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

Timothy A. Gilbertson and Huai Zhang

From the Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70808-4124

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, \(\beta\)-hydroxymercuribenzoate (\(\beta\)-HMB; 200 \(\mu\)M), 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 \(\beta\)-HMB. In sodium transport studies in fungiform taste bud-containing lingual epithelia, \(~\)40% of the change in short-circuit current (I\(_S\)) after addition of 500 mM NaCl to the mucosal chamber is amiloride sensitive (0.5 mM). \(\beta\)-HMB significantly enhanced mucosal NaCl-induced changes in these epithelia at mucosal Na\(^+\) concentrations of 50 mM and above. In contrast, the vallate-containing epithelia, which are insensitive to amiloride, showed no enhancement of I\(_S\) during \(\beta\)-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)\(^1\) 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 inhabitable 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-

\(^1\)Abbreviations used in this paper: ASSC, amiloride-sensitive sodium channel; I\(_S\), short-circuit current; KH, Krebs-Heinsleit; \(\beta\)-HMB, \(\beta\)-hydroxymercuribenzoate; TRC, taste receptor cells.
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 95% O₂/5% CO₂ 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. 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 tetrathyldiammonium 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-Heineleit (KH) buffer contained (mM): 118 NaCl, 25 NaHCO₃, 1.3 NaH₂PO₄, 6 KCl, 1.2 MgSO₄, and 5.6 glucose. KH buffer was bubbled with 95% O₂/5% CO₂ 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 MΩ when filled with intracellular solution. Scal resistances were typically in the tens of GΩs. In the perforated patch configuration, mean input resistance was 5.87 ± 1.02 (SD) GΩ, 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 3–5 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 tetrodotoxin-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):

\[
I = \frac{(V - P_{Na} A F^2 / RT)}{1 - e^{-(V/F)/RT}} - (N_{out} - N_{in}^\dagger) e^{(V/F)/RT},
\]

(1)

where I is the current measured at potential V = −100 mV, P_Na is the Na⁺ permeability in centimeters per second, N_{out} and N_{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 μF/cm², F is the Faraday constant, R is the gas constant, and T is the absolute temperature. N_{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 were averaged and analyzed by one-way ANOVA for significant effects of Na_{out} 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 cm². 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-to-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_v in symmetrical KH buffer of 4.8 ± 0.7 μA/cm² and the transepithelial resistance of 1.377 ± 0.86 Ω·cm² (n = 19 epithelia). In contrast, val- late-containing epithelia had a significantly higher transepithelial resistance (2.075 ± 141.2 Ω·cm²; n = 22 epithelia) than the fungiform-containing epithelia. This resistance, coupled with a measured I_v of 3.6 ± 0.8 μA/cm², 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 ($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_v 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 mamalian 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. 2 A, changing from 35 to 70 mM Na$^+$ extracellularly caused a time-dependent decrease in conductance. This decrease in conductance reached a maximum $\sim$15 s after complete solution change in the recording chamber. After accounting for the inherent delay in our perfusion system ($\sim$4 s), we estimated the time constant for the development of self-inhibition for the decrease in current resulting from switching from 35 to 70 mM Na$^+$ extracellularly (Fig. 2 B). Our results show that the time constant is $\sim$10 s (Fig. 2 B; $n = 10$ 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. 3 A. 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. 3 A, in the majority of TRCs the magnitude of the currents in 140 mM Na$^+$ were greater than in 35 mM Na$^+$. Fig. 3 B shows the relation-

![Figure 1](https://jgp.rupress.org/files/1-11340 FIG1.jpg)

**Figure 1.** Inhibition of whole-cell amiloride-sensitive sodium channels by extracellular sodium. Steady state currents in an isolated fungiform taste cell held at $-80$ mV during bath perfusion of saline solutions containing varying NaCl concentrations or amiloride (10 $\mu$M). N-methyl-d-glucamine was substituted equimolar for Na$^+$ in all solutions. Increasing current is oriented downward. Dotted line displays resting current in saline containing 70 mM NaCl.

![Figure 2](https://jgp.rupress.org/files/1-11340 FIG2.jpg)

**Figure 2.** Time course of self-inhibition. (A) Currents elicited by voltage ramps from $-110$ to $+50$ mV in a fungiform TRC that had amiloride-sensitive Na$^+$ currents (data not shown). Numbers to the right of traces refer to time after change from 35 to 70 mM Na$^+$. Times shown are corrected for perfusion delay. (B) Relative inhibition of ASSCs in fungiform TRCs after the change from 35 to 70 mM Na$^+$. Values are mean $\pm$ SD for eight cells showing an inhibitory effect of extracellular Na$^+$. Curve is the best fit to the data with a logistic function. Time constant for development of self-inhibition is $\sim$10 s.
ship between extracellular Na\(^+\) and amiloride-sensitive Na\(^+\) current, normalized to the current in 140 mM Na\(^+\) at −80 mV. Though the current magnitude was typically larger in 140 mM Na\(^+\) than 35 mM Na\(^+\) (Fig. 3 B), the relative permeability of Na\(^+\) ions through ASSCs (P_{Na}) was significantly greater in 35 mM Na\(^+\) than in either 70 or 140 mM Na\(^+\), highlighting the presence of self-inhibition at these concentrations. At −100 mV, the mean P_{Na} ± SD (Eq. 1) in 35 mM Na\(^+\) was 7.59 ± 2.01 × 10^{-10} cm/s (n = 10); for 70 mM Na\(^+\), P_{Na} was 2.78 ± 1.00 × 10^{-10} cm/s (n = 10); and for 140 mM Na\(^+\), P_{Na} was 3.30 ± 1.85 × 10^{-10} cm/s (n = 10). One-way analysis of variance revealed a significant effect of Na\(^+\) concentration on Na\(^+\) permeability (F[2.27] = 18.3, P < 0.001). Subsequent analysis using Tukey’s HSD post-hoc test showed that P_{Na} in 35 mM Na\(^+\) was significantly higher than P_{Na} in 70 and 140 mM Na\(^+\) (P < 0.001).

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 μM) 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_{Na} (Eq. 1) as a function of extracellular Na\(^+\). At −100 mV, in 35 mM Na\(^+\), the mean P_{Na} (±SD) was 6.69 ± 2.54 × 10^{-10} cm/s; in 70 mM Na\(^+\), P_{Na} was 7.42 ± 2.78 × 10^{-10} cm/s; in 140 mM Na\(^+\), P_{Na} was 5.75 ± 2.61 × 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_{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 μM) 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 μM 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-β-S.
Self-Inhibition in Taste Cells

(0.5 mM) in the patch pipette. Self-inhibition was unchanged under this condition (n = 5 cells; Fig. 5).

Epithelial Transport Experiments

Numerous studies have shown that 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) tongue is carried, in part, by Na\(^+\) movement through apical ASSCs into TRCs, where it is subsequently pumped out via ouabain-sensitive Na\(^+\)/K\(^+\) pumps. To determine the effects of sodium self-inhibition recorded at the cellular level on Na\(^+\) transport, we performed experiments on rat lingual epithelia containing either fungiform or vallate taste buds. Stepwise increases in NaCl concentration from 10 to 500 mM in the mucosal chamber caused net increases in Isc 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 Na\(^+\), only NaCl-induced changes in Isc 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...
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. Solid lines are best fits to the data points with a Boltzmann function. Data points are presented mean ± SD and reflect recordings from 20 (fungiform) and 21 (vallate) epithelia each.

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 Isc 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
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.

**D I S C U S S I O N**

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 other 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\(^+\) 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 \(\beta\)-HMB to reduce or eliminate \(\text{Na}\(^+\)\)-induced changes in the permeability of ASSCs is reflective of self-inhibition. There are no reports of \(\alpha\)-HMB altering the permeability effects of cytosolic \(\text{Na}\(^+\)\) on ASSCs. Moreover, in the present study, intracellular \(\alpha\)-HMB was ineffective in inhibiting the \(\text{Na}\(^+\)\)-induced inhibition of ASSC currents, suggesting that the site affected by \(\beta\)-HMB was extracellular, as has been proposed for the self-inhibition site in epithelial \(\text{Na}\) 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 \(\text{Na}\(^+\)\) 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 \(\text{Na}\(^+\)\) ions to inhibit the \(\text{Na}\(^+\)\) permeability of TRC membranes is linked with the presence of ASSCs. TRCs that do not possess functional ASSCs do not show self-inhibition. Furthermore, \(\beta\)-HMB had no effect on isolated TRCs nor on epithelial \(\text{Na}\(^+\)\) transport in cells or tissues that did not show amiloride sensitivity. In epithelial transport experiments on fungiform taste bud–containing epithelia, both amiloride and \(\beta\)-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 \(\text{Na}\(^+\)\) transport is that amiloride-insensitive (A-I) \(\text{Na}\(^+\)\) transport pathways are apparently not regulated in the same manner by extracellular \(\text{Na}\(^+\)\) concentrations. These A-I pathways may contribute from 20–100\% of the total \(\text{Na}\(^+\)\) 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 \(\text{Na}\(^+\)\) 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 \(\text{Na}\(^+\)\) 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 \(\text{Na}\(^+\)\) transport is regulated by \(\text{Na}\(^+\)\) 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 \(\text{La}\(^3+\)\) 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 \(\text{Na}\(^+\)\) 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 Norgren, 1990; Nakamura and Norgren, 1991) all display adaptation to \(\text{NaCl}\) 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-Sohraby and Benos, 1986). Because the apical membranes of taste receptor cells are constantly bathed with saliva, which in rats contains as much as 60 mM Na+ (Rehnberg 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. 3B 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; Rehnberg 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 3B. 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 (Sakuda and Avenet, 1998; 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 Ishizaka, 1972; Kitada, 1991). Thus, the concentration–response curve shown in Fig. 3B 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.

The authors thank Donna Gilbertson for the statistical analyses and W. Todd Monroe for his expert technical assistance. This work was supported by research grant DC-02567 (T.A. Gilbertson) from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

Original version received 22 January 1998 and accepted version received 12 March 1998.

REFERENCES


Gilbertson, T.A. 1993. The physiology of vertebrate taste receptor.
Kosten, T., and R.J. Contreras. 1985. Adrenalectomy reduced pe-
Lindemann, B. 1984. Fluctuation analysis of sodium channels in 

Gilbertson, T.A. 1995. Patch-clamping of taste cells in the hamster and 
rat. In Experimental Cell Biology of Taste and Olfaction. A.I. Spiel-
ton currents through amiloride-sensitive Na+ channels in hamster 
rents through amiloride-sensitive Na+ channels in isolated ham-
ster taste cells: enhancement by vasopressin and cAMP. Neuron. 10:931–942.
Gilbertson, T.A., and H. Zhang. 1996. Regulation of amiloride-sen-
sitive sodium channels by extracellular sodium ions: sodium self-
Hamill, O.P., A. Marty, E. Neher, B. Sakmann, and F.J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution cur-
rent recording from cell and cell-free membrane patches. Pflügers Arch. 391:85–100.
Heck, G.L., S. Mierson, and J.A. DeSimone. 1984. Salt taste trans-
duction occurs through an amiloride-sensitive sodium transport 
Herness, M.S. 1992. Aldosterone increases the amiloride-sensitivity 
of the rat gustatory neural response to NaCl. Comp. Biochem. Phys-
Hille, B. 1992. Ionic Channels in Excitable Membranes. 2nd ed.
Horn, R., and A. Marty. 1988. Muscarinic activation of ionic cur-
rents measured by a new whole-cell recording method. J. Gen.
Physiol. 92:145–159.
Ca2+ in water fibers of the frog glossopharyngeal nerve. Chem.
Senses. 16:95–104.
Control of the amiloride-sensitive Na+ current in salivary duct 
Komwatana, P., A. Dinudom, J.A. Young, and D.I. Cook. 1996b. Cy-
tosolic Na+ controls an epithelial Na+ channel via the GO gua-
USA. 93:8107–8111.
Kosten, T., and R.J. Contreras. 1985. Adrenalectomy reduced pe-
nipple peripheral responses to gustatory stimuli in the rat. Behav.
Sodium dependence of the epithelial sodium conductance ex-
transport through amiloride-blockable channels of frog skin epi-
Li, X.J., S. Blackshaw, and S.H. Snyder. 1994. Expression and loca-
ization of amiloride-sensitive sodium channels indicate a role for 
non-taste cells in taste perception. Proc. Natl. Acad. Sci. USA. 91:
1814–1818.
amiloride-sensitive sodium channel subunits and an alterna-
Lindemann, B. 1984. Fluctuation analysis of sodium channels in 
Na+ channels in A6 cells, a regulatory role for protein kinase C. 
ability of rabbit descending colon by sulphhydryl reagents. J. Phys-
Matsuo, R., and T. Yamamoto. 1992. Effects of inorganic constitu-
ents of saliva on taste responses of the rat chorda tympani nerve. 
Miers, S., M.M. Olson, and A.E. Tietz. 1996. Basolateral 
amiloride-sensitive Na+ transport pathway in rat tongue epithe-
Lingual epithelium of spontaneously hypertensive rats has de-
creased short-circuit current in response to NaCl. Hypertension. 
rons in the nucleus of the solitary tract of behaving rats. J. Neuro-
physiol. 66:1232–1248.
Nishio, H., and R. Norgren. 1990. Responses from parabrachial 
Nomura, H., and M. Ishizaki. 1972. Stimulation mechanism of wa-
ter response in the frog: roles of anions in the activity of a 
Okada, Y., T. Miyamoto, and T. Sato. 1990. Aldosterone increases 
gustatory neural responses elicited by NaCl and HCl. Comp. 
Okada, Y., R. Fujiyama, T. Miyamoto, and T. Sato. 1996. Vasop-
pressin modulates membrane properties of taste cells isolated from 
Sariban-Sohraby, S., and D.J. Benos. 1986. The amiloride-sensitive 
Transcellular and paracellular pathways in lingual epithelia and 
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 re-
sponse across the dorsal epithelium of frog tongue. Comp. Bio-
Turnheim, K. 1991. Intrinsic regulation of apical sodium entry in 
Van Driessche, W., and W. Zeiske. 1985. Ionic channels in epithel-
Wills, N.K., and A. Zwiefach. 1987. Recent advances in the charac-
terization of epithelial ion channels. Biochim. Biophys. Acta. 906: 
1–31.
sodium taste reception: resolution by voltage-clamp studies. Sci-
cence. 254:724–726.
1996. Characterization of sodium transport in fungiform, foli-
ate and vallate-containing epithelia from hamster and rat. Chem. 
Senses. 21:693. (Abstr.)
Physiol. Scand. 37:60–70.