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Characterization of Sodium Transport in Gustatory Epithelia from the Hamster and Rat

Timothy A. Gilbertson and Huai Zhang

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808-4124, USA

Correspondence to be sent to: Dr Timothy A. Gilbertson, Chemical Senses Laboratory, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124, USA. e-mail: tim.gilbertson@tasteful.com

Abstract

The transduction of sodium salts occurs through a variety of mechanisms, including sodium influx through amiloride-sensitive sodium channels, anion-dependent sodium movement through intercellular junctions and unidentified amiloride-insensitive mechanisms. Characterizations of sodium transport in lingual epithelium mounted in Ussing chambers have focused almost exclusively on epithelia containing only fungiform taste buds. In the present study we have investigated sodium transport by measuring NaCl-induced short-circuit current from lingual epithelia containing fungiform, foliate, vallate and palatine taste buds in the hamster and the rat. All areas show measurable sodium transport, yet significant differences were noted between the epithelia from the rat and the hamster and among the different epithelia within a single species in terms of current density, transepithelial resistance and mucosal amiloride sensitivity. In general, epithelia from the anterior tongue were of a lower resistance and transported sodium more effectively than from the posterior tongue. Moreover, fungiform- and vallate-containing epithelia in the rat had a greater current density than did the corresponding tissues in the hamster. Amiloride sensitivity also differed between the rat and the hamster. In the hamster all gustatory areas showed some amiloride sensitivity, while in the rat the vallate-containing epithelia were devoid of amiloride-sensitive sodium transport. The results are consistent with the interpretation that all chemosensitive areas may participate in the detection of salts but the degree of salt transport and the mechanism of transport is variable among different lingual epithelia and different species.

Introduction

The transduction of sodium salts in the peripheral gustatory system has been suggested to involve at least two distinct pathways for sodium movement. The most well described mechanism involves the influx of Na^+ ions through apical, amiloride-sensitive sodium channels (ASSCs). The influx of Na^+ ions directly depolarizes the taste cell, eventually leading to the release of neurotransmitter onto gustatory afferent nerve fibers (for review see Gilbertson and Kinnamon, 1996; Kinnamon and Margolskee, 1996; Lindemann, 1996). Consistent with this mechanism are numerous reports showing that amiloride inhibits NaCl-induced taste responses at the level of the taste receptor cell (Avenet and Lindemann, 1988; Gilbertson *et al.*, 1993; Doolin and Gilbertson, 1996), taste bud (Avenet and Lindemann, 1991; Gilbertson *et al.*, 1992), lingual epithelium (Heck *et al.*, 1984; Simon and Garvin, 1985; Mierson *et al.*, 1996), afferent nerve fiber (Heck *et al.*, 1984; Brand *et al.*, 1985) and central gustatory neuron (Scott and Giza, 1990; Giza and Scott, 1991). In addition to showing the involvement of ASSCs in salt taste transduc- tion, these reports also demonstrated that this mechanism could not fully explain salt detection in the periphery.

In addition to the transcellular model described above, there has also been hypothesized a paracellular route for Na^+ movement in the tongue (Ye *et al.*, 1991). That is, Na^+ ions, in an anion-dependent fashion, permeate the tight junctions between taste receptor cells where they may enter the cell via basolateral ASSCs (Simon *et al.*, 1993; Mierson *et al.*, 1996) or other unidentified submucosal sodium transport systems (for review see DeSimone and Heck, 1993). Thus, the picture that is emerging is one in which the whole lingual tissue may be viewed as a taste organ involving both the taste receptor cells and the non-taste epithelium in parallel.

The vast majority of the information about these salt-transducing pathways at the cellular level has come from experiments focused on fungiform taste buds or on the fungiform-containing lingual epithelium. In particular, the experiments that have detailed the biology of the lingual epithelium using Ussing chambers have recorded exclusively from the epithelium on the anterior tongue. Though afferent nerve recordings and patch-clamp studies have shown that the posterior tongue containing the foliate and vallate taste buds (Hanamori *et al.*, 1988; Formaker and Hill, 1991;

Doolin and Gilbertson, 1996), as well as taste buds in the soft palate (Harada *et al.*, 1991; Harada and Smith, 1992; Harada, 1994), transduces Na^+ ions, there have been no comparative studies of sodium transport in these areas. Clearly, understanding sodium transport in all areas of the oral cavity, not just the anterior tongue, would be prerequisite to any theory of peripheral salt taste transduction.

In the present study we have used isolated, voltage-clamped epithelia in an Ussing chamber to investigate the sodium transport properties of gustatory epithelia containing the fungiform, foliate, vallate and palatine taste buds in the rat and the hamster, two species about which a great deal of gustatory physiology is known. The results support the interpretation that all areas of the oral cavity are capable of significant sodium transport that, in turn, may contribute to sodium salt transduction. In general, the anterior tongue exhibits greater Na^+ transport than the posterior tongue. In recordings from the fungiform and vallate epithelia, rat epithelia had a greater sodium transport capacity than the comparable tissue in hamsters. Moreover, all areas of the hamster oral cavity contained apical ASSCs, while these channels were absent in rat vallate epithelia, consistent with a recent patch-clamp study (Doolin and Gilbertson, 1996). There was no evidence of basolateral ASSCs in rat epithelium, in contrast to a recent report (Mierson *et al.*, 1996). The tight junction inhibitor $LaCl_3$ reduced sodium transport in all epithelia in both species. Thus, there appears to be both tissue and species differences in the salt transport pathways in the rat and the hamster. Some of these results have appeared in abstract form (Zhang *et al.*, 1996).

Materials and methods

Tissue preparation and solutions

Lingual epithelia

Epithelia containing the fungiform, foliate and vallate papillae were isolated from 2- to 4-month-old male Sprague–Dawley rats and male Golden Syrian hamsters using methods described previously (Gilbertson, 1995; Doolin and Gilbertson 1996). Briefly, tongues were cut posterior to the vallate papilla and immersed in ice-cold Tyrode solution. Tongues were injected beneath the lingual epithelia with ~2–3 ml of Tyrode containing 3 mg/ml dispase (type II, Boehringer Mannheim Corp., Indianapolis, IN) and 1 mg/ml trypsin inhibitor (type I-S, Sigma Chemical Co., St Louis, MO). Amiloride was included in all dissociation solutions to protect against enzymatic degradation of ASSCs (Garty and Edelman, 1983; Gilbertson *et al.*, 1993). Injected tongues were incubated for 30 min in Tyrode solution that was bubbled continuously with O_2. Following the incubation period, the lingual epithelia containing the fungiform, foliate and vallate taste buds were removed from the underlying muscle layer and placed in a 35 mm Petri dish containing Tyrode. Tissues isolated in this manner were viable for at least 10 h and showed no electrophysiological decrement. Following isolation, the vallate-containing epithelia also contained intact von Ebner ducts, 1–2 mm in length, which may be expected to contribute significantly to Na^+ transport measurements. However, our methods of placing the serosal surface on a nylon support under slight positive pressure (see below) caused these ducts to be flattened against the support. The high resistance of the vallate epithelia (Table 1) argues to the efficacy of this technique. In most cases, we recorded from two of the four tissue types from a single animal in each experiment. Typically, an experiment consisted of recording from a fungiform-containing epithelium and one of the other three types of epithelia, which accounts for the comparatively higher number of fungiform-containing epithelia analyzed in the present study.

Palatine epithelia

For isolation of the soft palate, the jaw and cheek were cut and an incision was made with a scapel in the epithelium between the hard and soft palates. The sublingual frenulum and the underside of the tongue were cut until the epiglottis was visible. An incision was made through the epiglottis and continued through the muscle layer underlying the soft palate. Once the original incision between the hard and soft palates was reached, the tongue, a portion of the epiglottis and the soft palate could be removed. Similar to the isolation of the lingual epithelia, the palate was injected with enzyme solution between the muscle and epithelial layers. Other procedures were identical to those described above.

Modified Krebs–Heinseleit (KH) buffer contained (in mM): NaCl, 118; $NaHCO_3$, 25; NaH_2PO_4, 1.3; KCl, 6; $CaCl_2$, 2; $MgSO_4$, 1.2; and glucose, 5.6. 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. Amiloride and $LaCl_3$ were added to either the NaCl-containing solutions (for mucosal application) or to KH buffer (serosal applications). All chemicals were obtained from Sigma Chemical Co. unless indicted otherwise.

Transepithelial current recording

Isolated epithelia containing either the vallate, foliate, palatine or fungiform taste buds were mounted in a bi-partitioned Ussing chamber (World Precision Instruments, Sarosota, 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 (pore size ~300 μm, Small Parts, Miami Lakes, FL) covering the opening to the serosal chamber to prevent damage to the tissue. The two sides of the chamber were lightly coated with vacuum grease and the chamber was assembled and filled with

Table 1 Electrophysiological properties of gustatory epithelia in the rat and the hamster

		Fungiform	Foliate	Vallate	Palate
Resistance ($\Omega \cdot cm^2$)	Rat	1155 ± 58 [41][b]	1590 ± 133 [17]	1320 ± 49 [18]	1479 ± 102 [9]
	Hamster	1859 ± 159 [44]	2036 ± 179 [10]	2040 ± 130 [21]	1580 ± 175 [8]
I_{SC} ($\mu A/cm^2$)	Rat	5.4 ± 0.8	4.8 ± 1.0	6.2 ± 0.6	4.7 ± 0.9
	Hamster	4.1 ± 0.9	4.4 ± 0.6	4.0 ± 0.7	4.3 ± 0.8
Potential difference[a] (mV)	Rat	6.1 ± 1.0	7.7 ± 1.4	8.2 ± 0.8	7.0 ± 1.2
	Hamster	7.2 ± 1.2	8.8 ± 0.7	8.2 ± 0.7	6.8 ± 1.1

Measurements of resistance and short-circuit current (I_{SC}) were made in symmetrical KH buffer. Values are given as mean ± SEM.

[a]Potential difference was calculated from resistance and I_{SC} by Ohm's Law.

[b]Numbers in brackets in the top rows refers to the number of epithelia and are applicable to all values listed.

KH buffer and allowed to equilibrate for 30 min. Solutions were maintained at 34°C in the Ussing chamber 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). This arrangement allowed solution change without disturbing 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 (personal observation). The mucosal solution was perfused at a rate roughly 1.5× that of the serosal solution. This kept the tissue flat against the nylon support membrane and prevented tissue movement and potential edge damage.

Transepithelial currents were recorded by a dual voltage clamp (model DVC-1000; WPI) 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 for prior to the introduction of an epithelium with symmetrical KH buffer. Short-circuit current (I_{SC}) was recorded on a two-channel PCM recorder (model 200; A.R. Vetter, Rebersburg, PA) at a frequency of 44.1 kHz and displayed on a strip chart recorder (model RS3200, Gould, Valley View, OH). Transepithelial resistance was monitored by –20 mV pulses delivered by a stimulator (model S-900; Dagan, 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 apex to the basolateral side of the tissue.

Results

In the present study we recorded transepithelial ionic currents from >130 epithelia containing fungiform, foliate, vallate or palatine taste buds. These experiments represent the first report of transepithelial current flow in areas containing taste buds other than those from the fungiform papillae. To be included in the subsequent analysis, epithelia had to demonstrate stable properties over the entire time course of the experiment. Epithelia that had a >10% change in I_{SC} induced by mucosal 500 mM NaCl measured at the beginning and end of an experiment were excluded.

Basic characteristics of rat and hamster gustatory epithelia

The basic electrical properties of the gustatory epithelia were measured when the various epithelia were bathed with KH buffer in both the mucosal and the serosal chambers and measured with an open circuit (i.e. no voltage clamp). Transepithelial resistance, measured in each of the epithelia by –20 mV voltage steps, and the open circuit current were used to calculate the open circuit potential difference by Ohm's Law. Table 1 lists the electrophysiological parameters for each of the four types of gustatory epithelia in both the hamster and the rat. In general, there was a trend indicating that the anterior (e.g. fungiform-containing) epithelia had a lower resistance than the more posterior epithelia. Epithelia from the rat were also of typically lower resistance than in the hamster. The epithelia with the greatest resistance were the hamster vallate and foliate, which had transepithelial resistance >2000 $\Omega \cdot cm^2$. One-way analysis of variance (ANOVA) revealed a main effect of tissue type on transepithelial resistance [$F(7,160) = 6.0$, $P < 0.001$]. Though there were significant differences in the resistance among the various types of epithelia between rat and hamster determined by Dunnett's T3 post-hoc test for unequal variance (see below; Table 2), there were no significant differences within each species. There were no significant differences in either I_{SC} or potential difference within tissue types or between species. Moreover, these values reflect that the tissues from each of these four areas were viable and point to the applicability of using these techniques to study gustatory epithelia in addition to those from the anterior tongue.

Concentration-dependence of NaCl-induced changes in I_{SC}

Increases in mucosal NaCl concentrations (10–500 mM)

Table 2 Statistical comparison among the various epithelia in terms of magnitude of I_{SC} induced by 500 mM NaCl in the mucosal chamber (top right) and the transepithelial resistance measured in symmetrical KH buffer (bottom left)

Transepithelial resistance	Transepithelial current (500 mM NaCl mucosal)							
	Rat fungiform	Rat foliate	Rat vallate	Rat palate	Hamster fungiform	Hamster foliate	Hamster vallate	Hamster palate
Rat fungiform		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**
Rat foliate	0.146		0.996	0.997	**0.009**	0.411	**0.039**	0.894
Rat vallate	0.587	0.771		1.000	0.088	0.153	**0.013**	0.996
Rat palate	0.333	1.000	0.978		**0.016**	0.058	**0.002**	0.980
Hamster fungiform	**<0.001**	0.996	0.058	0.748		**<0.001**	**<0.001**	0.699
Hamster foliate	**0.015**	0.714	**0.050**	0.305	1.000		0.938	0.187
Hamster vallate	**<0.001**	0.445	**0.001**	0.068	1.000	1.000		0.078
Hamster palate	0.527	1.000	0.950	1.000	0.977	0.818	0.638	

Values represent the level of significance determined by Dunnett's T3 post-hoc test for unequal variance following a one-way ANOVA. Significant differences are denoted in bold at a level of $P < 0.05$. See text for additional details.

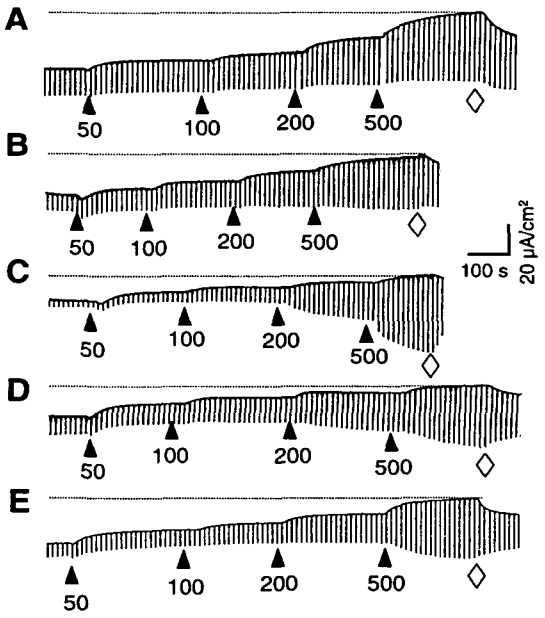

Figure 1 Short-circuit currents induced by stepwise increases in mucosal NaCl concentration (in mM) in rat gustatory epithelia containing fungiform **(A)**, foliate **(B, C)**, vallate **(D)** or palatine **(E)** taste buds. Foliate-containing epithelia apparently fall into two groups: those which are similar to fungiform (type I, B) and those which are more similar to vallate (type II, C) based upon electrophysiological and kinetic analyses (see text). Initial mucosal solution was 10 mM NaCl and KH buffer was in the serosal chamber. Increases in I_{SC} are reflected upward and transient deflections are current responses to brief 20 mV hyperpolarizations of the epithelia to monitor transepithelial resistance. ◇, NaCl solutions were replaced with mucosal KH buffer.

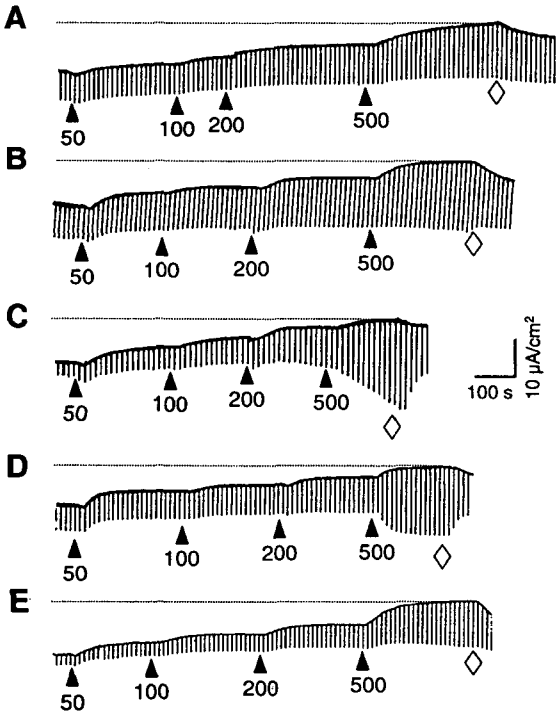

Figure 2 Short-circuit currents induced by stepwise increases in mucosal NaCl concentration (in mM) in hamster gustatory epithelia containing fungiform **(A)**, foliate **(B, C)**, vallate **(D)** or palatine **(E)** taste buds. Other details are identical to those given in Figure 1.

caused corresponding increases in I_{SC} in all epithelia tested in the present study. Concomitant with the increase in I_{SC} was a decrease in transepithelial resistance in both rat (Figure 1) and hamster (Figure 2) epithelia. In each of the four types of epithelia, the NaCl-induced change in I_{SC} was inhibited by serosal application of ouabain (1 mM) for 20–30 min (data not shown), suggesting that there are common mechanisms for sodium transport in all epithelia.

Among the various types of epithelia there were differences both in the magnitude of current change (see below) and in the kinetics of the resistance change caused by increasing mucosal NaCl. In all fungiform- and most palatine-containing epithelia, the change in resistance closely paralleled the change in I_{SC} during NaCl stimulation

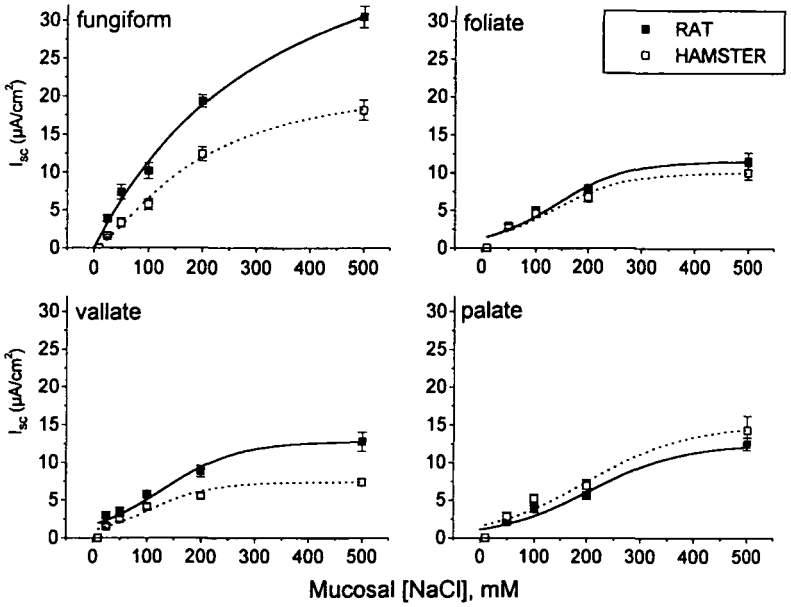

Figure 3 Comparison of steady-state sodium transport in the gustatory epithelia of the hamster and rat. Values of I_{SC} are shown ± SEM and each point represents 8–44 individual epithelia.

(Figures 1A and 2A,E). However, the vallate-containing epithelia exhibited more progressive, time-dependent changes in resistance despite little continued change in I_{SC} in both species (Figures 1D and 2D). Though the underlying cause of this difference was not determined, these respective kinetic profiles are readily defining characteristics of these epithelia. Interestingly, the foliate-containing epithelia typically fall into one of two categories. They resemble those from either the fungiform (type I; Figures 1B and 2B) or the vallate epithelia (type II; Figures 1C and 2C) in terms of the time course of the resistance change. Roughly half of the foliate-containing epithelia in the rat and the hamster were classified as type I (9 /17 and 4/10 respectively). Though this distinction was clear by examining the current records, we have no information regarding the physiological basis, if any, for these kinetic distinctions. Thus, we have pooled the data from each of the two subtypes of foliate epithelia in subsequent analyses, except where noted otherwise.

The concentration–response curves for NaCl-induced changes in I_{SC} showed saturation at concentrations >200 mM NaCl (Figure 3). In all epithelia, except those from the soft palate, there was a trend indicating that sodium transport was greater in the rat than in the hamster. One-way ANOVA revealed significant differences between I_{SC} with mucosal 500 mM NaCl measured in the different tissues [$F(7,160) = 32.6$, $P < 0.001$]. Since Levine's test for equality of variances showed significant differences ($P < 0.05$), Dunnett's T3 post-hoc test for unequal variance was used. Table 2 shows the results of post-hoc analysis using Dunnett's T3 test comparing the 500 mM NaCl-induced changes in I_{SC} in the four types of epithelia in the rat and the hamster. This analysis revealed that sodium transport was greater in rat fungiform-containing epithelia than in all other epithelia in both species. Within rats, there was no significant difference among the other three types of epithelia; the foliate, vallate and palate all had similar magnitudes of I_{SC} in 500 mM NaCl. Within the hamster epithelia, the fungiform-containing epithelia had significantly greater sodium transport than the vallate ($P < 0.001$) and foliate ($P < 0.001$). Comparing between species revealed significantly greater sodium transport in the rat fungiform- ($P < 0.001$) and vallate-containing ($P = 0.013$) epithelia than in the corresponding tissues in the hamster. All the tissues in the rat had significantly greater sodium transport than the hamster vallate epithelia (Table 2). Clearly, the magnitude and kinetics of sodium transport in gustatory epithelia are dependent upon the type of tissue and show marked species differences.

Mechanisms of NaCl-induced changes in I_{SC}

One of the most well described mechanisms for transepithelial movement of sodium ions in anterior (i.e. fungiform-containing) taste tissue is via Na^+ influx through apical ASSCs and subsequent pumping out through basolateral ATP-dependent Na^+/K^+ pumps (DeSimone *et al.*, 1981; Simon and Garvin, 1985; Mierson *et al.*, 1996). Consistent with this pathway, we have found that ouabain inhibits NaCl-induced I_{SC} in all types of gustatory epithelia (see above). To determine if apical ASSCs were contributing to the sodium transport in all gustatory epithelia, we measured I_{SC} during stimulation with mucosal NaCl in the absence and presence of 0.5 mM amiloride. As shown in Figure 4A–D, application of amiloride in the mucosal chamber reduced 500 mM NaCl-induced I_{SC} in all epithelia

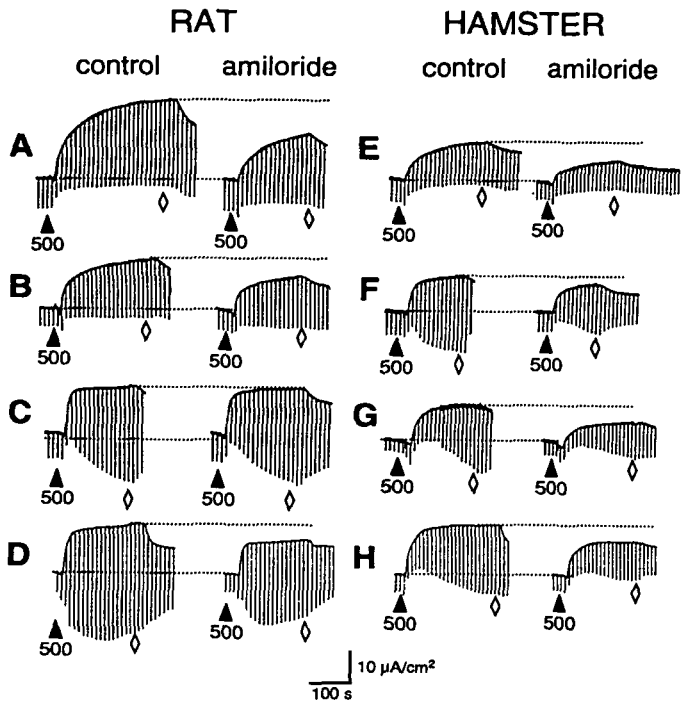

Figure 4 Effect of mucosal amiloride on I_{SC} induced by 500 mM NaCl. Data are shown as the change in I_{SC} following replacement of 10 mM NaCl in the mucosal chamber with 500 mM NaCl in the absence or presence of 0.5 mM amiloride. Serosal solution contained KH buffer. Dotted lines show the difference in I_{SC} from the baseline to the peak of the response to 500 mM NaCl. Representative examples are shown for each of the four gustatory epithelia in the rat and the hamster respectively: fungiform **(A, E)**; foliate **(B, F)**; vallate **(C, G)** and palate **(D, H)**. Epithelia A and C were from the same rat. ◇, NaCl solutions were replaced with mucosal KH buffer. All the effects of amiloride were reversible within 20 min of washing with symmetrical KH buffer.

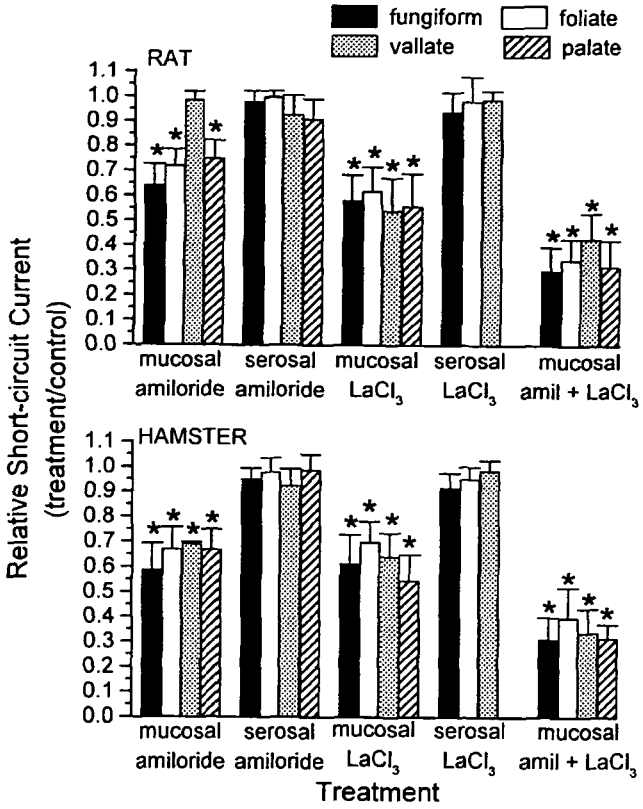

Figure 5 Effects of amiloride and the tight junction blocker, $LaCl_3$, are limited to the apical membrane in the gustatory epithelia of rat and hamster. Relative steady-state I_{SC} (treatment/control) ± SEM recorded in 500 mM NaCl during application of amiloride (0.5 mM) or $LaCl_3$ (6 mM) in the mucosal or serosal chamber. For mucosal applications, amiloride and $LaCl_3$ were added to 500 mM NaCl; for serosal applications they were added to KH buffer. Each bar represents 5–20 epithelia (n = 3 for rat taste bud-free epithelia). Asterisks denote significant reductions in I_{SC} compared with control records ($P < 0.05$, Student's t-test). No TBs, taste bud-free epithelia.

from the rat except those containing the vallate taste buds. This is consistent with patch-clamp recordings from isolated taste buds that have shown that ASSCs are present in fungiform, foliate (Doolin and Gilbertson, 1996) and palatine TRCs (Zhang *et al.*, 1997), but not vallate TRCs (Doolin and Gilbertson, 1996). In contrast, I_{SC} in all four gustatory epithelia in the hamster were sensitive to mucosal amiloride (Figure 4E–G). Preliminary patch-clamp experiments have demonstrated that functional ASSCs are present in all four types of taste buds in the hamster (Gilbertson *et al.*, 1997), consistent with these findings. Thus, there is a good correlation between mucosal amiloride-sensitive sodium transport and the presence of ASSCs revealed by patch-clamp recording. Amiloride inhibited only a portion (~25–40%) of the I_{SC} in any of the affected epithelia and, with the exception of the rat vallate-containing epithelia, there were no significant differences in the sensitivity to amiloride among the tissue types examined. All the effects of mucosal amiloride in the present study were reversible following a return to amiloride-free solutions for ~15–20 min.

A recent report has demonstrated that in Wistar rats there appear to be ASSCs located on both the apical and basolateral membranes of fungiform-containing taste tissue (Mierson *et al.*, 1996). Though the role of basolateral ASSCs in taste transduction is unclear, we tested the sensitivity of NaCl-induced I_{SC} to applications of amiloride (0.5 mM) in KH buffer in the serosal chamber in a manner similar to that described above. However, for applications as long as 30 min, serosal amiloride had no significant effect upon I_{SC} in any of the epithelia in either species (Figure 5).

To determine the relative contribution of paracellular movement of NaCl to I_{SC} (Ye *et al.*, 1991; DeSimone and Heck, 1993; Simon *et al.*, 1993), we examined effects of the tight junction inhibitor $LaCl_3$ (Simon *et al.*, 1993) on Na^+ transport. In all types of epithelia in hamster and rat, addition of 6 mM $LaCl_3$ reduced I_{SC} by ~40% compared with control levels (Figure 5). The effects of mucosal $LaCl_3$ could not be attributed to other reported actions of $LaCl_3$ on transport proteins, notably voltage-activated Ca^{2+} (Gelli and Blumwald, 1997) and K^+ channels (Tytgat and

Daenens, 1997). Mucosal applications of the Ca^{2+} channel antagonist nifedipine (25 μM) and the K^+ channel blocker TEA (20 mM) did not significantly affect 0.5 M NaCl-induced changes in I_{SC} (n = 5 epithelia; data not shown).

There were no significant differences either among the types of epithelia or between species, suggesting that this paracellular pathway contributes to all areas in a quantitatively similar manner. $LaCl_3$-mediated inhibition of I_{SC} was completely reversible following rinsing with KH buffer mucosally for 20–30 min. $LaCl_3$ was effective only from the apical side of the epithelia. In fungiform-, foliate- and vallate-containing epithelia, serosal application of 6 mM $LaCl_3$ in KH buffer had no significant effect upon the change in I_{SC} elicited by 500 mM NaCl in the mucosal chamber (Figure 5). Palatine-containing epithelia were not tested with serosal $LaCl_3$ in these experiments. Addition of both amiloride (0.5 M) and $LaCl_3$ (6 mM) to the mucosal chamber could not completely inhibit NaCl-induced changes in I_{SC} (Figure 5).

Amiloride sensitivity of KCl-induced changes in I_{SC}

In order to try to understand more about the differences between amiloride-sensitive transport pathways in the anterior and posterior tongue, we performed several experiments that were designed to determine if KCl-induced changes in I_{SC} were sensitive to mucosal amiloride. Addition of 500 mM KCl to the mucosal chamber leads to an increase in I_{SC} in all three types of lingual epithelia in rat and hamster (Figure 6). In contrast to sodium transport, there was no significant difference between the fungiform-, foliate- and vallate-containing epithelia in terms of the magnitude of I_{SC} induced by 500 mM KCl. In rat, mucosal amiloride (0.5 mM) had no effect upon KCl-induced changes in I_{SC} in any of the three lingual epithelia. In hamster, however, amiloride inhibited >30% of the KCl-induced change in I_{SC} in the vallate-containing epithelia (Figure 6D,E), while having no effect in the fungiform-containing epithelia (Figure 6C). Thus, it would appear that potassium transport might be mediated, in part, via amiloride-sensitive pathways. When taken together, there was no significant effect of amiloride on I_{SC} in the hamster foliate epithelia. However, when the foliate epithelia were broadly classified into type I or type II on the basis of the response to 500 mM NaCl (cf. Figures 1 and 2) and re-analyzed, it became apparent that potassium transport in type II (vallate-like) foliate-containing epithelia was amiloride-sensitive (Figure 5C) while in type I it was not. Clearly, the elucidation of the mechanism of this amiloride-sensitive potassium transport will require the use of more direct electrophysiological methods, such as patch-clamp recording.

Discussion

There is a significant amount of information on the sodium

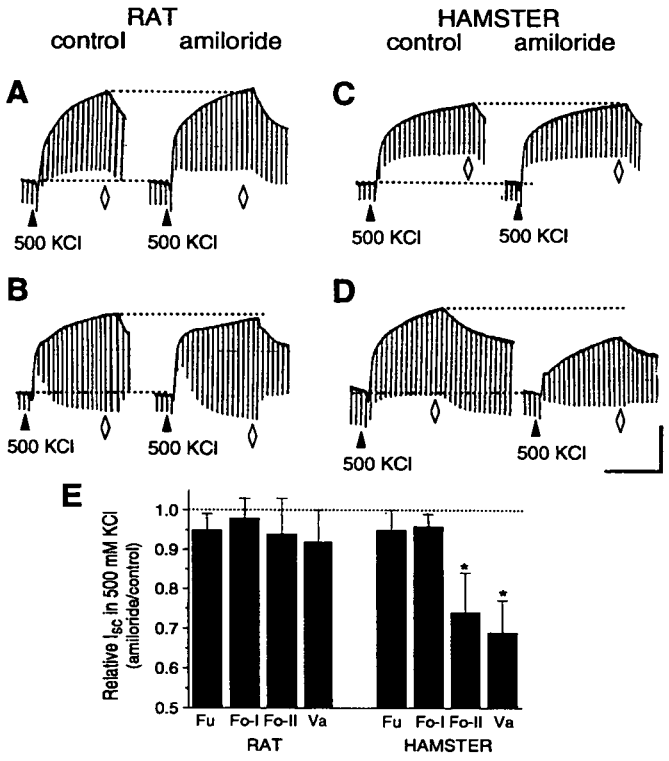

Figure 6 Effects of amiloride on KCl-induced changes in I_{SC}. Representative current records for epithelia from rat and hamster, respectively, in the fungiform- **(A, C)** and vallate-containing **(B, D)** epithelia. Amiloride (0.5 mM) was applied in the mucosal chamber. ◇, KCl solutions were replaced with mucosal KH buffer. Scale bars, 10 μA/cm² and 200 s. **(E)** Inhibition of KCl-induced I_{SC} by amiloride in the various lingual epithelia is limited to a subset of hamster epithelia. Data are shown mean ± SEM and represent 5–8 epithelia per point. Asterisks denote a significant effect of amiloride on I_{SC} compared with control (P < 0.05, Student's t-test).

transport properties in the lingual epithelium from the anterior tongue. These experiments have demonstrated that the basic mechanism for NaCl-induced changes in I_{SC} in lingual tissue involves both transcellular and paracellular routes for sodium movement which are coupled to an ouabain-sensitive Na^+/K^+ ATPase transporter. There is good evidence for sodium responsiveness in the glossopharyngeal (Hanamori *et al.*, 1988; Formaker and Hill, 1991) and greater superficial petrosal nerves (Harada *et al.*, 1991; Harada and Smith, 1992; Harada, 1994), which innervate the vallate and posterior foliate taste buds and the palatine taste buds respectively. Surprisingly, however, there has been no investigation of the sodium transport properties of the posterior lingual epithelia containing the vallate and foliate taste buds or of the epithelium containing taste buds from the soft palate. In the present study we have compared the basic properties of the lingual epithelia containing either fungiform, foliate, vallate or palatine taste buds and shown that there are significant differences between them in terms of transepithelial resistance, the

ability to transport sodium and the mechanism of NaCl-induced I_{SC}.

Furthermore, in these studies we have compared sodium transport in two species that have been the focus of considerable gustatory research, the rat and the hamster. Though the rat has been extensively studied using Ussing chambers to elucidate sodium transport pathways (Mierson *et al.*, 1988, 1996), there is no similar analysis of the hamster gustatory epithelia. This is despite the fact that the disparate behavioral responses in the rat and the hamster to NaCl (Gilbertson and Gilbertson, 1994) suggest there may be underlying mechanistic differences between the physiology of salt-transducing pathways in these two species. We have compared the physiology of the four gustatory epithelia in the hamster and the rat and have shown that there are, in some types of epithelia, differences in the sodium transport pathways between the species. In general, hamster epithelia were of a higher resistance and transported less sodium than rat epithelia. Moreover, the presence of apparent apical ASSCs in hamster vallate epithelia, which are absent in the rat, point to a fundamental difference in the salt-transducing pathways. These physiological differences may be important for understanding the physiological basis for the differences in the behavioral responsiveness these animals have to NaCl.

Comparison of sodium transport among gustatory epithelia

Within each species there were consistent and significant differences in the transport of sodium ions from the mucosal to the serosal chamber. This variability could not be attributed directly to differences between the basic electrical properties of the epithelia. The transepithelial resistance, open circuit I_{SC} and potential difference (Table 1) of the fungiform, foliate, vallate and palatine epithelia were not significantly different from one another.

In the rat, the fungiform-containing epithelia had significantly greater sodium transport, determined by I_{SC} in 500 mM NaCl, than the other three taste bud-containing epithelia (Figures 1 and 3, Table 2). This difference is intriguing given the fact that the anterior tongue is significantly more responsive to NaCl than the posterior (i.e. glossopharyngeal-innervated) tongue (Travers *et al.*, 1987; Spector and Grill, 1992). This may be reflective of the potential importance in the basic properties of the lingual epithelia in the detection and transduction of sodium salts in the mammalian tongue. A similar finding was detected in the hamster, which had significantly greater sodium transport in the fungiform than in the vallate and foliate epithelia (Table 2). The presence of significant NaCl-induced I_{SC} in the palates of the hamster and the rat is likely a contributing mechanism which may allow the animals to respond to NaCl even following bilateral transection of both the chorda tympani and glossopharyngeal nerves (Sollars and Bernstein, 1994).

In both species there was a consistent difference in the kinetics of the NaCl-induced changes in I_{SC} between the fungiform and vallate-containing epithelia. This was most evident when comparing the NaCl response to hyperosmotic NaCl stimuli (200 and 500 mM NaCl; Figures 1, 2 and 4). In fungiform-containing epithelia the change in I_{SC} and transepithelial resistance closely paralleled one another. In the vallate-containing epithelia, however, there was a progressive decrease in resistance despite no continued change in I_{SC}. The physiological significance of this difference, if any, is not clear, though it might be expected to contribute to the time course of NaCl responses. Several possible causes for this difference may be in the anatomical differences between the fungiform- and vallate-containing epithelia, differences in the sodium transport pathways themselves or differences in the surrounding epithelial (non-taste) cells.

Though the apical membranes of fungiform taste buds lie on the surface of the gustatory epithelia and those from vallate taste buds lie in deep crypts, we find it unlikely that this alone accounts for the kinetic differences seen. If this were the case, one would anticipate that the expected perfusion delay in the vallate epithelia would affect both I_{SC} and resistance, not resistance alone. Moreover, the finding that the foliate-containing epithelia, which anatomically closely resemble the vallate-containing epithelia, fall into two groups would argue against this point. Second, this kinetic difference between fungiform and vallate epithelia is not likely due to the amiloride- or $LaCl_3$-sensitive transport pathways. This difference was maintained even in the presence of maximal concentrations of mucosal amiloride and $LaCl_3$. Though we cannot rule out that these kinetic differences were due to the unidentified amiloride- and $LaCl_3$-insensitive transport mechanisms, the fact that this pathway occurs in both the fungiform and vallate epithelia makes this explanation speculative at best. It remains possible, as well, that the microenvironment around the taste buds is different in these two areas. To determine the source and role of the kinetic differences between the fungiform (and type I foliate) and vallate (and type II foliate) epithelia was beyond the scope of the present study. Additional experiments will be required to investigate in greater detail the three (or more) sodium transport pathways in gustatory epithelia.

Based upon the results in the present study there appears to at least three distinct mechanisms underlying the NaCl-induced changes in I_{SC}. The ability of amiloride in the mucosal chamber to inhibit sodium transport is consistent with the contribution of sodium entry through apical ASSCs in this process. Inhibition of I_{SC} by $LaCl_3$, which blocks ion permeation through intercellular tight junctions (Simon *et al.*, 1993), is reflective of the contribution of paracellular sodium movement to sodium transport. There also appears to be a third, though unidentified, contributor to sodium transport. Amiloride- and $LaCl_3$-sensitive mechanisms cannot fully explain all sodium transport and when

added together do not block all changes in I_{SC} caused by mucosal NaCl (Figure 5). Thus, there is another mechanism that is apparently independent of ASSCs and $LaCl_3$-sensitive transport that contributes to sodium salt detection in the oral cavity. This mechanism may be especially important for sodium transduction in the rat vallate epithelia, which, while $LaCl_3$-sensitive, apparently lack apical ASSCs. All of these mechanisms, however, rely upon active sodium extrusion from the taste tissue as the Na^+/K^+ pump inhibitor ouabain completely blocks NaCl-induced changes in I_{SC}. Another implication from the data showing complete amiloride-insensitivity in the rat vallate-containing epithelia is that the non-taste cells (epithelial cells) themselves do not contain apical or basolateral ASSCs (data not shown). Thus, if these cells contribute to the NaCl-induced changes in I_{SC}, they do so independently of ASSC activity.

With the exception of the rat vallate-containing epithelia, there is a remarkable consistency in the relative contributions of these different sodium pathways to the total I_{SC}. All the other epithelia examined were sensitive to mucosal amiloride and $LaCl_3$, and in roughly equivalent amounts. Serosal applications of these compounds had no significant effect upon sodium transport, suggesting that the sites of action of both amiloride and $LaCl_3$ are limited to the mucosal (e.g. apical) surface of the epithelia. The finding that there was no basolateral amiloride sensitivity in either the rat or the hamster in any of the different epithelia is in contrast to a recent report which showed that serosal amiloride could completely inhibit NaCl-induced changes in I_{SC} in anterior, fungiform-containing epithelia from Wistar rats (Mierson *et al.*, 1996). In this report, the amiloride sensitivity on the basolateral membrane was approximately two orders of magnitude less sensitive than functional ASSCs in the apical membrane of the intact tongue (Heck *et al.*, 1984; Brand *et al.*, 1985; Avenet and Lindemann, 1991; Gilbertson *et al.*, 1992) and in isolated taste receptor cells (Gilbertson *et al.*, 1993; Doolin and Gilbertson, 1996). Moreover, it was not clear from this study how basolateral ASSCs could completely inhibit sodium transport, including that presumably generated by sodium entry through apical ASSCs. The finding in the present study of a segregation of ASSCs apically and Na^+/K^+ pumps basolaterally is consistent with the vast majority of other sodium-transporting epithelia. Though the reason for this discrepancy is not clear at present, methodological or species differences could potentially be responsible. Whatever the reason, there have been no reports at the cellular level of another class of functional ASSCs in taste tissue with a reduced amiloride sensitivity (higher inhibition constant). Thus, if these basolateral channels exist in the two species in the present study they must be especially labile. Clearly, to rectify this apparent controversy may require innovative approaches since the presence of ASSC mRNA or protein by *in situ* hybridization and immunocytochemistry respectively (Simon *et al.*, 1993; Li and Snyder, 1994; Li *et al.*, 1994) is not necessarily correlated with the presence of functional amiloride-sensitive sodium channels (Doolin and Gilbertson, 1996).

Comparison of sodium transport between rat and hamster

Though rat lingual epithelia have been studied extensively, there is little or no information on the sodium transport properties of epithelia from the hamster, a species whose gustatory system otherwise has been extensively investigated. Given that hamsters find sodium salts aversive (Hettinger and Frank, 1990; Gilbertson and Gilbertson, 1994) and rats find the same compound appetitive (Hill *et al.*, 1990, Breslin *et al.*, 1993), one might speculate that there were underlying physiological differences in the salt-transducing pathways of these two species.

We have described the basic properties of four of the gustatory epithelia containing taste buds in the male Golden Syrian hamster. By combining these data with data from rat epithelia in the same study, we are able to compare directly the properties of sodium transport in these two species. As in the rat, sodium transport in the hamster seems to involve both transcellular, via apical ASSCs, and paracellular transport pathways, as well as an unidentified amiloride- and $LaCl_3$-insensitive mechanism. These mechanisms are all coupled to the action of a basolateral Na^+/K^+ pump since ouabain blocks all NaCl-induced changes in I_{SC} in both the hamster and the rat.

The most significant differences between the rat and the hamster were found by comparing the properties of the fungiform and vallate epithelia. In both these epithelia, the tissues from the hamster were of a significantly higher resistance and transported sodium less effectively than those from the rat. If, as we proposed above, the increased resistance of the lingual epithelia would tend to favor the transcellular over the paracellular route for sodium movement, then these transcellular pathways, presumably via apical ASSCs, should be relatively more important in the hamster than in the rat. In other words, in the rat, with its less resistive epithelia, the contribution of the paracellular pathway to sodium transduction may be significantly greater, reducing the relative importance of the transcellular pathway. One might speculate, then, that in the hamster an individual taste bud might play a greater role in taste transduction than in the rat. It is intriguing, given this hypothesis concerning the relationship between transepithelial resistance and relative contribution of transcellular to paracellular pathways in taste transduction, that hamsters have roughly half the number of taste buds of rats (Miller, 1977; Miller and Smith, 1985).

Another significant difference between the rat and the hamster lies in the distribution of ASSCs in the various taste bud-containing epithelia. In the rat, mucosal amiloride was able to inhibit, to a degree, sodium transport in the fungiform, foliate and palatine, but not the vallate, epithelia

(Figures 4 and 5). These findings are consistent with the reported distribution of ASSCs in isolated taste cells from these areas using patch-clamp recording (Doolin and Gilbertson, 1996; Zhang *et al.*, 1997). In contrast, all four hamster epithelia showed amiloride-sensitive sodium transport (Figures 4 and 5). In a preliminary report, functional ASSCs have been shown to be present in isolated taste receptor cells from all these areas in the hamster as well, using patch-clamp recording (Gilbertson *et al.*, 1997). Given that hamsters find NaCl to be aversive (Hettinger and Frank, 1990; Gilbertson and Gilbertson, 1994) and that the glossopharyngeal nerve is generally believed to be more responsive to aversive stimuli than the chorda tympani (Harada and Smith, 1992; Ninomiya *et al.*, 1994), the finding that these channels are in the vallate-containing taste tissue may be important for understanding the physiology underlying the disparate behavioral responses of rats and hamsters to NaCl.

The amiloride-sensitive sodium transport pathways in the hamster vallate and type II (vallate-like) foliate epithelia may be unlike that found in the rat and other areas of the hamster oral cavity. KCl-induced changes in I_{SC} in these two epithelia were sensitive to mucosal amiloride (Figure 6). The obvious implication from this is that ASSCs found in these areas have a significant potassium permeability, unlike ASSCs from other gustatory areas (for review see Lindemann, 1996). ASSCs with high potassium permeability have been reported in frog taste cells (Avenet and Lindemann, 1988). Moreover, the relative potassium (to sodium) permeability of ASSCs in renal cells, which are similar to the ASSCs found in mammalian taste receptor cells, is greatly enhanced by phosphorylation and ADP ribosylation (Benos *et al.*, 1997). It is consistent, then, that such a channel is found in the hamster vallate taste receptor cells. The elucidation of the source of the amiloride-sensitive KCl response will require more direct experiments at the cellular level.

In summary, we have shown that one can apply the technique of transepithelial current recording using Ussing chambers to a variety of gustatory epithelia. This approach has allowed us to compare the different taste bud-containing tissues in the oral cavity of rats and hamsters to gain the first insights in the physiology of sodium transport in gustatory areas other than the anterior (i.e. fungiform-containing) tongue. Clearly understanding the contribution of both transcellular and paracellular sodium transport in these areas in both of these two species will be important in elucidating the mechanisms of sodium salt transduction in the peripheral gustatory system and their role in the behavioral response to salts.

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