The Active Ion Transport Properties of Canine Lingual Epithelia In Vitro

Implications for Gustatory Transduction

JOHN A. DESIMONE, GERARD L. HECK, SHEELLA MIERSON, and SHIRLEY K. DESIMONE

From the Department of Physiology and Biophysics, Virginia Commonwealth University, Medical College of Virginia, Richmond, Virginia 23298

ABSTRACT The electrophysiological properties of the dorsal and ventral canine lingual epithelium are studied in vitro. The dorsal epithelium contains a special ion transport system activated by mucosal solutions hyperosmotic in NaCl or LiCl. Hyperosmotic KCl is significantly less effective as an activator of this system. The lingual frenulum does not contain the transport system. In the dorsal surface it is characterized by a rapid increase in inward current and can be quantitated as a second component in the time course of either the open-circuit potential or short-circuit current when the mucosal solution is hyperosmotic in NaCl or LiCl. The increased inward current (hyperosmotic response) can be eliminated by amiloride (10^{-4} M). The specific location of this transport system in the dorsal surface and the fact that it operates over the concentration range characteristic of mammalian salt taste suggests a possible link to gustatory transduction. This possibility is tested by recording neural responses in the rat to NaCl and KCl over a concentration range including the hyperosmotic. We demonstrate that amiloride specifically blocks the response to NaCl over the hyperosmotic range while affecting the KCl response significantly less. The results suggest that gustatory transduction for NaCl is mediated by Na entry into the taste cells via the same amiloride-sensitive pathway responsible for the hyperosmotic response in vitro. Further studies of the in vitro system give evidence for paracellular as well as transcellular current paths. The transmural current-voltage relations are linear under both symmetrical and asymmetrical conditions. After ouabain treatment under symmetrical conditions, the short-circuit current decays to zero. The increase in resistance, though significant, is small, which suggests a sizeable shunt pathway for current. Flux measurements show that sodium is absorbed under symmetrical conditions. Mucosal solutions hyperosmotic in various sugars also induce an amiloride-sensitive inward current. In summary, this work provides evidence that the sodium taste receptor is most probably a sodium transport system, specifically adapted to the dorsal surface of the tongue. The transport paradigm of gustation also suggests a simple model for electric taste and possible mechanisms for sweet taste.

Address reprint requests to Dr. John A. DeSimone, Dept. of Physiology and Biophysics, Medical College of Virginia, P.O. Box 551, MCV Station, Richmond, VA 23298.

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INTRODUCTION

Although the dorsal surface of the tongue has long been recognized as containing the major peripheral sensory apparatus for gustation in mammals, little was known of its transepithelial properties until recently. These would seem to be of some importance because two of the major classes of gustatory stimuli, salts and acids, are ionic, and under a variety of physiological conditions, they regularly contact the lingual surface over a wide range of concentrations. The prevailing view has been that the lingual epithelium, and by extension the taste bud cells, are impermeable toward most substances, including small ions (Beidler, 1967; Mistretta, 1971; Kamo et al., 1974; DeSimone and Heck, 1980; Sato et al., 1982). This view has largely dominated thinking on peripheral gustatory mechanisms. The surface potential theory of Kamo et al. (1974) was based on the assumption that the lingual epithelium is ion impermeable and attempted to rationalize this assumption using Millipore membranes impregnated with large quantities of phospholipids. Other mechanisms have invoked mechanochemical changes caused by both stoichiometric and nonstoichiometric interactions of the ionic tastant with the apical processes of taste bud cells (Beidler and Gross, 1971; DeSimone and Heck, 1980). Mistretta's work (1971) is often cited as supporting the assumption of ion impermeability (cf. Kurihara, 1974; Sato et al., 1982). However, this work on passive nonelectrolyte transport across rat lingual epithelia does not directly confront the question of ion transport. While it is possible to conceive of chemical gustatory stimulation without ion penetration, the same cannot be said for gustatory stimulation by means of a direct current (Bujas et al., 1979). Such electric taste experiments in fact imply the existence of transepithelial ion pathways and their involvement in the gustatory mechanism.

Indirect evidence of active sodium absorption by a mammalian tongue was first obtained by Hallbäck et al. (1979), although Hayashi (1978) had already reported that gustation in the frog was significantly influenced by potassium penetration from the oral cavity. Hallbäck et al. (1979) demonstrated an osmotic gradient increasing from the base toward the tip of feline fungiform papillae. Maintenance of the gradient appeared to depend on the presence of sodium in the isotonic bathing medium and a countercurrent exchanger in the papillary vasculature similar to those of the intestinal villus and renal medulla. The first direct evidence of active ion transport in the lingual epithelium was obtained by us (DeSimone et al., 1981) using canine tongues. With the in vitro Ussing preparation (Ussing and Zerahn, 1951), we showed that both the dorsal and ventral lingual surfaces are engaged in active ion transport. With Krebs-Henseleit buffer placed symmetrically across the tissue, ouabain caused the short-circuit current to decay to zero. In addition, the dorsal surface gave an unusual response to hyperosmotic NaCl placed in the mucosal bath. Both the short-circuit current and open-circuit potential increase nonlinearly over the concentration range of interest from the standpoint of mammalian gustation. As a function of NaCl concentration, the response describes a sigmoidal saturating curve. The integrated chorda tympani response of the rat is also sigmoidal and saturating as a function of the stimulating NaCl concentration as shown herein and elsewhere.

The saturating character of the neural response was first noted by Beidler (1954). From these observations emerged the adsorption theory of transduction. Later, Mooser (1981) reported sigmoidal behavior from which he formulated an allosteric model of transduction. We now present results which indicate that the characteristic form of the neural response function is closely connected with a special ion transport system activated under conditions of mucosal hypertonicity and critical in the early transduction events in NaCl taste.

The hyperosmotic response of the dorsal surface can be eliminated by ouabain. It is not observed in the ventral surface. It also differs from the hyperosmotic effect reported in gallbladder (Bindslev et al., 1974; Reuss and Finn, 1977) in that the transepithelial potential changes in the opposite direction. Exposure of the lingual epithelium to NaCl or KCl concentrations as high as 1 M produces no irreversible changes in tissue function. This is in marked contrast to degenerative changes observed in frog skin (Motokawa, 1935) or toad urinary bladder (DiBona and Civan, 1973) under conditions of mucosal hypertonicity.

Earlier we observed that amiloride significantly reduced the short-circuit current across the dorsal surface under symmetrical conditions of Krebs-Henseleit buffer (DeSimone et al., 1981) and we subsequently proposed that amiloride-sensitive ion pathways in cell membranes making contact with the oral cavity might be important mediators of gustation (DeSimone et al., 1982). Evidence in support of this hypothesis was obtained by Schiffman et al. (1983), who showed that perception of saltiness in humans is reduced for NaCl and LiCl but not for KCl after amiloride treatment of the tongue. They also showed that amiloride applied to rat tongues selectively reduced the response of single units in the nucleus tractus solitarius to NaCl without affecting the response to KCl.

In this paper we show that the large inward currents observed in vitro when hyperosmotic NaCl solutions are placed in contact with the mucosal lingual surface are eliminated by amiloride. We further show that amiloride significantly reduces the rat chorda tympani response over the same hyperosmotic range of NaCl concentrations. Moreover, we show that in the in vitro system the inward current caused by KCl is significantly less affected by amiloride treatment. This is also reflected in the neural recordings where the KCl response after amiloride is much closer to control values than that of NaCl.

In addition to investigating the effects of hyperosmotic salt solutions on the inward current in vitro, we show that D-glucose can also induce an inward amiloride-sensitive current when placed in the mucosal reservoir. However, unlike the salt-induced response, the resistance remains essentially constant as a function of the stimulus concentration.

MATERIALS AND METHODS

In Vitro Preparation

DISSECTION Dogs were anesthetized with sodium pentobarbital and killed by exsanguination. The tongue was then removed caudal to the circumvallate region, preserving as much of the lingual frenulum as possible. The excised tissue was washed in cold (5°C) Krebs-Henseleit buffer (K-H) consisting of 118 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.2

mM $MgSO_4$, 1.3 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 5.6 mM glucose. The tongue was then placed dorsal surface down on a hard rubber dissecting board previously rinsed in K-H buffer. It was pinned along its periphery and the frenulum was extended with forceps and separated from the adherent connective tissue with a scalpel. After sufficient area had been exposed, the double-layered frenulum was itself further dissected by peeling back the inner layer of tissue, leaving the single layer that contacts the oral cavity as one surface and the side normally in contact with extracellular fluid as the opposing surface. To obtain the dorsal epithelium, most of the striated muscle was grossly dissected away. The remaining fibers were removed by cutting through the connective tissue that anchors the muscle to the epithelium. The most effective technique involved freeing a section of muscle at the posterior end of the tongue by cutting transversely across the muscle-epithelium interface using round-nosed scissors. Once started, continued transverse cuts resulted in a peeling of the muscle layer from posterior to anterior. The freed epithelium with an adherent layer of connective tissue was then washed in cold K-H buffer. In a few cases the remaining layer of connective tissue was removed using collagenase. This did not change the steady state properties of the tissue.

POTENTIAL, CURRENT, AND RESISTANCE A medial-to-anterior section of dorsal epithelium was placed between two Lucite chambers each with a 7-ml volume. The tissue was held between silicone rubber gaskets. Sufficient axial pressure was applied to the chamber halves with a turn-screw brace in order to just achieve a leak-proof seal. The exposed geometrical area of 1.77 cm^2 was used to calculate current densities.

Each chamber contained a well housing a magnetic, Teflon-coated spin-fin which provided vigorous stirring. The temperature was maintained at 34°C by an electric heating pad placed under the chambers. The temperature was monitored by a sensor submerged in the solution bathing the interior surface of the epithelium. Power was fed to the pad through a variable-duty cycle controller which held the temperature to within ±0.5°C of the set point. Oxygenation was maintained by bubbling a 95% O_2, 5% CO_2 gas mixture into each chamber using hypodermic needles to direct the gas stream toward the tissue. In K-H buffer, this produced a pH of 7.4. Alkali metal chloride solutions were unbuffered.

Potential differences were measured across the tissue using conventional agar/0.15 M NaCl salt bridges in conjunction with a Keithley (Cleveland, OH) 610C electrometer (input resistance $> 10^{14}$ Ω). Potentials obtained with agar/1.5 M NaCl bridges were not significantly different. To estimate the magnitude of the junction potential error at the bridge-solution interfaces under asymmetrical conditions, a few experiments were done with agar-saturated KCl bridges. Both KCl and NaCl bridges produced the same qualitative features of the transepithelial potential or short-circuit current time course when the mucosal NaCl concentration was varied between 0.01 and 1 M. With adequate stirring, both types of bridges gave stable potentials. Below 0.15 M NaCl, NaCl bridges gave potentials 2–3 mV more negative than KCl bridges, except at 0.01 M, where the difference was 7.5 mV. Above 0.15 M NaCl, both types of bridges gave results that did not differ significantly. To minimize further possible bridge artifacts, comparisons were made between two states of the same system as in the ouabain pre- and post-treated dorsal surface, or between two different tissues under the same transepithelial concentration differences in the case of the dorsal surface and the frenulum. Potentials were recorded on a Gould/Brush (Cleveland, OH) model 105 strip chart recorder. Current was passed across the tissue via sintered Ag/AgCl electrodes. Resistances were measured by ramping a current across the tissue and recording the resulting variation in transepithelial potential. Ramp times were typically <10 s. Potential-measuring salt bridge electrodes were always situated 2 or 3 mm from the tissue to minimize the contribution of the solution resistance to the transepithelial resistance. Under symmetrical conditions in K-H buffer this intro-

duces a correction of <10 Ω cm^2. A Hewlett-Packard (Palo Alto, CA) 7044A *X-Y* plotter was used to obtain the resistance with transepithelial potential as the ordinate and a potential proportional to the applied current as the abscissa. *I-V* curves were linear over the usual range of interest, i.e., between −60 and +60 mV of transepithelial potential. The negative of the intersection of *I-V* curve with the current axis was taken as the short-circuit current. This term is used for both symmetrical and asymmetrical solution configurations.

All experiments were begun with a symmetrical K-H configuration. When an asymmetrical configuration was subsequently used, the interior solution was always K-H buffer. As a test of tissue viability, the epithelium was restored to symmetrical conditions periodically during the course of an experiment. Symmetrical control parameters (potential, current, and resistance) could invariably be re-established following exposure of the mucosal surface to NaCl, KCl, or LiCl for several minutes over the concentration range of 1 mM to 1 M. In a few cases, mannitol was added to the hyposmotic NaCl solutions to make them isosmotic with the interior K-H medium. This produced negligible effects on the electrical parameters including the symmetrical controls.

In Vivo Preparation

For the neurophysiological investigation, adult female Sprague-Dawley rats were anesthetized with sodium pentobarbitol. The heads were mounted in a non-traumatic headholder and the anterior two-thirds of the tongue was drawn into a flow chamber, isolating it from the oral cavity by a rubber dam. The chorda tympani nerve was exposed with conventional microsurgical techniques and hung over a platinum hook electrode. Electrical signals from this electrode were amplified, half-wave rectified, and integrated with a time constant of 5 s. The result of this integration was recorded on a strip chart recorder and serves as a measure of the neural response.

The tongue was stimulated by injecting 5-ml aliquots of solution into the flow chamber at a rate of 1 ml/s and through a cluster of seven hypodermic needles (0.37 mm ID). These needles were closely packed, six about one, and oriented perpendicular to the surface of the tongue. Excess stimulus was free to drain from the tongue, but the tongue was not rinsed for at least 60 s.

Ascending concentration series of NaCl and KCl were applied to the tongue to characterize the responsiveness of the preparation and to serve as controls against which the effects of amiloride could be evaluated. Each stimulus in the series was followed by a water rinse.

The tongue was treated with 10^{-4} M amiloride (in water) by flowing it over the tongue as a normal stimulus, but with the drain of the flow chamber blocked. The tongue was thus totally immersed for the 5-min treatment period. The chamber was then emptied and the tongue was thoroughly rinsed.

The effect of the amiloride treatment was assessed by re-stimulating with the three highest concentrations of NaCl and KCl.

Chemicals and Drugs

All chemicals were reagent grade. Ouabain octahydrate (0-3125) was obtained from Sigma Chemical Co. (St. Louis, MO) and amiloride hydrochloride was the gift of Dr. E. G. Cragoe of Merck, Sharp and Dohme, West Point, PA.

RESULTS

Symmetrical Conditions

The canine dorsal lingual epithelium satisfies all the thermodynamic require-

ments for active ion transport (Essig and Caplan, 1968). Under symmetrical conditions a steady open-circuit potential, V_{oc}, develops. Mean values (± SEM, n = 20) in K-H buffer for the potential, short-circuit current, I_{sc}, and the resistance, R, are V_{oc} = 18.1 ± 0.7 mV, I_{sc} = 36.1 ± 1.9 μA/cm^2, and R = 517 ± 29 Ω cm^2. The mucosal reservoir is taken as reference and the positive direction of current flow is from mucosa toward the interior. The potential and short-circuit current are oxygen dependent, decaying ~80% in 30 min after N_2 replaces O_2. Approximately 80% of the control potential can be restored in 12 min. Fig. 1 shows a typical I-V relation illustrating its linearity. Preliminary flux experiments using ^{22}Na have demonstrated active Na^+ transport in the symmetrical configuration (K-H) under voltage clamp conditions. For matched pairs of tissues, with an average value of I_{sc} of 1.32 ± 0.12 μeq/cm^2 h, the net sodium absorption was 0.80 ± 0.22 μeg/cm^2 h (n = 6). Sodium absorption accounted for 59 ± 13% of the short-circuit current. The remaining current carriers in the symmetrical configuration and the distribution of the current under hyperosmotic conditions

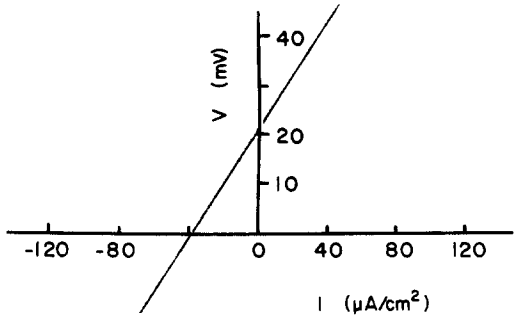

FIGURE 1. A typical current-voltage relation for the canine dorsal lingual epithelium in symmetrical Krebs-Henseleit medium. I-V curves are linear in general. This particular preparation gives: V_{oc} = 20.2 mV, I_{sc} = 36.7 μA/cm^2, R = 550 Ω cm^2.

are areas under investigation and will be treated in a subsequent paper, along with details of the flux experiments.

All of the short-circuit current, recorded under symmetrical conditions, can be eliminated by ouabain. Fig. 2 shows a typical time course for both the decline in I_{sc} and the increase in R regularly observed under ouabain. In this experiment the interior bathing medium was replaced by K-H buffer containing 9 × 10^{-4} M ouabain at time zero. The decline in current commences immediately and is approximately exponential with a half-time of ~15 min. The increase in resistance, although usually <20%, is statistically significant (cf. Fig. 7) and is complete within the first current half-time.

If the mucosal compartment is replaced with K-H buffer containing 10^{-4} M amiloride, there is an immediate decline in I_{sc}. However, the final percent reduction of the current is only ~50% on the average, as shown in Table I. The reduction in mean current is accompanied by a minor increase in resistance. The time course of the current decline is exponential with a half-time of 2–3 min.

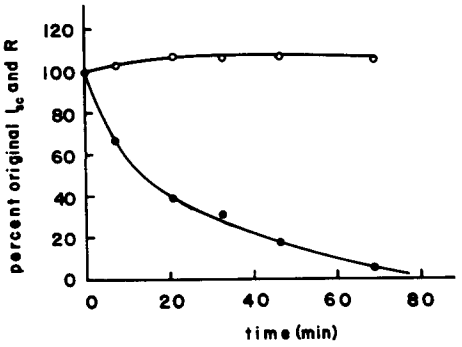

FIGURE 2. The percentage decline in short-circuit current (●) following the introduction of 9×10^{-4} M ouabain into the inside compartment. In most preparations the short-circuit current declines to zero in 60–70 min. Ouabain in the mucosal compartment is ineffective. The percentage increase in resistance is illustrated by the other curve (○).

Thus, unlike ouabain, amiloride does not eliminate all the short-circuit current under isosmotic conditons.

Asymmetrical Conditions

Unlike the epithelia of the lower GI tract, the lingual epithelium experiences wide variations in the ionic strength, pH, osmotic pressure, and temperature under physiological conditions. We have accordingly studied its electrophysiological response to NaCl over a series of concentrations and have found that the dorsal epithelium displays unusual properties in the hyperosmotic range. Fig. 3 shows the time course of the potential with changes in mucosal NaCl. Similar changes are noted in the short-circuit current if the experiment is done under voltage clamp. Four concentrations are displayed (0.15, 0.25, 0.75, and 1.00 M), each separated by a 30 mM NaCl wash. Fig. 3 shows that increasing the mucosal NaCl concentration always causes a positive potential deflection. Such changes include the hyperosmotic range and are reversible. Increases in potential caused by hyperosmotic NaCl are unusual for most transporting epithelia. The frog skin, for example, shows a rapid and largely irreversible decline in potential with

TABLE I

Effect of Amiloride (10^{-4} M) Under Symmetrical Conditions

Experiment number	Initial I_{sc}	Final I_{sc}	Percent reduction in I_{sc}	Percent increase resistance
	$\mu A/cm^2$	$\mu A/cm^2$		
1	34.6	16.2	53	12
2	24.0	15.5	35	−1
3	43.8	15.5	65	10
4	35.3	12.7	64	13
5	33.2	19.1	42	4
Mean ± SD			52 ± 13	8 ± 6

hypertonicity (Motokawa, 1935). There are several characteristic phases in the potential profile, depending on mucosal NaCl concentration. With 0.03 M NaCl as reference concentration, increasing NaCl up to 0.15 M results in a rapid rise in potential followed by a steady state. At 0.25 M a new characteristic is observed, i.e., an inflection point in the profile. Beyond 0.25 M the time course develops

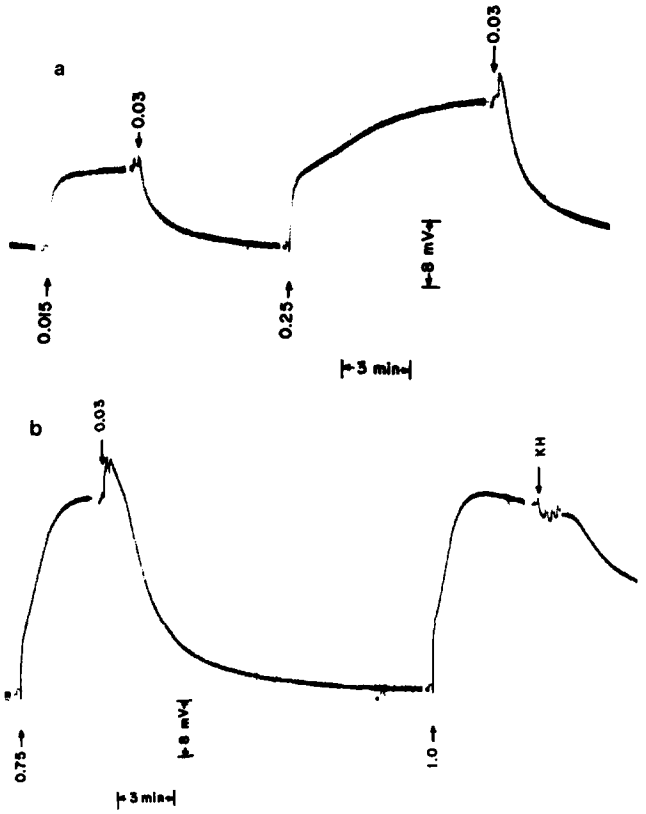

FIGURE 3. (*a*) The time course of the potential on changing the mucosal NaCl concentration from 0.03 to 0.15 M and then back to 0.03 M. At NaCl concentrations below 0.15 M the potentials rises smoothly to a new steady state. At hyperosmotic NaCl concentrations a second potential component appears as is seen when 0.25 M NaCl is added. (*b*) The second component is fully developed following an inflection point. This particular preparation also shows a rapid increase in potential when 0.03 M NaCl replaces a more concentrated solution.

as: (*a*) an initial rising phase, (*b*) an inflection point, (*c*) a second rising phase, and (*d*) either an asymptotic approach to a steady state or an overshoot and decline toward a steady state. In many (but not all) preparations a dilution response is observed. This is a rapid positive deflection in potential when 0.03 M NaCl follows a more concentrated solution. Note that when the wash solution was K-H buffer following 1 M NaCl the dilution response was not observed. (In those

tissues that show this behavior, it can be recorded with either NaCl or KCl salt bridges.) The dilution response may be due to different relaxation rates in the tissue resistance and current. When 0.03 M NaCl replaces a more concentrated solution, R must rise and I must fall. If the former process is faster, the potential will transiently rise before relaxing.

The increase in potential (and current) produced by hyperosmotic NaCl is essentially abolished by ouabain. Fig. 4 shows a typical set of *I-V* relations for the dorsal surface. The set was obtained starting with a symmetrical configuration

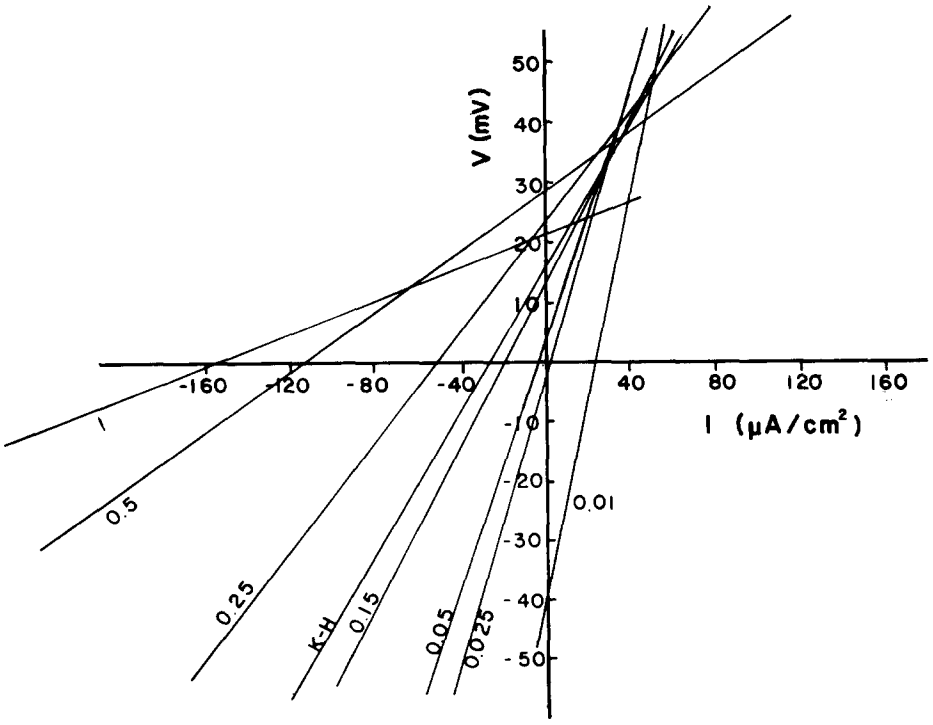

FIGURE 4. The typical set of *I-V* relations for the dorsal lingual surface parameterized by the indicated concentration of NaCl in the mucosal bath. The series was begun under symmetrical conditions (K-H). The mucosal bath was then changed in turn from 0.01 to 1 M. At a given concentration the potential was allowed to reach a steady state before obtaining the *I-V* relation.

(cf. curve K-H, Fig. 4). Each remaining curve was obtained by replacing the mucosal solution with the indicated concentration of NaCl, beginning with the most dilute and proceeding to the most concentrated. Each *I-V* curve was obtained after the open-circuit potential reached a steady state. Typically the resistance decreases with increasing NaCl concentration. After oubain has eliminated the short-circuit current under symmetrical conditions, the system no longer produces a hyperosmotic response of increased potentials and currents. The resistance, however, changes in the same manner with NaCl concentration. Fig. 5

shows the *I-V* relations for the same tissue after ouabain treatment. As expected, the curve for 0.15 M NaCl goes through the origin. However, the response in the hyperosmotic range is attenuated relative to the control curves. This is more clearly seen in Fig. 6, where we compare the mean short-circuit current as a function of the NaCl concentration for both control and ouabain-treated tissues. In the hyperosmotic range there is roughly a 90% reduction in the short-circuit current. The remaining curve in Fig. 6 is the typical response of the lingual frenulum. Like the dorsal surface it exhibits a short-circuit current under

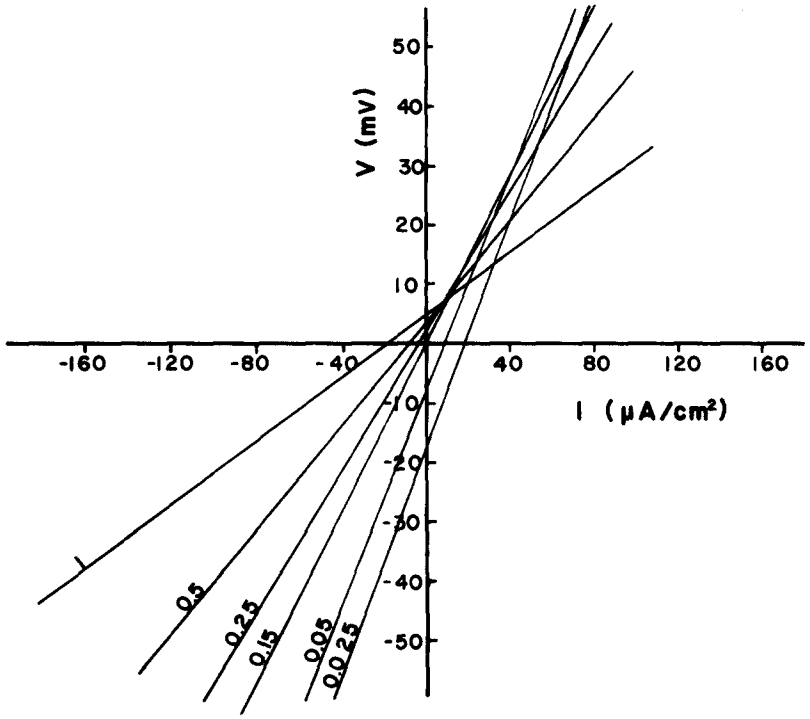

FIGURE 5. *I-V* relations under the same conditions as Fig. 4 except that the short-circuit current was allowed to decay to zero with ouabain in the inside medium under symmetrical conditions before changing the mucosal medium. Note the compression of the curves along the current axis.

symmetrical conditions. However, it lacks the hyperosmotic response observed in the dorsal surface. The sigmoidal character of the curve for the dorsal surface is clearly the result of a current-producing process or pathway which is ouabain sensitive, available only under hyperosmotic conditions, and evidently characteristic of the dorsal lingual surface alone.

Fig. 7 compares the resistances of the dorsal surface as a function of NaCl concentration for control and ouabain-treated tissues. Resistances after ouabain tend to be higher and, at 0.15 M NaCl and higher, the differences are statistically significant. The curves show the same concentration dependence, but the ouabain

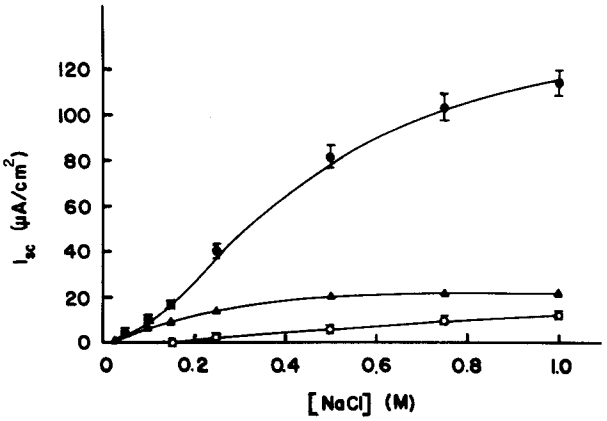

FIGURE 6. The short-circuit current as a function of the mucosal NaCl concentration for the dorsal surface (●), the dorsal surface following ouabain (○), and the lingual frenulum (▲). For the control dorsal surface each point is a mean ± SEM (n = 22). Following ouabain each point is a mean ± SEM (n = 6). Ouabain eliminates >90% of the dorsal hyperosmotic current. The frenulum is without a hyperosmotic response.

curve is displaced from the control by nearly a constant amount. The former may be obtained from the latter using:

$$R_o(c) = R(c) + \Delta R, \tag{1}$$

where $R_o(c)$ is the resistance at a NaCl concentration, c, under ouabain, $R(c)$ is the control resistance at the same concentration, and ΔR is a constant independent of concentration. If $c > 0.15$ M, $\Delta R = 182 \pm 62\ \Omega\ cm^2$.

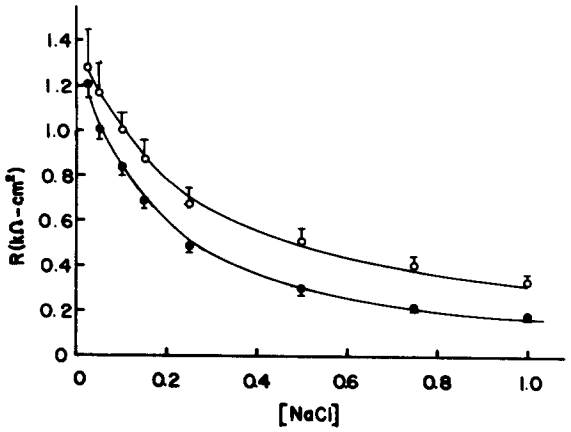

FIGURE 7. The mean resistance of the dorsal lingual surface as a function of the mucosal NaCl concentration: (●) control (mean ± SEM, n = 22); (○) following ouabain (mean ± SEM, n = 6). At NaCl concentrations above 0.15 M, the differences are significant.

The effects of ouabain are far more evident on the current than on the resistance. For example, at 0.25 M NaCl, the resistance shows a 27% increase, whereas the current has declined by 95%. One possible explanation for this observation is that the net resistance is dominated by a low-resistance paracellular shunt. This is further supported by the linearity of the *I-V* curves in symmetrical and asymmetrical solutions and in control as well as ouabain-treated tissues. Linearity of this sort is characteristic of epithelia with prominent paracellular shunts (Moreno and Diamond, 1975). The increased resistance seen after ouabain might then be attributable to cell swelling and collapse of lateral intercellular spaces, perhaps similar to that observed in gallbladder following ouabain (Ericson and Spring, 1982*a*). The linearity of the short-circuit current vs. NaCl concentration over the hyperosmotic range is an additional factor consistent with the existence of a single, thin transport barrier with weak cation selectivity. This can be illustrated as follows. The current, I, is dominated by Na^+ and Cl^- transport; i.e.,

$$I = F(J_{Na} - J_{Cl}), \tag{2}$$

where J_{Na} and J_{Cl} are the flows per unit area of Na^+ and Cl^-, respectively, in the ouabain-treated tissue, and F is the Faraday constant. If ion flow is due to electrodiffusion across a single barrier:

$$J_i = P_i(a_{ib}e^{z_i\phi} - a_{im}), \tag{3}$$

where P_i is the permeability coefficient, a_{ib} and a_{im} are the activities of Na^+ and Cl^- on the blood side and mucosal side, respectively, z_i is +1 for Na^+ and −1 for Cl^-, and ϕ is the normalized potential given by FV/RT, where each symbol has its usual meaning. Under short-circuit conditions Eqs. 2 and 3 give:

$$I_{sc} = FP_{Cl}\,(a_{Clb} - a_{Clm}) - FP_{Na}(a_{Nab} - a_{Nam}). \tag{4}$$

Replacing individual ion activities by mean salt activites results in:

$$I_{sc} = F(P_{Na} - P_{Cl})\,(a_m - a_b). \tag{5}$$

In general, the permeability coefficients are state dependent, varying with the potential profile. The effect of the potential profile can be viewed as a correction on the path length (DeSimone, 1977). If fixed charges contribute to the profile, their influence will diminish as the ionic strength increases. Under these conditions, P_{Na} and P_{Cl} will asymptotically approach constant values. Thus, Eq. 5 is a reasonable representation of the system's behavior at high mucosal ionic strength following ouabain. With $a_m > a_b$, P_{Na} must exceed P_{Cl}; i.e., the passive barrier is moderately cation selective.

There is characteristically a significant difference in the hyperosmotic responses of NaCl and KCl. This can be seen in Fig. 8, where the potential transients evoked in turn by 0.5 M NaCl and 0.5 M KCl are compared (in the same preparation). NaCl always evokes a large second component relative to that for KCl. The behavior of LiCl parallels that of NaCl. The large second component in the case of NaCl accounts for the high short-circuit current. After ouabain treatment the current is substantially reduced because the second

component is eliminated. If there were no resistance changes, the ouabain-labile or ouabain-sensitive current at any given mucosal salt concentration would be simply the difference between the currents in the control and ouabain-treated cases. Such a definition would also eliminate error introduced by liquid junction potentials at the bridge-solution interface. With a resistance change the same end can be achieved by defining the ouabain-sensitive part of the current, I_a, as:

$$I_a = I_{sc} - \frac{R_o}{R} I_{osc}, \tag{6}$$

where I_{sc} is the short-circuit current at a given concentration in the control state,

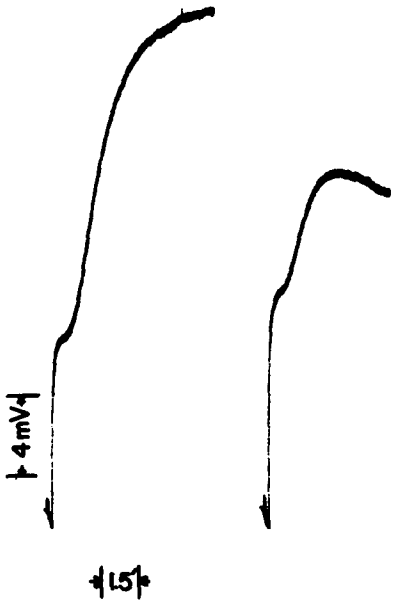

FIGURE 8. The time course of the potential following a change from 0.03 M NaCl to 0.5 M NaCl (left) and a change from 0.03 M NaCl to 0.5 M KCl (right). The second component is always greater for NaCl. This particular result was obtained with saturated KCl salt bridges.

and I_{osc} is the short-circuit current at the same concentration following ouabain. Fig. 9 shows I_a for NaCl and KCl over a series of salt concentrations. Up to 0.15 M, their respective ouabain-sensitive currents are not significantly different (no second components). At concentrations above 0.25 M, NaCl produces the greater ouabain-sensitive current (large NaCl second component, smaller for KCl). The large NaCl and LiCl second components are affected by amiloride in a similar manner, as discussed below.

Amiloride Action on the Hyperosmotic Response

The left-hand record in Fig. 10 shows a normal hyperosmotic response upon replacing a mucosal solution containing 1 mM NaCl with 0.5 M NaCl. The right-

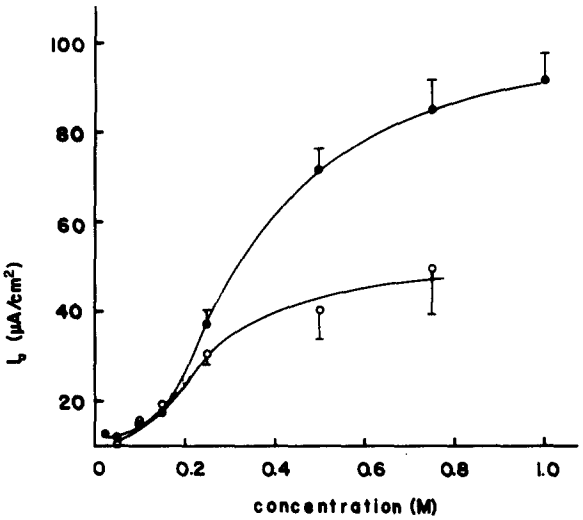

FIGURE 9. The ouabain-sensitive current, I_a, defined by Eq. 6 as a function of mucosal NaCl concentration (●) and KCl concentration (○). The differences between the salts are significant in the hyperosmotic range.

hand record shows the response obtained when 0.5 M NaCl replaces 1 mM NaCl to which 10^{-4} M amiloride has been added. In the control case, the first and second components are clearly distinguishable, the second component comprising a sizeable proportion of the potential (and therefore the short-circuit current). Following amiloride exposure, the first component is truncated and the second component is completely eliminated. The extent to which amiloride causes the

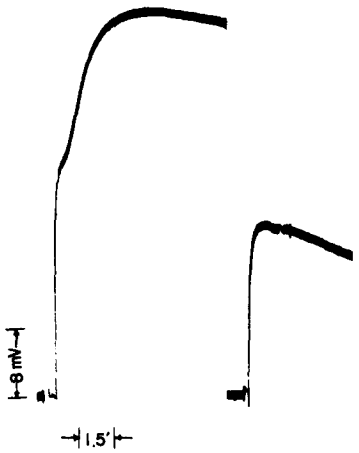

FIGURE 10. The time course of the potential change caused by replacing 1 mM NaCl with 0.5 M NaCl. The left-hand curve is the control. The right-hand curve is the limiting curve following amiloride exposure. Note the elimination of the second potential component.

potential (and current) to decline depends on the particular salt under consideration and the length of exposure to amiloride. These factors were evaluated by studying the hyperosmotic response to NaCl, LiCl, or KCl after various times of exposure to amiloride.

The procedure for estimating the extent of decline in the hyperosmotically induced short-circuit current after a given period of amiloride exposure was as follows. We first established that the hyperosmotic response was stable in the absence of amiloride. Tissues for which stability could not be established were discarded. Fig. 11, curves *a–c,* shows three repeated control responses to 0.5 M LiCl. At the arrows, 1 mM LiCl was replaced by 0.5 M LiCl. Between trials baseline conditions were re-established in 1 mM LiCl. (Control curves for NaCl were characteristically similar to those for LiCl.) Curves *e–g* show three control curves for KCl in a different preparation. Here 1 mM KCl was replaced by 0.5 M KCl. The associated current and resistance did not differ significantly for each set of control curves. These constitute zero-time exposure conditions. At this point a baseline solution containing 1 mM salt and 10^{-4} M amiloride was introduced. After a given exposure period, the hyperosmotic response was again measured. For example, curve *d* shows the effect of 60 s of amiloride exposure on the 0.5 M LiCl response. As expected, the second component was significantly reduced. In the case of KCl (curve *h*), amiloride exposure had a relatively small effect on the overall response.

The given system was returned to baseline conditions (in 1 mM salt) and the process of alternate amiloride exposure and measurement of the hyperosmotic response was continued. The percent of the control current remaining after the indicated cumulative exposure time to amiloride is shown in Fig. 12. Amiloride has virtually the same effect on the hyperosmotic response of NaCl and LiCl. These salts have relatively large second potential and current components, which are significantly truncated within a minute of amiloride exposure. On the other hand, the overall sensitivity of the KCl response to amiloride is significantly less. This is because the second component always represents the smaller proportion of the potential profile (e.g., Fig. 9), and in some instances it is almost absent (e.g., Fig. 11, *e–g*).

In the case of NaCl and LiCl, almost 50% of the current was lost in 1 min, and 60% was lost in 3 min, with 70% being the limiting value of the decline. The KCl current never declined by as much as 50%. For each salt the decline in current was accompanied by a small (<15%) increase in resistance.

Correlation with Neurophysiology

As noted earlier, there are several factors regarding the hyperosmotic response which suggest a relation to gustatory transduction, viz., its unique association with the anterior dorsal lingual surface, its coincidence with the range of maximum neural and psychophysical reaction to salt, and the grouping of the responses of Na and Li as distinct from that of K. The action of amiloride on the hyperosmotic response in vitro suggests a means of testing the hypothesis that NaCl taste is mediated by an influx of Na ions via specific pathways in the lingual epithelium. Although this hypothesis cannot be proved categorically, it is

strengthened considerably if it can be shown that amiloride placed on the tongue specifically blocks the gustatory response to NaCl in the hyperosmotic range while affecting KCl responses to a significantly smaller extent. Earlier indications that this might be the case were obtained by Schiffman et al. (1983). In this

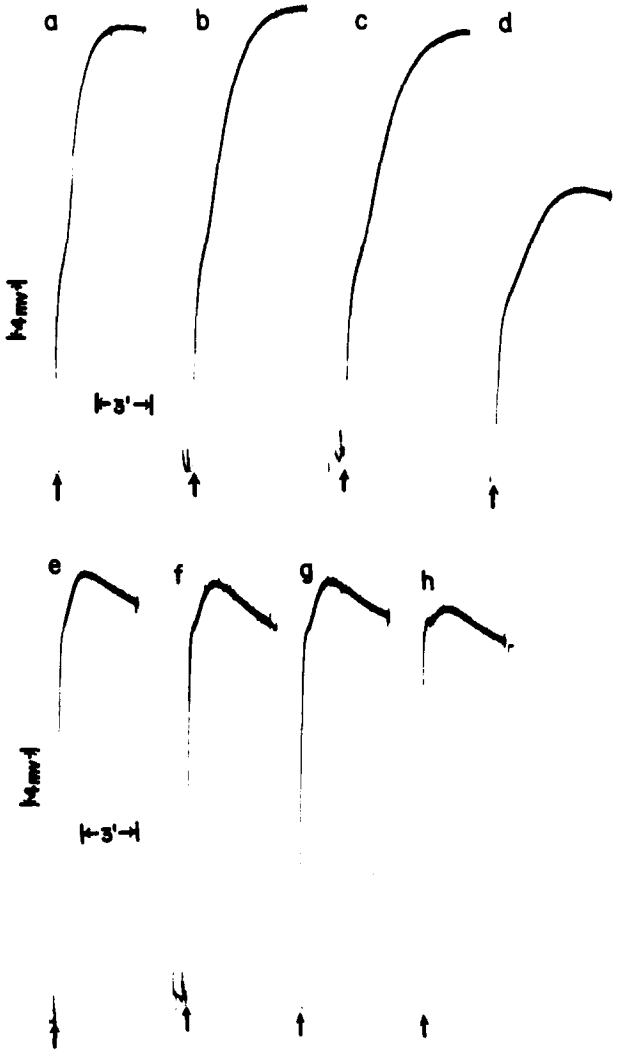

FIGURE 11. Curves *a–c* show the effect on the potential of three consecutive applications of 0.5 M LiCl to the mucosal side of the same dorsal preparation. Baseline conditions in 1 mM LiCl were re-established between each trial. Repeated application did not itself diminish the response. Curve *d* shows the effect on the response to 0.5 M LiCl of removing the baseline solution and replacing it with 1 mM LiCl plus 0.1 mM amiloride for 60 s before testing the response. Curves *e–g* are successive controls for KCl in another tissue. Curve *h* shows the effect of a 60-s amiloride exposure on the KCl response.

paper we use the integrated whole chorda tympani response of the rat as a measure of taste response. In Fig. 13, records *a* and *c* show control responses to 0.5 M and 1 M NaCl. Using the canine response in vitro as a guide, Fig. 12 indicates that exposure of the mucosal lingual surface to 10^{-4} M amiloride for <5 min should result in nearly maximal suppression of the NaCl response. The results of a 5-min exposure are shown in records *b* and *d* for 0.5 M and 1 M NaCl, respectively. Fig. 14 shows the entire response-concentration relation for the control chorda tympani responses and those following amiloride. Here the response is defined as the mean displacement of the integrated signal above

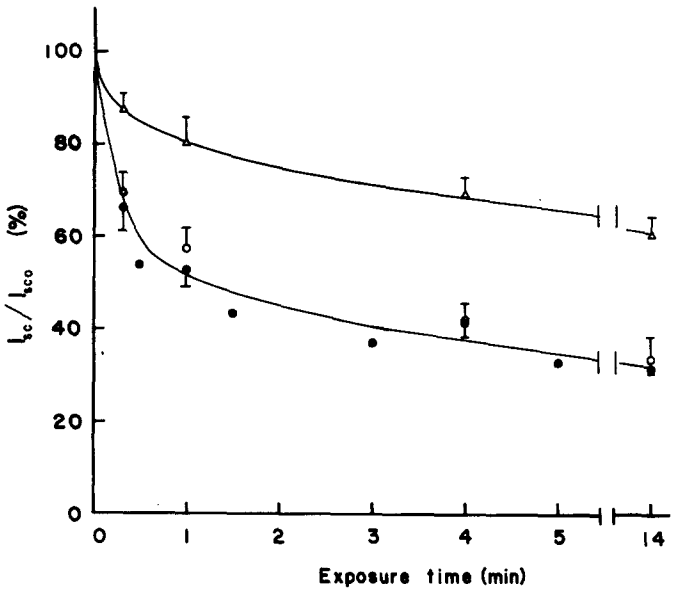

FIGURE 12. The short-circuit current elicited by 0.5 M NaCl (●), 0.5 M LiCl (○), and 0.5 M KCl (△) applied to the mucosal surface of the in vitro preparation following exposure to 10^{-4} M amiloride for the indicated cumulative time period. The currents are expressed relative to their control (pre-amiloride) responses. There is no significant difference between the results for NaCl and LiCl. The KCl results are significantly different (mean ± SEM, $n = 4$, except for four NaCl time points where $n = 1$).

baseline 15 s after stimulus application. All responses are scaled to that of the 1 M NaCl control. It is clear that amiloride has significantly reduced the neural response to NaCl. It is striking to compare these data with in vitro canine results presented over the same concentration range. This is done in Fig. 15. Here we note that amiloride suppresses the short-circuit current over the hyperosmotic range in vitro about as much as the neural response is blocked. Finally, Fig. 16 shows the corresponding response-concentration relation for KCl. At 1 M, the response is 80% of control, compared with 18% for NaCl. In sum, these data strongly support the hypothesis that the same amiloride-sensitive pathway that

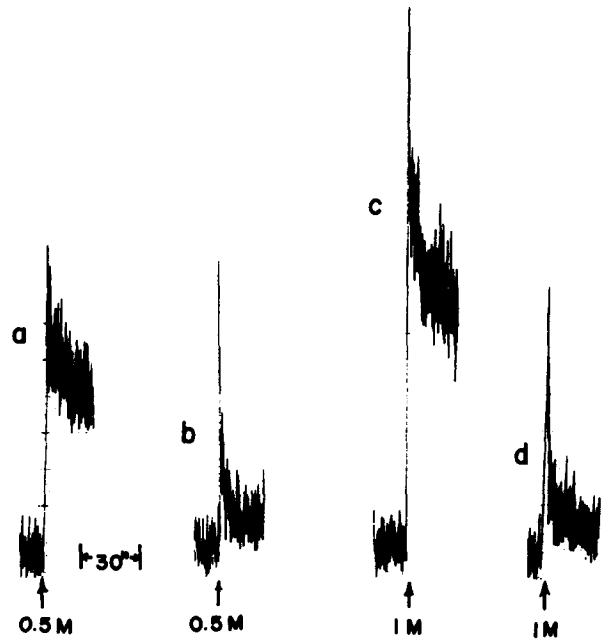

FIGURE 13. Records *a* and *c* are integrated rat chorda tympani responses to 0.5 M and 1 M NaCl, respectively, following adaptation in water. Curves *b* and *d* are the corresponding responses following exposure to 10^{-4} M amiloride for 5 min.

subserves the in vitro hyperosmotic response is involved in some of the early events in NaCl taste transduction.

Hyperosmotic Response Caused by D-Glucose

Glucose and other sugars give a hyperosmotic response in vitro when presented to the mucosal lingual surface. However, unlike the response to salts, the increase

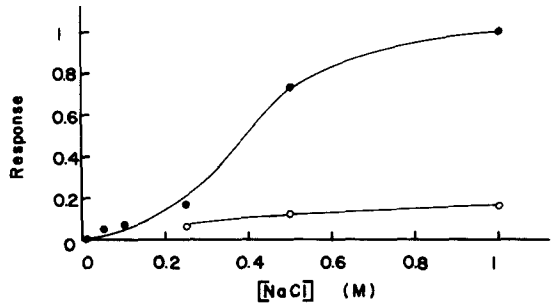

FIGURE 14. The neural response concentration curve for NaCl. A response is defined as the mean displacement above baseline in the chorda tympani record 15 s after stimulation. All responses are normalized to that of 1 M NaCl under control conditions. Closed circles (●) are control results. Open circles (○) are responses following amiloride exposure.

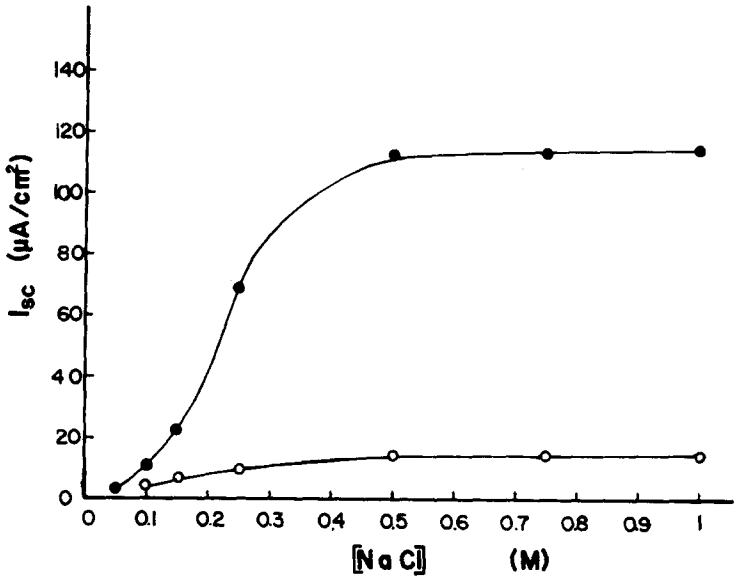

FIGURE 15. The short-circuit current of the canine dorsal preparation as a function of the mucosal NaCl concentration; (●) control; (○) after amiloride. In this experiment 10^{-4} M amiloride was added to the mucosal solution under symmetrical conditions. After the decline in current, the amiloride K-H solution was removed and the open circle data were obtained.

in current occurs at essentially constant resistance. Fig. 17 shows the effect on the current of increasing the glucose concentration in the mucosal K-H buffer. Thus, each successive point represents an increase in nonelectrolyte concentration at constant and symmetrical ionic strength. The three curves represent responses in different tissues. Although there is some individual variation, the effect is clearly demonstrated. The mean (± SEM) resistance of the three tissues at 0.01 glucose was 477 ± 15 Ω cm^2 and 429 ± 32 Ω cm^2 at 0.25 M. The mean (± SEM) over all concentrations was 451 ± 12 Ω cm^2. Maintenance of constant

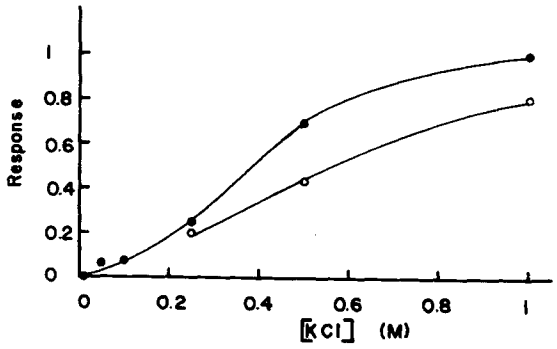

FIGURE 16. The neural response concentration curve for KCl (see Fig. 14 for details). Note that KCl responses are much less affected by amiloride.

resistance seems to be characteristic of sugars, at least when the supporting electrolyte is K-H buffer. Sugar-induced inward current can also be significantly reduced by amiloride, which suggests that sodium carries at least part of it via a transcellular route.

DISCUSSION

The hyperosmotic response appears to involve the induction of a sodium-linked transport system when the mucosa is hyperosmotic in NaCl. LiCl is a suitable substitute for NaCl, but KCl is not. The system is absent from the lingual frenulum, and the high potentials (blood-side positive) and large inward currents that characterize it are not present in other epithelial preparations known to us. The transient induction of ion exchange systems by hyperosmotic solutions is,

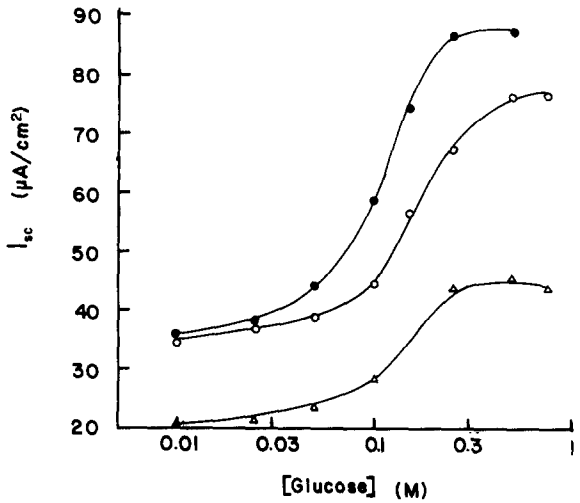

FIGURE 17. The effect of D-glucose on the short-circuit current of three canine dorsal preparations. The glucose was dissolved in K-H buffer and each curve was generated by successively increasing the glucose concentration.

however, well documented in other epithelial preparations, such as *Necturus* gallbladder (Ericson and Spring, 1982*b*), and in single cell preparations such as *Amphiuma* red blood cells (Cala, 1980). Both of these respond to mucosal hypertonicity with the induction of an amiloride-sensitive Na-H exchange believed to be involved in cell volume regulation. However, because the gallbladder and red blood cells do not ordinarily experience sudden and large changes in osmolarity, Spring and Ericson (1982) have suggested that rather than volume regulation, the inducible transport systems may function physiologically as part of hormonally regulated ion transport systems. Sudden and large changes in osmolarity do occur under normal physiological conditions in the case of the lingual epithelium. The detailed characteristics of its hyperosmotic response have not as yet been fully explored. It is possible, however, that the inducible transport system of the dorsal lingual surface may serve a dual purpose: that of volume

regulator and that of specific Na input to the gustatory system over the hyperosmotic concentration range.

The discovery of the active transport characteristics of the lingual epithelium (DeSimone et al., 1981) has led directly to parallel discoveries in the taste system. One of the most important of these was the discovery of the first specific inhibitor for NaCl taste, viz., amiloride. Schiffman et al. (1983) were the first to show that amiloride suppresses NaCl and LiCl taste in humans without substantially affecting KCl taste. Single-unit recordings in the rat nucleus tractus solitarius were consistent with those findings. In this paper we used integrated chorda tympani responses as a gustatory measure. They support the hypothesis that the specific amiloride-sensitive transport system discovered in vitro subserves a gustatory function. While all of the in vitro data presented here are from the dog, we have shown that similar data can be obtained from a rat in vitro preparation, albeit with greater technical difficulty (DeSimone et al., 1983). Schiffman et al. (1983) also noted that amiloride reduced the sweetness of sugars in humans. To what extent our observations on glucose-induced inward current in the in vitro preparation bear on this remains to be explored. They do show, however, that nonelectrolyte taste stimuli are capable of stimulating significant transcellular ion flow, a necessary condition for any gustatory mechanism.

The classic model of taste proposes the presence of various classes of receptors in the microvilli of the taste bud cells (Beidler and Gross, 1971). The present work suggests that, at least in the case of simple salts, ion receptors are identical to or closely allied with specific ion channels or other transport pathways. The fact that the ion recognition sites have transport properties has important consequences. Because the classic receptor did not include ion penetration, it required some form of transduction whereby the binding of an ion was decoded so as to cause a depolarization of the taste cell. There has been much speculation about the presumed nature of this transduction (Beidler and Gross, 1971; Kamo et al., 1974; DeSimone and Heck, 1980). There is no independent evidence that it occurs, however. In the transport paradigm, the need for this undefined process is removed once an ion enters a specific transport pathway, because the ionic stimulus may then move along its electrochemical potential gradient, carrying current into the cells. The ionic stimulus itself can therefore become the immediate agent of cell depolarization.

The transport theory can account for electric taste (Bujas et al., 1979). However, the receptor theory, in its original form, has difficulties. If the taste buds are ion impermeable, they are of necessity high-resistance pathways and therefore conduct no current. As such they can never be stimulated. The fact that they are stimulated is itself evidence that taste-sensitive regions of the tongue are ion permeable. The transport model suggests further that the applied potential supplies the energy required to redirect the cation flow through the shunt and through the cells, so that it resembles that which obtains under chemical stimulation at higher salt concentrations. This can be done by passing anodal current, a well-documented stimulus to electric taste (Bujus et al., 1979).

One may seek alternative explanations for the observations involving the effects of amiloride in vitro on transport and in vivo on the taste system, but

each requires additional assumptions for which there is presently little support. For example, it may be argued that the taste buds are not directly affected by amiloride and that they are rather reacting to effects on the epithelial cells around them. This, however, requires the taste buds to be coupled electrically to the surrounding epithelium. Miller (1972) has shown that the neural response of a given taste papilla can be modulated by stimulation of the surrounding epithelium. However, this observation is subject to a variety of interpretations. Thus, before this more complex explanation can be considered, evidence for electrical coupling must be compelling. Similarly, it may be argued that the lingual epithelium transports ions, except for the taste buds, which have instead an equilibrium receptor system. However, this receptor system would have to have many of the same properties of a transport system to be affected by amiloride in the specific way that it is. While this could be possible, there is no reason to suppose that amiloride is involved in a new effect which is in all respects indistinguishable from its effects on Na transport. In sum, the simplest model that accounts for the data is that the taste bud cells contain specific ion transport pathways mediating gustation.

A transport function for the taste bud cells was suggested by Scalzi (1967) on the basis of ultrastructural analogies between taste bud cells and those of transporting epithelia such the gallbladder, gastric mucosa, and intestine. Given further the close correlation between lingual papillary blood flow and that of the villus (Hallbäck et al., 1979), it seems that at the peripheral level the taste system may have as much in common with absorbing epithelia as with the visual or auditory systems to which it is often compared. The latter systems are fundamentally different in that their stimuli are not electrochemical and therefore must be transduced before sensory processing can occur. In the taste system, the common ionic stimuli are already electrochemical, and, provided that they may permeate the cells, transduction need not occur at the level of the apical membrane. Changes in cell ion composition (and perhaps that of the shunt) may then serve to depolarize the cell activating ion pumps, voltage-sensitive Ca channels, or other events associated with the basal parts of the taste bud cells that lead to transmitter release and neural excitation (Nagahama et al., 1982).

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