inhibition seen. Voltage-activated Na\(^+\) and K\(^+\) channels were inhibited pharmacologically as described in Methods. (B) Concentration–response curve for the relative Na\(^+\) current (normalized to the current in 140 mM Na\(^+\)) under different extracellular Na\(^+\) concentrations. Data are mean ± SEM and represent 8–14 cells per point. Dotted line is a spline fit to the data points.

Approximately one third of rat fungiform TRCs and all vallate TRCs lack ASSCs (Doolin and Gilbertson, 1996). To determine if sodium self-inhibition was solely a property of ASSCs, we looked for evidence of sodium self-inhibition in TRCs lacking ASSCs. 25 of 66 (38%) fungiform TRCs in the present study did not respond to amiloride (10 \(\mu\)M) with a decrease in conductance and were classified as amiloride insensitive. Of the amiloride-insensitive fungiform TRCs, 88% (\(n = 22\) cells) showed no apparent self-inhibition; that is, currents were larger in 70 mM than 35 mM Na\(^+\) (Fig. 4 A). For fungiform TRCs that were amiloride insensitive and were able to be tested with each of the three concentrations of extracellular Na, we found no significant difference in \(P_{\text{Na}}\) (Eq. 1) as a function of extracellular Na\(^+\). At \(-100\) mV, in 35 mM Na\(^+\), the mean \(P_{\text{Na}}\) (±SD) was 6.69 ± 2.54 \(\times\) \(10^{-10}\) cm/s; in 70 mM Na\(^+\), \(P_{\text{Na}}\) was 7.42 ± 2.78 \(\times\) \(10^{-10}\) cm/s; in 140 mM Na\(^+\), \(P_{\text{Na}}\) was 5.75 ± 2.61 \(\times\) \(10^{-10}\) cm/s (\(n = 8\) cells each). Analysis of variance (one-way ANOVA) revealed a lack of any significant effect of Na\(^+\) concentration on \(P_{\text{Na}}\) (\(F[2,21] = 0.80, P = 0.462\)). There were, however, three amiloride-insensitive cells that showed apparent self-inhibition, though this was too few cells to permit a meaningful analysis. Similarly, TRCs from vallate taste buds were completely amiloride insensitive (\(n = 18\) cells), in agreement with a previous report (Doolin and Gilbertson, 1996). These cells showed no signs of sodium self-inhibition (Fig. 4 B).

Because numerous reports have suggested that sodium self-inhibition or saturation of ASSCs may be reduced or abolished by sulfhydryl compounds in other cell types (Li and Lindemann, 1983; Kroll et al., 1991; Turnheim, 1991), we tested \(p\)-hydroxymercuribenzoate in patch clamp recording conditions to determine if it could remove sodium self-inhibition in TRCs. Treatment of TRCs by addition of \(p\)-HMB (200 \(\mu\)M) for 10 min caused a marked increase in conductance (Fig. 5). In all cells showing apparent sodium self-inhibition (\(n = 11\) cells), \(p\)-HMB significantly enhanced currents in response to voltage ramps and removed self-inhibition. Conversely, \(p\)-HMB had no effect on TRCs that were classified as amiloride insensitive (Fig. 5, inset; \(n = 9\) cells). The effects of \(p\)-HMB were nearly completely reversible by treatment of TRCs with 10 mM cysteine for a period of 15–20 min (data not shown). Inclusion of 200 \(\mu\)M \(p\)-HMB in the patch pipette did not remove self-inhibition nor affect Na\(^+\) permeability estimates (\(n = 4\) cells; Fig. 5), suggesting that this compound acts at an extracellular site. To test for the involvement of G protein activation in the effects of extracellular Na\(^+\) on the permeability of ASSCs (Komwatana et al., 1996b), we included the G protein activation inhibitor GDP-\(\beta\)-S.
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(0.5 mM) in the patch pipette. Self-inhibition was unchanged under this condition \( (n = 5 \text{ cells}; \text{Fig. 5}) \).

**Epithelial Transport Experiments**

Numerous studies have shown that \( \text{Na}^+ \) transport across the lingual epithelia from rat (Mierson et al., 1988, 1996), hamster (Zhang et al., 1996), and dog (DeSimone et al., 1984; Heck et al., 1984; Simon and Garvin, 1985) is carried, in part, by \( \text{Na}^+ \) movement through apical ASSCs into TRCs, where it is subsequently pumped out via ouabain-sensitive \( \text{Na}^+/\text{K}^+ \) pumps. To determine the effects of sodium self-inhibition recorded at the cellular level on \( \text{Na}^+ \) transport, we performed experiments on rat lingual epithelia containing either fungiform or vallate taste buds. Stepwise increases in \( \text{NaCl} \) concentration from 10 to 500 mM in the mucosal chamber caused net increases in \( \text{I}_{\text{sc}} \) in both fungiform- and vallate-containing epithelia. In general, fungiform-containing epithelia have a greater current density than epithelia containing vallate taste buds (Fig. 6), consistent with their differences in transepithelial resistance (see Methods). Though both epithelia transport \( \text{Na}^+ \), only \( \text{NaCl} \)-induced changes in \( \text{I}_{\text{sc}} \) in the fungiform-containing epithelia were amiloride sensitive (Gilbertson and Zhang, 1996), consistent with the absence of functional ASSCs in isolated rat vallate TRCs (Doolin and Gilbertson, 1996). To test for evidence of

![Figure 4](image4.png)

**Figure 4.** Taste cells that do not contain amiloride-sensitive sodium channels do not display sodium self-inhibition. Relative whole-cell current as a function of extracellular \( \text{Na}^+ \) concentration in fungiform (A) and vallate (B) TRCs lacking amiloride sensitivity. Note ineffectiveness of amiloride (10 \( \mu \text{M} \)) in inhibiting currents in 35, 70, and 140 mM \( \text{Na}^+ \). Currents were normalized to the current in 70 mM \( \text{Na}^+ \) at \(-80\) mV. Unlike cells with ASSCs (Figs. 1 and 3 B), current increases with increasing \( \text{Na}^+ \) concentration from 35 to 70 mM. The TRCs were not tested with voltage-activated \( \text{Na}^+ \) and \( \text{K}^+ \) channels inhibited; hence, some of the current shown may be through other types of channels as well. Data are mean \( \pm \) SEM and represent from 11–22 cells per point.

![Figure 5](image5.png)

**Figure 5.** Self-inhibition may be removed by treatment with the sulfhydryl-modifying reagent, \( \text{p}-\text{hydroxymercuribenzoate (200} \mu\text{M)} \). Normalized currents in rat fungiform taste receptor cells recorded in varying extracellular \( \text{Na}^+ \) concentration in the presence of amiloride (10 \( \mu\text{M} \)) or \( \text{pHMB} \). \( \text{pHMB} \) had no effect upon the current in 35 mM \( \text{Na}^+ \), but significantly enhanced currents in 70 and 140 mM \( \text{Na}^+ \) \((n = 11 \text{ cells}) \). Currents in \( \text{pHMB} \)-treated cells appeared qualitatively similar to the current in amiloride-insensitive TRCs (Fig. 4). Intracellular GDP-\( \beta\)-\( \text{S} \) (0.5 mM) and intracellular \( \text{pHMB} \) (200 \( \mu\text{M} \)) had no effect on this apparent self-inhibition seen in 70 mM \( \text{NaCl} \) (insf) TRCs that lack ASSCs are insensitive to \( \text{pHMB} \). In 70 mM \( \text{Na}^+ \), normalized currents in a rat fungiform TRC that was amiloride-insensitive also did not respond to \( \text{pHMB} \) \((n = 9 \text{ cells}) \). Data were normalized to the current in 70 mM \( \text{Na}^+ \) at \(-80\) mV and are presented as mean \( \pm \) SEM. *Significant differences from the control condition within a given concentration group (Student’s \( t \) test, \( P < 0.05 \)).
self-inhibition at the tissue level, epithelia were treated with \( p \)-HMB for 10 min before applying a series of NaCl concentrations (also containing \( p \)-HMB) in the mucosal chamber and KH buffer in the serosal chamber. As illustrated in Fig. 7 C, \( p \)-HMB increased the magnitude of changes in I\(_c\) caused by increasing mucosal NaCl concentration in fungiform-containing epithelia over control levels (Fig. 7 A). In contrast, \( p \)-HMB had no effect on Na\(^+\) transport in vallate-containing epithelia (Fig. 7 D), reflective of the lack of functional ASSCs in that tissue. Moreover, \( p \)-HMB, like amiloride, had no effect in either epithelia when applied in the serosal chamber (\( n = 8 \) epithelia each), suggesting that ASSCs, if present, are apically restricted (data not shown).

The ability of \( p \)-HMB to increase I\(_c\) and, hence, remove self-inhibition at the tissue level was only evident at mucosal NaCl concentrations of 50 mM and above (Fig. 8 A). This is consistent with isolated cell recordings in the present study that showed that in 35 mM NaCl there was little or no self-inhibition, while in 70 mM NaCl self-inhibition was apparent (e.g., Figs. 1, 3, and 5). At mucosal NaCl concentrations of 100 mM and above, addition of \( p \)-HMB increased I\(_c\) roughly 35–40% above control levels. At all NaCl concentrations, \( p \)-HMB had no effect on Na\(^+\) transport in vallate-containing epithelia (Fig. 8 A).

In fungiform-containing epithelia, \( \sim 40\% \) of the Na\(^+\) transport (mucosal, 500 mM; serosal, KH buffer) is inhibited by 0.5 mM amiloride in the mucosal chamber (Fig. 8 B). Amiloride applied in the serosal chamber had no effect on I\(_c\) in the present study. The tight junction blocker, lanthanum chloride (6 mM; Simon et al., 1993), when applied mucosally, also inhibited \( \sim 40\% \) of the transepithelial Na\(^+\) transport, consistent with the presence of paracellular pathways for Na\(^+\) movement (Simon et al., 1993; Ye et al., 1991). Curiously, however, application of both lanthanum chloride and amiloride together did not produce additive effects, the combination inhibited only \( \sim 55\% \) of the Na\(^+\) transport in fungiform-containing epithelia (Fig. 8 B). This amiloride- and lanthanum-insensitive current suggests the presence of another still unidentified route for Na\(^+\) transport that is independent of apical ASSCs and the

**Figure 6.** Concentration-response functions for the magnitude of changes in I\(_c\) as a function of mucosal NaCl concentration in fungiform- and vallate-containing epithelia. Zero current was arbitrarily defined as the current with 10 mM NaCl in the mucosal chamber and KH buffer in the serosal chamber. Solid lines are best fits to the data points with a Boltzmann function. Data points are presented mean \( \pm \) SD and reflect recordings from 20 (fungiform) and 21 (vallate) epithelia each.

**Figure 7.** Short-circuit currents display evidence of sodium self-inhibition. I\(_c\) measured during increasing mucosal NaCl concentrations (arrowheads, millimolar) in fungiform- (A) and vallate-containing (B) epithelia. Response of the same fungiform (C) and vallate (D) epithelia during treatment with 200 \( \mu \)M \( p \)-HMB mucosally. The fungiform and vallate epithelia were from the same rat. Increasing absorption of cations (apical to basolateral) is reflected upward. Brief vertical deflections are current responses to 20-mV pulses across the epithelia to monitor transepithelial resistance. Note difference in scale between fungiform and vallate epithelia. Only the I\(_c\) measured in fungiform-containing epithelia is enhanced by \( p \)-HMB treatment. This is consistent with reports in isolated TRCs (Doolin and Gilbertson, 1996) and lingual epithelia (Gilbertson and Zhang, 1996), which show that vallate taste receptor cells lack functional ASSCs.

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from nine pairs of epithelia. (B) Channels, ing only vallate taste buds, which lack amiloride-sensitive sodium in epithelia containing fungiform taste buds. In epithelia containing increases I_sc paracellular pathway. At 500 mM NaCl, p-HMB increases I_sc ~40% over control levels. However, when amiloride was applied in the presence of p-HMB, amiloride was not as effective at inhibiting I_sc as in control conditions. This may reflect that the binding sites for amiloride and Na^+ (at the self-inhibition site) may overlap, as has been previously suggested (Li and Lindemann, 1983), or, alternatively, that conformational changes in ASSCs induced by p-HMB may alter amiloride binding. Nonetheless, it is clear that self-inhibition is evident at the tissue level as well as in isolated TRCs.

**Discussion**

The involvement of amiloride-sensitive sodium channels in the transduction of NaCl in taste receptor cells has been well established. There is, however, little understanding of the regulation of the function of ASSCs in the taste system. The ability of salt-sensitive pathways in TRCs to respond to hormonal cues has recently been reported in a variety of species. For example, the hormones aldosterone (Kosten and Contreras, 1990; Okada et al., 1990; Herness, 1992) and arginine vasopressin (Okada et al., 1991, 1996; Gilbertson et al., 1993), which contribute to the regulation of salt and water balance, have been shown to regulate salt responsiveness in the peripheral gustatory system. The present study describes another process that regulates ASSC function, the ability of Na^+ ions to inhibit their own permeation through ASSCs, known as sodium self-inhibition.

Sodium self-inhibition in ASSCs is most evident in patch clamp recordings from isolated rat fungiform TRCs as a decrease in current when the extracellular solution is changed from 35 to 70 mM Na^+ while all others ions are held constant. Increasing extracellular Na^+ to 140 mM leads to increases in whole-cell current in most cells, due to the increase in driving force for Na^- entry. Estimates of Na^- permeability using Eq. 1, however, show that P_Na in 35 mM Na^+ is significantly greater than in 140 mM Na, consistent with the presence of self-inhibition at the higher concentration. Moreover, the ability of p-HMB to enhance Na^- currents both at the cellular and epithelial levels is consistent with this ability of Na^- ions to modulate the permeability of ASSCs.

Though we have not ruled out that changes in cytosolic Na^- ions may be mediating, in part, the effects of Na^- on ASSCs, it is clear that this feedback inhibition mechanism cannot fully explain the present results (see below). Moreover, it is unlikely that a change in Na^- driving force was responsible for the effects since in most cases (i.e., Fig. 2) there was little or no shift in the zero current (reversal) potential for the Na^- current during development of inhibition.

Several factors point to the presence of sodium self-inhibition as being largely responsible for the effects seen. One, the time course of the response is significantly more rapid than reported for cases of feedback inhibition. Changes in whole-cell currents and Na^- permeability in rat TRCs were evident within a few seconds and typically complete within 15–30 s. This is consistent with other reports of self-inhibition in epithelial Na^- channels (Fuchs et al., 1977; Kroll et al., 1991), unlike that reported for cytosolic Na^-induced changes in Na^- permeability, which takes several minutes to develop (Lindemann, 1984; Ling and Eaton, 1989). The time course seen was too slow to be due to saturation of the
Na\textsuperscript{+} binding site within the pore of the ASSC (Hille, 1992; Garty and Palmer, 1997). As shown in Fig. 2, changes in the current were seen seconds after the bath solution had been completely replaced. Two, the ability of sulfhydryl reagents like \(p\)-HMB to reduce or eliminate Na\textsuperscript{+}-induced changes in the permeability of ASSCs is reflective of self-inhibition. There are no reports of \(p\)-HMB altering the permeability effects of cytosolic Na\textsuperscript{+} on ASSCs. Moreover, in the present study, intracellular \(p\)-HMB was ineffective in inhibiting the Na\textsuperscript{+}-induced inhibition of ASSC currents, suggesting that the site affected by \(p\)-HMB was extracellular, as has been proposed for the self-inhibition site in epithelial Na channels (Luger and Turnheim, 1981; Li and Lindemann, 1983). Three, inhibition of G protein activation by GDP-\(\beta\)-S did not remove self-inhibition, suggesting that this was unlike the feedback inhibition seen in mouse mandibular gland ASSCs (Komwatana et al., 1996b). Nonetheless, we have not eliminated the possibility that changes in intracellular Na\textsuperscript{+} also contribute to the effects seen in the present study nor that saturation of the channel pore may have occurred before the development of self-inhibition. It is clear that sodium self-inhibition does affect the permeability of ASSCs in taste receptor cells.

The ability of Na\textsuperscript{+} ions to inhibit the Na\textsuperscript{+} permeability of TRC membranes is linked with the presence of ASSCs. TRCs that do not possess functional ASSCs do not show self-inhibition. Furthermore, \(p\)-HMB had no effect on isolated TRCs nor on epithelial Na\textsuperscript{+} transport in cells or tissues that did not show amiloride sensitivity. In epithelial transport experiments on fungiform taste bud–containing epithelia, both amiloride and \(p\)-HMB were effective only when applied to the mucosal chamber. Taken together, it is clear that the phenomenon of sodium self-inhibition is a property of ASSCs in taste tissue.

Another implication from the finding that self-inhibition is seen only in amiloride-sensitive Na\textsuperscript{+} transport is that amiloride-insensitive (A-I) Na\textsuperscript{+} transport pathways are apparently not regulated in the same manner by extracellular Na\textsuperscript{+} concentrations. These A-I pathways may contribute from 20–100\% of the total Na\textsuperscript{+} transport in rat taste tissue depending upon the type of taste bud (Doolin and Gilbertson, 1996). In vallate TRCs, for example, there is a complete lack of amiloride-sensitive Na\textsuperscript{+} transport (Formaker and Hill, 1991; Doolin and Gilbertson, 1996; Zhang et al., 1996). Though the presence of ASSCs in vallate taste tissue has been identified using immunocytochemistry and in situ hybridization (Li et al., 1994; Li and Snyder, 1994; Simon et al., 1993), it’s been suggested that these channels, if present in the membrane, lack the capability to be inhibited by amiloride (Doolin and Gilbertson, 1996). These putative vallate ASSCs that cannot be inhibited by amiloride may reflect an alternatively spliced form or unique subunit arrangement of the channel that has been suggested to contribute to the amiloride-insensitive Na\textsuperscript{+} currents in these cells (Doolin and Gilbertson, 1996).

From the present study, it would also appear that these putative ASSCs in vallate taste buds also lack the site(s) that is responsible for mediating sodium self-inhibition. Thus, the ASSCs in rat vallate TRCs are functionally very different from those in the fungiform taste buds. It is not clear from the present study if the total A-I Na\textsuperscript{+} transport is regulated by Na\textsuperscript{+} ions in an alternative manner that does not involve the process of self-inhibition described here.

The inability of lanthanum and amiloride, when applied together, to completely inhibit sodium transport has several possible explanations. One, there may be additional transport mechanisms for sodium ions that do not involve ASSCs and paracellular pathways. Though there is presently no direct evidence in support of such a mechanism, it has been reported that there may be multiple forms of epithelial sodium channels (ENaC), some of which have a greatly reduced ability to be inhibited by amiloride that arises by a unique combination of the \(\alpha\), \(\beta\), and \(\gamma\) subunits that comprise the functional ENaC (see Benos et al., 1997). This channel, if present in taste tissue, may rectify the conflicting evidence that shows the presence of ASSC protein and mRNA in the posterior rat tongue (Li and Snyder, 1994; Simon et al., 1993) with the physiological evidence that is consistent with the lack of amiloride sensitivity in these areas (Doolin and Gilbertson, 1996; Fig. 4). A second possibility is that the binding of the trivalent cation La\textsuperscript{3+} may affect the ability of amiloride to bind to the ASSC, or vice versa. Third, we have used a single concentration of lanthanum in these experiments that, while generally higher than is typically used (Simon et al., 1993), may nonetheless not completely inhibit paracellular transport on sodium ions. Further experiments will be needed to distinguish among these possibilities.

Implications of Sodium Self-Inhibition in Taste Receptor Cells

Though the role of sodium self-inhibition in mammalian TRCs is presently unclear, this regulatory mechanism may play a role in several processes related to taste transduction. Self-inhibition of ASSCs by extracellular Na\textsuperscript{+} ions may account, at least in part, for the adaptation seen during sodium salt stimulation. Recordings from taste buds in situ (Avenet and Lindemann, 1991; Gilbertson et al., 1992), afferent nerve fibers (Frank et al., 1983; Matsuo and Yamamoto, 1992), and central gustatory neurons (Smith et al., 1975; Nishio and Norrgren, 1990; Nakamura and Norrgren, 1991) all display adaptation to NaCl stimulation over a time course similar to that described for the development of sodium
self-inhibition in the present study. Given the magnitude of the decrease in permeability with increasing extracellular sodium concentration seen in the present study, self-inhibition would clearly participate in the adaptation of the amiloride-sensitive sodium transport pathways. However, because the amiloride-insensitive salt transport pathways do not show self-inhibition, this process alone cannot explain NaCl adaptation at the cellular level in total. It is unclear if adaptation occurs in these as yet undefined salt-transducing pathways.

Sodium self-inhibition has been proposed to be one mechanism by which sodium-transporting cells conserve resources during chronic sodium loads (Wills and Zwiefach, 1987; Sariban-Sohrab and Benos, 1986). Because the apical membranes of taste receptor cells are being constantly bathed with saliva, which in rats contains as much as 60 mM Na\(^+\) (Rehberg et al., 1992), the ability of extracellular sodium ions to inhibit their own influx may be reflective of a mechanism by which taste receptor cells limit Na\(^+\) entry through ASSCs. Since increases in intracellular Na\(^+\) ions are rapidly pumped out via the ATP-dependent Na\(^+\)/K\(^+\) pumps on the basolateral membrane, limiting Na\(^+\) entry may be one mechanism by which the taste receptor cells conserve their energy stores. Given this theory, it is interesting that the nadir of the concentration–response curve shown in Fig. 3 B occurs at concentrations near that found in rat saliva. Further investigations of sodium self-inhibition in other species, like the hamster, that have comparatively low salivary sodium levels (~6 mM; Rehberg et al., 1992) should help clarify this relationship.

A third potential implication of the role that sodium self-inhibition may play in taste transduction is illustrated in the records shown in Figs. 1 and 3 B. That is, in the rat, changes in extracellular Na\(^+\) concentrations from 70 to 35 mM actually caused an increase in the magnitude of amiloride-sensitive Na\(^+\) currents in fungiform TRCs. This would imply that decreases in apical (extracellular) Na\(^+\) concentrations from that found in saliva might actually lead to a depolarization and activation of the TRC. Thus, it might be postulated that sodium self-inhibition may participate in the gustatory response to water. Since vertebrate “water receptors” are apparently not osmoreceptors (Soeda and Sakudo, 1988; Zotterman, 1956), the activation of taste receptor cells elicited by decreases in extracellular Na\(^+\) concentration may mediate part of this transient response to water. In other words, it may not be solely water that is the stimulus for water taste, but the system may also be activated by a lack of Na\(^+\) ions. Consistent with this interpretation is the finding that extracellular sodium ions competitively inhibit the water response (Nomura and Sakada, 1963; Nomura and Ishizaka, 1972; Kitada, 1991). Thus, the concentration–response curve shown in Fig. 3 B would be indicative of a gustatory system that is tuned to detect both increases and decreases in Na\(^+\) concentration from that found in the saliva. Whatever the relative role of sodium self-inhibition is in the transduction of sodium salts, it is clear that this regulatory mechanism will likely be important for understanding the gustatory processing of sodium salt taste.

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REFERENCES

Avenet, P. 1992. Role of amiloride-sensitive sodium channels in taste. In Sensory Transduction. D.P. Corey and S.D. Roper, editors. The Rockefeller University Press, New York. 271–280.

Avenet, P., and B. Lindemann. 1991. Noninvasive recording of receptor cell action potentials and sustained currents from single taste buds maintained in the tongue: the response to mucosal NaCl and amiloride. J. Membr. Biol. 105:245–255.

Benos, D.J., M.S. Asayda, B.K. Berdiev, A.L. Bradford, C.M. Fuller, O. Senyk, and I.I. Ismailov. 1996. Diversity and regulation of amiloride-sensitive Na\(^+\) channels. Kidney Int. 49:1632–1637.

Benos, D.J., C.M. Fuller, V.G. Shlyonsky, B.K. Berdiev, and I.I. Ismailov. 1997. Amiloride-sensitive Na\(^+\) channels: insights and outlooks. News Physiol. Sci. 12:55–61.

DeSimone, J.A., G.L. Heck, S. Mierson, and S.K. DeSimone. 1984. The active ion transport properties of canine lingual epithelia in vitro. Implications for gustatory transduction. J. Gen. Physiol. 83:635–656.

Doolin, R.E., and T.A. Gilbertson. 1996. Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. J. Gen. Physiol. 107:545–554.

Formaker, B.K., and D.L. Hill. 1991. Lack of amiloride sensitivity in SHR and WKY glossopharyngeal taste responses to NaCl. Physiol. Behav. 50:765–769.

Frank, M.E., R.J. Contreras, and T.P. Hettinger. 1983. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. J. Neurophysiol. 50:941–960.

Fuchs, W., E.H. Larsen, and B. Lindemann. 1977. Current–voltage curve of sodium channels and concentration dependence of sodium permeability on frog skin. J. Physiol. (Lond.). 267:137–166.

Garty, H., and D.J. Benos. 1988. Characteristics and regulatory mechanisms of the amiloride-blockable Na\(^+\) channel. Physiol. Rev. 68:309–373.

Garty, H., and L.G. Palmer. 1997. Epithelial sodium channels: function, structure and regulation. Physiol. Rev. 77:359–396.

Gilbertson, T.A. 1993. The physiology of vertebrate taste reception.

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Gilbertson, T.A. 1995. Patch-clamping of taste cells in the hamster and rat. In Experimental Cell Biology of Taste and Olfaction. A.I. Spielman and J.G. Brand, editors. CRC Press, Boca Raton, FL. 317–328.

Gilbertson, T.A., J.H.Y. Li, and B. Lindemann. 1993. Proton currents through amiloride-sensitive Na⁺ channels in isolated hamster taste cells: enhancement by vasopressin and cAMP. J. Gen. Physiol. 103:931–942.

Gilbertson, T.A., and S.C. Kinnamon. 1996. Making sense of chemicals. Chem. Biol. (Lond.). 3:233–237.

Gilbertson, T.A., and H. Zhang. 1996. Regulation of amiloride-sensitive sodium channels by extracellular sodium ions: sodium self-inhibition. Chem. Senses. 21:606. (Abstr.)

Gilbertson, T.A., and S.D. Roper. 1985. Adrenalectomy reduced peripheral neural responses to gustatory stimuli in the rat. Pflügers Arch. 391:85–100.

Heck, G.L., S. Mierson, and J.A. DeSimone. 1984. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science. 223:403–405.

Horn, R., and A. Marty. 1988. Muscarinic activation of ionic currents measured by a new whole-cell recording method. J. Gen. Physiol. 92:145–159.

Kinnamon, S.C., and R.F. Margolskee. 1996. Mechanisms of taste transduction. Curr. Opin. Neurobiol. 6:506–513.

Komwatana, P., A. Dinudom, J.A. Young, and D.I. Cook. 1996a. Control of the amiloride-sensitive Na⁺ current in salivary duct cells by extracellular sodium. J. Membr. Biol. 150:133–141.

Korn, S.J., J.A. Marty, A. Dinudom, J.A. Young, and D.I. Cook. 1996b. Cytosolic Na⁺ controls an epithelial Na⁺ channel via the GO guanine nucleotide-binding regulatory protein. Proc. Natl. Acad. Sci. USA. 93:8107–8111.

Korn, S.J., A. Marty, J.A. Connor, and R. Horn. 1991. Perforated patch recording. Methods Neurochem. 4:364–373.

Kroll, B., S. Bremer, B. Tümmler, G. Kottra, and E. Frömter. 1991. Sodium dependence of the epithelial sodium conductance expressed in Xenopus oocytes. Pflügers Arch. 419:101–107.

Li, J.H.Y., and B. Lindemann. 1985. Chemical stimulation of Na transport through amiloride-blockable channels of frog skin epithelium. J. Membr. Biol. 75:179–192.

Li, X.J., S. Blackshaw, and S.H. Snyder. 1994. Expression and localization of amiloride-sensitive sodium channels indicate a role for non-taste cells in taste perception. Proc. Natl. Acad. Sci. USA. 91:1814–1818.

Li, X.J., and S.H. Snyder. 1994. Heterologous expression of amiloride-sensitive sodium channel subunits and an alternatively spliced form in taste tissues. Soc. Neurosci. Abstr. 20:1472. (Abstr.)

Lindemann, B. 1984. Flowchart analysis of sodium channels in epithelia. Annu. Rev. Physiol. 46:497–515.

Lindemann, B. 1996. Taste Reception. Physiol. Rev. 76:719–766.

Ling, B.N., and D.C. Eaton. 1989. Effects of luminal Na⁺ on single Na⁺ channels in A6 cells, a regulatory role for protein kinase C. Am. J. Physiol. 256:F1094–F1103.

Luger, A., and K. Turnheim. 1981. Modification of cation permeability of rabbit descending colon by sulphhydryl reagents. J. Physiol. (Camb.). 317:49–66.

Matsuo, R., and T. Yamamoto. 1992. Effects of inorganic constituents of saliva on taste responses of the rat chorda tympani nerve. Brain Res. 583:71–80.

Miersson, S., M.M. Olson, and A.E. Tietz. 1996. Basolateral amiloride-sensitive Na⁺ transport pathway in rat tongue epithelium. J. Neurophysiol. 76:1297–1309.

Miersson, S., M.E. Welter, C. Gennings, and J.A. DeSimone. 1988. Lingual epithelium of spontaneously hypertensive rats has decreased short-circuit current in response to NaCl. Hypertension. 11:519–522.

Nakamura, K., and R. Norgren. 1991. Gustatory responses of neurons in the nucleus of the solitary tract of behaving rats. J. Neurophysiol. 66:1232–1248.

Nishio, H., and R. Norgren. 1990. Responses from parabrachial gustatory neurons in behaving rats. J. Neurophysiol. 63:707–724.

Nomura, H., and S. Sakada. 1965. On the “water response” of frog’s tongue. Jpn. J. Physiol. 15:433–443.

Okada, Y., T. Miyamoto, and T. Sato. 1990. Aldosterone increases gustatory neural response to NaCl in the frog. Comp. Biochem. Physiol. A. 97:535–536.

Okada, Y., T. Miyamoto, and T. Sato. 1991. Vasopressin increases frog gustatory neural responses elicited by NaCl and HCl. Comp. Biochem. Physiol. A. 100:693–696.

Okada, Y., R. Fujiyama, T. Miyamoto, and T. Sato. 1996. Vasopressin modulates membrane properties of taste cells isolated from bullfrogs. Chem. Senses. 21:739–745.

Rehagen, B.G., T.P. Hettinger, and M.E. Frank. 1992. Salivary ions and neural taste responses in the hamster. Chem. Senses. 17:179–190.

Sariban-Sohraby, S., and D.J. Benos. 1986. The amiloride-sensitive sodium channel. Am. J. Physiol. 250:C175–C190.

Simon, S.A., and J.L. Garvin. 1985. Salt and acid studies on canine lingual epithelium. Am. J. Physiol. 249:C308–C408.

Simon, S.A., V.F. Holland, D.J. Benos, and G.A. Zamphigi. 1993. Transcellular and paracellular pathways in lingual epithelia and their influence in taste transduction. Microsc. Res. Tech. 26:196–208.

Smith, D.V., J.W. Steadman, and C.N. Rhodine. 1975. An analysis of the time course of gustatory neural adaptation in the rat. Am. J. Physiol. 229:1134–1140.

Soeda, H., and F. Sakudo. 1988. Characteristics of the water response across the dorsal epithelium of frog tongue. Comp. Biochem. Physiol. A. 99:683–691.

Turnheim, K. 1991. Intrinsıc regulation of apical sodium entry in epithelia. Physiol. Rev. 71:429–445.

Van Driessche, W., and W. Zeiske. 1985. Ionic channels in epithelial cell membranes. Physiol. Rev. 65:833–903.

Wills, N.K., and A. Zwiefach. 1985. Recent advances in the characterization of epithelial ion channels. Biochem. Biophys. Acta. 906:1–31.

Ye, Q., G.L. Heck, and J.A. DeSimone. 1991. The anion paradox in sodium taste reception: resolution by voltage-clamp studies. Science. 254:724–726.

Zhang, H., D.M. Gilbertson, W.T. Monroe, and T.A. Gilbertson. 1996. Characterization of sodium transport in fungiform, foliate-and vallate-containing epithelia from hamster and rat. Chem. Senses. 21:693. (Abstr.)

Zotterman, Y. 1956. Species differences in the water taste. Acta Physiol. Scand. 37:60–70.