Direct measurement of translingual epithelial NaCl and KCl currents during the chorda tympani taste response

Gerard L. Heck, Krishna C. Persaud, and John A. DeSimone
Department of Physiology, Virginia Commonwealth University, Richmond, Virginia 23298-0551

ABSTRACT We have measured the NaCl or KCl currents under voltage clamp across the dorsal lingual epithelium of the rat and simultaneously the response of the taste nerves. Under short-circuit conditions a NaCl stimulus evoked an inward current (first current) that coincided with excitation of the chorda tympani. This was followed by a slower inward current (second current) that matched the kinetics of taste nerve adaptation. The peak first current and the coincident neural response satisfied the same saturating NaCl concentration dependence. Both first and second currents were partially blocked by amiloride as were the phasic and tonic components of the neural response. The NaCl-evoked second current was completely blocked by ouabain. Investigation of the NaCl-evoked current and the neural response over a range of clamped voltages showed that inward negative potentials enhanced the inward current and the neural response to 0.3 M NaCl. Sufficiently high inward positive potentials reversed the current, and made the neural response independent of further changes in voltage. Therefore, one of the NaCl taste transduction mechanisms is voltage dependent while the other is voltage independent. A KCl stimulus also evoked an inward short-circuit current, but this and the neural response were not amiloride-sensitive. The data indicate that neural adaptation to a NaCl stimulus, but not a KCl stimulus, is mediated by cell Na/K pumps. A model is proposed in which the connection between the NaCl-evoked second current and cell repolarization is demonstrated.

INTRODUCTION

The mammalian dorsal lingual epithelium in vitro actively absorbs sodium ions over a concentration range that coincides with the range of sensitivity to NaCl of the gustatory nerves (DeSimone et al., 1981, 1984; Mierson et al., 1985). If the taste receptor cells are among those capable of sodium absorption, then the peripheral mechanism for excitation of the taste nerves by sodium ions could be initiated directly through depolarization of the taste cells by sodium ions crossing a cell apical membrane. This hypothesis has been supported by both whole nerve and single fiber recordings from the taste nerves of various mammals. These studies show that amiloride, a well-known blocker of epithelial sodium ion transport pathways, applied to the tongue is a selective inhibitor of taste nerve responses to NaCl (Schiffman et al., 1983; Heck et al., 1984; DeSimone et al., 1984; Brand et al., 1985; DeSimone and Ferrell, 1985; Hill and Bour, 1985; Hettinger and Frank, 1987; Herness, 1987; Hellekant et al., 1988). Independent investigation of ion transport across the canine lingual epithelium in vitro has shown that sodium absorption can be similarly inhibited by amiloride (DeSimone et al., 1981, 1984; Mierson et al., 1985; Simon and Garvin, 1985; Simon et al., 1988).

Until now establishing a relation between the transport of ions across the dorsal mucosa of the tongue in vitro and the response of the taste nerves to ionic stimuli has depended on the outcome of parallel pharmacological studies in different experimental systems. While this continues to be a useful approach, it leaves open the question of the temporal relation between the stimulus-evoked current and the response of the taste nerves. This is a point of some importance because the kinetic response of the lingual epithelium in vitro to changes in NaCl concentration is usually far slower than the corresponding response of the taste nerves to NaCl stimulation. In this paper we introduce a new experimental approach, using the rat, that allows for the simultaneous measurement of NaCl-induced transepithelial current under voltage clamp across a defined area of tongue and the taste response as monitored via chorda tympani fibers innervating the same lingual region. With this approach we find that stimulation with NaCl at zero transepithelial voltage results in a fast initital inward current followed by a slower second inward current. Both currents are correlated with the temporal development of the neural response to the NaCl stimulus. The initial current coincides with the onset and rise in neural activity; the second current coincides with the slow course of neural adaptation.

We further investigate the concentration dependence, voltage dependence, and amiloride sensitivity of both the stimulus-evoked current and the neural response. The

0006-3495/89/05/843/15 $2.00

results show that the NaCl gustatory response of the rat consists of two processes: one of these varies with the magnitude and polarization of the transepithelial clamping voltage; the second is voltage independent. We conclude that the voltage dependence of the neural response results from modulation of the driving force for Na ions through an apical membrane ion channel in the receptor cells, and that amiloride sensitivity results from modulation of that channel's ion permeability. This view is supported by direct measurement of the NaCl-evoked inward current under voltage clamp. This is the first evidence that the time course of NaCl-evoked current is sufficently fast to support the view that NaCl taste transduction follows from the direct entry of a Na-depolarizing current of stimulus origin across the apical membranes of taste cells (DeSimone et al., 1981, 1984; Heck et al., 1984; Simon et al., 1988). Smith et al. (1975, 1978) proposed a taste cell mechanism with exponential time course as the principal source of neural adaptation to NaCl stimulation. The coincidence of the NaCl-evoked slow current and neural adaptation, and the sensitivity to ouabain of the slow current component in vitro suggest that the cellular basis of adaptation of the neural response to NaCl postulated by Smith et al. is via a repolarizing current mediated by the Na/K pump. Preliminary versions of this work appear in Heck et al. (1985), DeSimone et al. (1987), and DeSimone et al. (1989).

MATERIALS AND METHODS

Surgical preparation

Female Sprague-Dawley rats weighing 150–250 g were anesthetized by interperitoneal injection of sodium pentabarbital at a dose of 60 mg/kg. The trachea was cannulated to facilitate free breathing, and the head was immobilized by clamping the upper jaw in a nontraumatic headholder (Erickson, 1966). Body temperature was maintained by resting the rat's body on a pad containing a substance with a solid/liquid phase transition temperature of 39°C (Braintree Scientific Inc., Braintree, MA). The left chorda tympani nerve was surgically exposed and cut just after its exit from the petrotympanic fissure. The nerve was freed of overlying tissue and suspended from a platinum hook recording electrode. A semiviscous mixture of petrolatum and mineral oil was placed around the nerve/electrode preparation to prevent drying and assist electrical isolation of the nerve. A second platinum electrode was placed in contact with exposed muscle tissue to serve as a reference. The lower jaw was secured in an open position with a loop of thread so that the tongue was freely accessible for placement of the stimulation chamber.

Stimulation chamber and solution delivery

A stimulation chamber as illustrated in Fig. 1 was placed over the anterior tongue. A vacuum applied through the outer annulus effectively sealed the chamber to the tongue. In this way a hydraulic and electrical seal was created about a circular region of anterior tongue of 5.96 mm diameter. Stimulating and rinse solution volumes of 3 ml were infused into the chamber with hypodermic syringes at the rate of 1 ml/s. The solutions entered the chamber through a central orifice of 1.12 mm diameter. The hydrodynamic flow profile was radially out from the orifice and over the surface of the tongue. (For an analysis of the hydrodynamics of the chamber see DeSimone and Heck, 1980.) Along the periphery of the circumscribed tongue region, the solution was collected through a second annular ring in the roof of the chamber and directed to an outflow port. At the end of an infusion the solution remaining in the chamber was allowed to bathe the tongue to prevent drying of the mucosa and to insure equilibration of the adapting solution with the tongue. The interior height of the chamber was 1.05 mm so the volume of fluid in the cylindrically shaped chamber at any time was 0.029 ml. A Ag/AgCl pellet electrode, making contact with the interior contents of the stimulating chamber, was used in measuring the lingual transepithelial potential relative to a second Ag/AgCl pellet electrode imbedded in the tongue muscle. An additional pair of Ag/AgCl electrodes, one placed in the chamber, the other in the tongue muscle, was used to pass current across the epithelial patch.

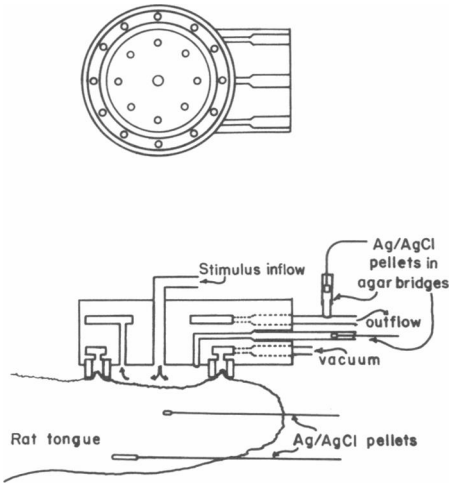

FIGURE 1 (*Top*) Chamber for stimulus delivery to rat tongue. Outer ring is used to apply a vacuum that permits both hydrodynamic and electrical isolation. Stimulus inflow port is at the center of the chamber. Outflow is along the periphery of the fluid-containing part of the chamber. (*Bottom*) Illustration of chamber placement on anterior dorsal tongue of rat, stimulus flow path, and potential and current electrode arrangement. Diameter of effective stimulating area is 5.96 mm. Volume of contained fluid is 0.029 ml.

Electrical measurements

A model VCC600 voltage-current clamp (Physiologic Intruments, Houston, TX) was used to measure and clamp transepithelial voltages and currents. It was configured with the current passing electrode in the chamber as virtual ground. This configuration insured that only current passing across the epithelial patch was collected and measured. The preparation was isolated from all other electrical connections except a battery-operated, optically isolated preamplifier which was used for recording chorda tympani activity. The preparation therefore floated electrically at the potential required to drive the programmed current through the lingual area circumscribed by the chamber. The clamp was operated in either a voltage or current clamp mode. In the former case the clamp drove sufficient current via the current electrodes so that the

differential voltage measured across the epithelium matched a programmed reference voltage. The reference voltage was usually zero (short-circuit) but in some experiments the clamping voltage was varied within a range of ±120 mV. In current clamp a fixed current was passed and the resulting differential voltage measured. Zero current clamp yielded the equivalent of an open-circuit potential. During most phases of experimentation a periodic biphasic perturbation of either the clamped voltage or current was used to obtain a running measure of changes in transepithelial conductance. The signal from the preamplifier representing the massed chorda tympani activity was bandwidth-limited, rectified, and integrated with a time constant of 10 s. The neural signal along with signals from the tissue clamp representing transepithelial current and voltage were displayed on a Linseis TYP 2045 strip chart recorder. In voltage clamp studies inward currents (mucosa to submucosa) are displayed as upward pen deflections from baseline.

Data analysis

Records were routinely digitized and stored for further analysis. Where appropriate, data sets were fit to nonlinear functions by choosing parameters which minimized the sum of square deviations. This was carried out on a laboratory microcomputer using a simplex method (Nicol et al., 1986).

In vitro methods

Experiments treating ouabain effects were carried out on isolated lingual preparations using both canine and rat tongues. The dissection of the dorsal lingual epithelium has been extensively described (DeSimone et al., 1984; Mierson et al., 1985). Briefly, the animals were killed by an interperitoneal injection of sodium pentobarbital. The tongues were surgically removed and placed dorsum down on a dissecting board. Most of the muscle was removed by blunt dissection. The remaining muscle was removed by cutting through the connective tissue linking muscle and epithelium. Anterior sections of epithelium were placed in Ussing chambers. Tissues were initially bathed symmetrically in Krebs-Henseleit (KH) buffer maintained at 34°C. Potential differences across the tissue were measured by calomel electrodes connected to the solution by 3% agar/saline bridges. Current was passed with sintered Ag/AgCl electrodes in 0.15 M NaCl delivered to the tissue via a second set of salt bridges. The short-circuit current and resistance were measured with single-channel voltage clamps (Physiologic Instruments VCC600 series) and monitored on a strip-chart recorder. Resistance was determined continuously by pulsing a known perturbation voltage about the clamping voltage and observing the excursions in the short-circuit current. In KH buffer the series solution resistance was automatically compensated.

TABLE 1 Electrical parameters of the rat dorsal lingual epithelium

	I_{sc}	R	V_{oc}	n
	$\mu A/cm^2$	$\Omega\,cm^2$	mV	
In vitro*	8.2 ± 0.9	1686 ± 147	13.4 ± 0.7	14
In vivo	10.4 ± 1.6	1495 ± 81	16.0 ± 3.2	5

Values are expressed as the mean ± SEM. In vitro the bathing medium on both sides of the tissue was Krebs-Henseleit buffer. In vivo Krebs-Henseleit buffer was placed in the flow chamber while the submucosal medium was the animal's extracellular fluid.
*From Heck et al., 1984.

Solutions and chemicals

All chemicals were reagent grade. Ouabain octahydrate 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. Krebs-Henseleit buffer consisted 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. When bubbled with 95% O_2–5% CO_2, the pH was 7.4.

RESULTS

Steady-state electrical parameters

The steady-state electrical properties of the rat lingual epithelium in vivo bathed in KH buffer were not significantly different from the corresponding in vitro values. Table 1 shows that in both cases there exists an open-circuit potential, V_{oc}. The submucosa is electropositive and the magnitudes of V_{oc} do not differ significantly. Similarly in each case an inwardly directed short-circuit current (I_{sc}) exists. Assuming KH buffer reasonably approximates the ion composition of extracellular fluid, so that transepithelial ion gradients are negligible, it appears that the steady-state active ion current density in vivo is comparable to that of the well-oxygenated in vitro preparation.

Time course of response in vivo

In vitro studies have shown that the rat lingual epithelium responds to increasing concentrations of NaCl with increasing short-circuit current (Heck et al., 1984; Simon et al., 1988). Fig. 2 shows the relation of this current to the neural response to NaCl in vivo. In this experiment the tongue was adapted to 0.01 M NaCl and then stimulated under short-circuit conditions with successively higher NaCl concentrations. The stimulus-evoked current occurred in two distinct phases: (*a*) an initial rapid rise to a maximum (first current) followed by (*b*) a slower rising current (second current) that tended toward a steady state asymptotically. Both phases were characterized by increasing conductance with the larger rate of increase in conductance occurring during the first current. The simultaneous monitor of the integrated neural response showed that the first current coincided with the phasic rise in neural activity, while the slower second current coincided with neural adaptation. Upon rinsing the tongue there was a rapid decline in current temporally correlated with the decline in neural activity.

Concentration dependence

Both the neural response and I_{sc} increased with NaCl concentration. Fig. 3 shows a plot of the initial maximum

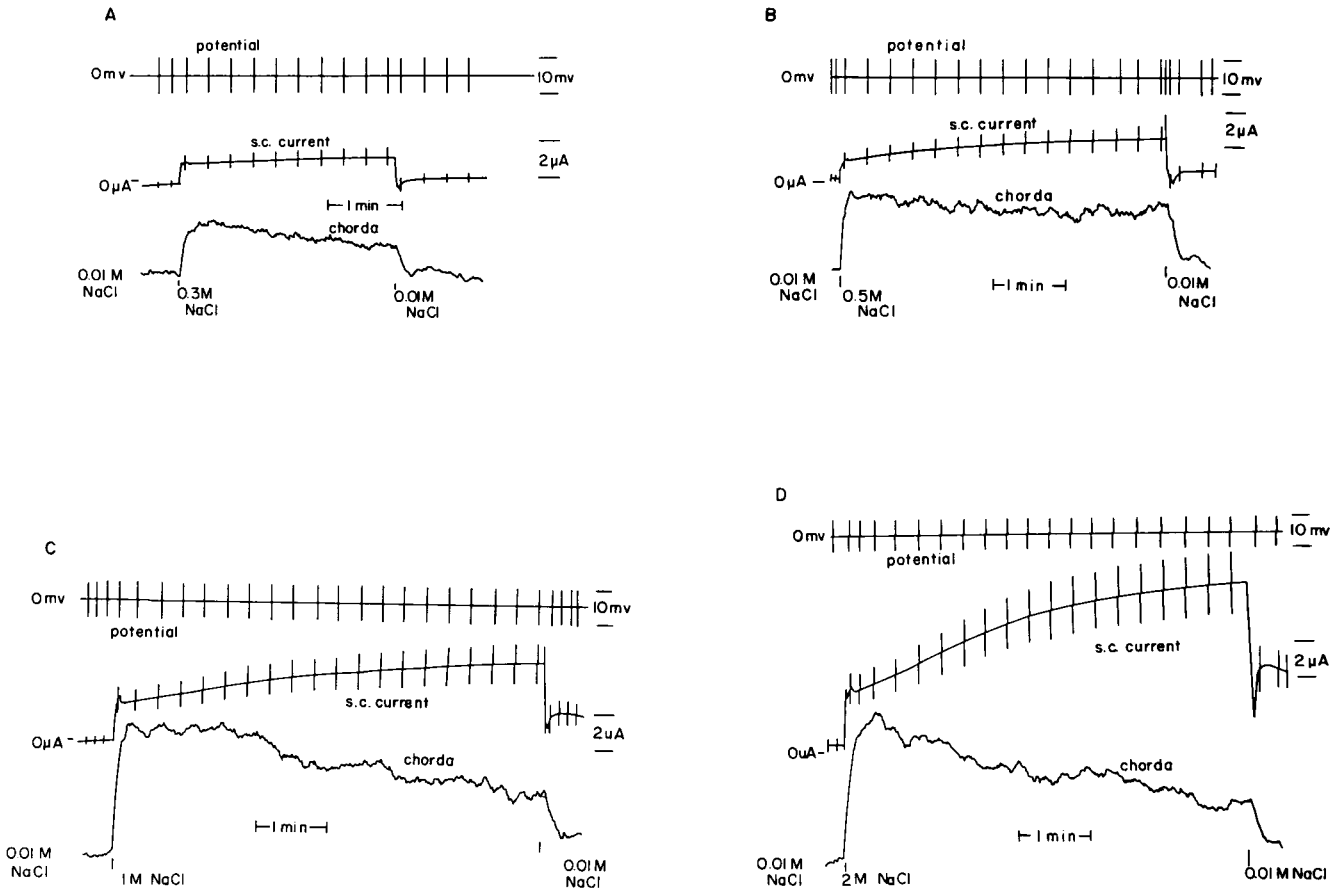

FIGURE 2 Records of the change in the current, recorded at zero transepithelial potential and the chorda tympani response to NaCl: (*A*) 0.3 M; (*B*) 0.5 M; (*C*) 1 M; (*D*) 2 M. Adapting and rinse solutions were 0.01 M NaCl. The first current accompanies the initial rise in neural activity. The second current follows the time course of neural adaptation. The transepithelial conductance also increases upon stimulation. The current changes and neural responses increase with concentration and the rate of relaxation in both current and neural response is similar at a given concentration.

value of I_{sc} (first current) normalized according to:

$$\Delta I = [I_{sc}(c) - I_{sc}(0.01)]/[I_{sc}(1) - I_{sc}(0.01)],$$

where $I_{sc}(c)$ is the value of the short-circuit current at a NaCl concentration, c. $I_{sc}(0.01)$ and $I_{sc}(1)$ are the values of the short-circuit current at 0.01 M NaCl and 1 M NaCl, respectively. In Fig. 3, I is shown as solid diamonds. Open squares represent the chorda tympani response above baseline taken at the same time as peak current and also normalized to the 1 M NaCl response. The chorda tympani response of the rat to NaCl has been demonstrated to have a slowly saturating concentration dependence (Beidler, 1954; Smith et al., 1975). Fig. 3 shows that the NaCl-evoked short-circuit current has the same concentration dependence as the chorda tympani response when both are normalized to the same scale. Both the current and the neural response are scaled to unity at 1 M NaCl, however, they also do not differ significantly at the other concentrations. The curve represents a fit of both data sets to a Hill equation of the form

$$\Delta I = \mathrm{CT} = Ac^n/(c^n + K^n) \quad (1)$$

where ΔI is the normalized current, CT is the chorda tympani response, and c is the NaCl concentration. A, n, and K are constants having values: 1.18, 1.38, and 0.31, respectively. These results offer further evidence that the NaCl-evoked current is the stimulus for the neural response over the entire gustatory range. It is also clear that the rat is more sensitive to NaCl concentration differences in the low part of the gustatory range.

The second current and neural adaptation

The chorda tympani response of the rat to NaCl slowly declines over the course of a stimulus presentation, a characteristic often termed adaptation (Smith et al., 1975, 1978). Adaptation in the neural response is also

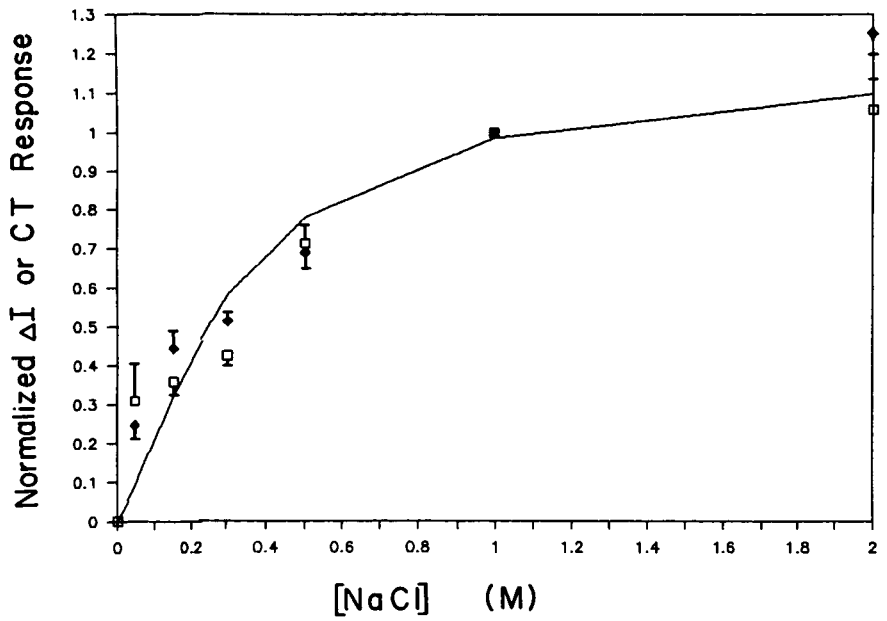

FIGURE 3 The concentration-response curve of the normalized current (*solid diamonds*, mean ± SEM, $n = 4$), and the chorda tympani response (*open squares*, mean ± SEM, $n = 5$). The solid curve is a least-squares fit of both data sets to a saturating function of concentration (see text).

evident under short-circuit conditions (cf Fig. 2). Adaptation is asymptotically an exponential decay process (Smith et al., 1975). Analysis of the kinetics of the second current shows that it also relaxes exponentially, and that furthermore at a given NaCl stimulus concentration, the second current and the neural response relax with the same rate constant. Fig. 4 shows that the relaxation rate constants for the second current and the neural responses do not differ significantly, however, at NaCl concentrations below 0.3 M the relaxation in both the current and neural response is faster. Both data sets can be described by the following function of stimulus NaCl concentration.

$$r = A - [Bc^n]/[c^n + K^n], \quad (2)$$

where r is the rate constant in seconds^{-1}, c is the molar NaCl concentration, $n = 3.6$, $A = 0.050$ seconds^{-1}, $B = 0.045$ s^{-1}, and $K = 0.165$ M. The concentration dependence of r may be a reflection of the saturability of the cell Na pump. This possibility is developed further in the Appendix using a mathematical model.

Effect of ouabain on the second current

The effect of ouabain on the second current was investigated in vitro. Because ouabain is effective only from the submucosal side, it could not be easily utilized in vivo. It has been previously shown that ouabain greatly suppresses the NaCl-evoked short-circuit current across both the canine (DeSimone et al., 1981, 1984; Mierson et al., 1985) and the rat (Mierson et al., 1988*a*; Simon et al., 1988) lingual epithelium. Fig. 5 shows specifically that the second current is virtually eliminated after ouabain treatment. In this experiment canine tissue was used. After the establishment of steady-state conditions in symmetrical K-H buffer, a baseline short-circuit current was reestablished with 0.025 M NaCl placed only in the mucosal reservoir. The control in Fig. 5 shows the effect of replacing 0.025 M NaCl with 0.5 M NaCl. The first and second current components are clearly visible. After return to symmetrical K-H buffer, 0.1 mM ouabain was placed in the submucosal reservoir and in 1 h the short-circuit current was reduced to zero. This is a typical time course for the total elimination of the short-circuit current (DeSimone et al., 1984; Simon and Garvin, 1985). The decline in the short-circuit current begins in 2–3 min after ouabain addition. This latency probably represents the time required for ouabain to diffuse to its first sites of action. The slow approach to equilibrium may be due in part to a relaxation current resulting from the equilibration of the cell sodium concentration and/or to pump sites that may be more remote and are therefore inactivated more slowly than more superficial sites. When the response to 0.5 M NaCl was retested, the first current was truncated and the second current entirely eliminated (Fig. 5). It appears reasonable to conclude the second current depends on the maintenance of ion gradients

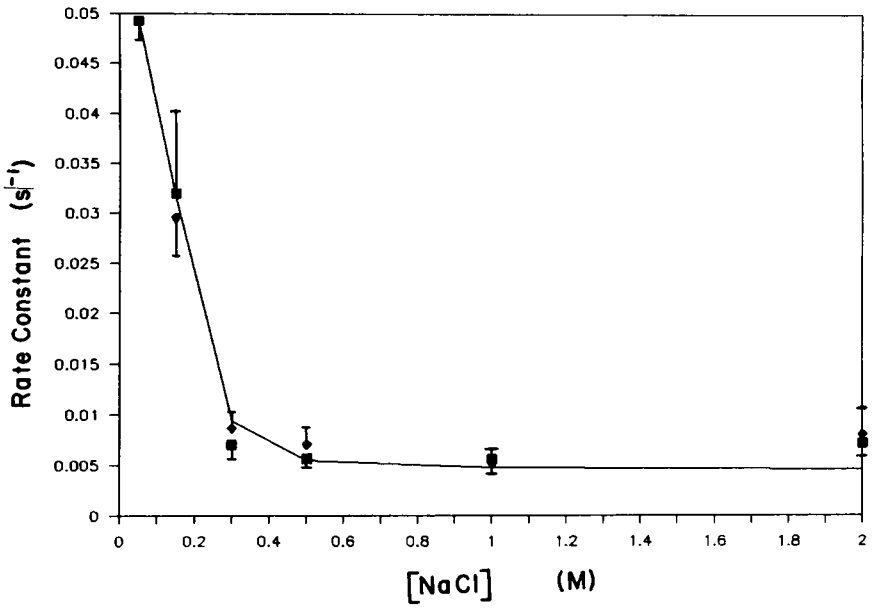

FIGURE 4 Dependence of the relaxation rate constant on stimulating NaCl concentration for the short-circuit current (*diamonds*, mean ± SEM, $n = 4$, except for 1 M where $n = 12$), and for the chorda tympani response (*squares*, mean ± SEM, $n = 5$, except for 1 M where $n = 8$). The solid curve is a least-squares fit of both data sets to a saturating function of concentration. The data show that adaptation takes longer at NaCl concentrations above 0.15 M.

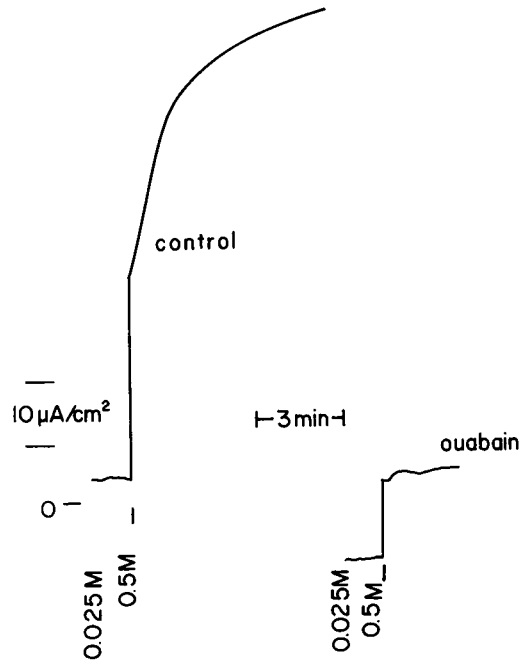

FIGURE 5 Effect of ouabain on the NaCl-evoked short-circuit current in vitro across the canine lingual epithelium. The control response (*left*) shows the first and second currents for 0.5 M NaCl stimulation. After ouabain treatment (1 h with 0.1 mM ouabain in the submucosal bath) the response at the right was obtained. Note that the first current is truncated and the second current eliminated.

across the apical membranes of transporting cells which are in turn dependent on basolateral membrane Na/K-pumps.

Effect of amiloride on NaCl and KCl responses

Replacing the 0.01 M NaCl adapting solution in the chamber with 0.01 M NaCl containing 0.1 mM amiloride caused a significant and parallel reduction in both the short-circuit current and the chorda tympani response to 1 M NaCl (cf Fig. 6). In these experiments the tongue was bathed in amiloride for 1 min and then stimulated with 1 M NaCl that also contained amiloride. As seen in Fig. 6 both the first and second currents were reduced as were both the phasic and tonic parts of the chorda tympani response. In three experiments the peak first current response was inhibited by 41.9 ± 8.1% and the peak chorda tympani response was inhibited by 57.8 ± 11.3%. The difference was not significant, however, in each case the neural response was inhibited more than the corresponding peak current. This could be due to small paracellular shunt currents, which are unaffected by amiloride (DeSimone et al., 1984). The percent suppression of both the current and the neural response at later times was always greater than the suppression of the peak response (DeSimone and Ferrell, 1985). In sharp contrast, when the experiment was repeated with 1 M KCl as

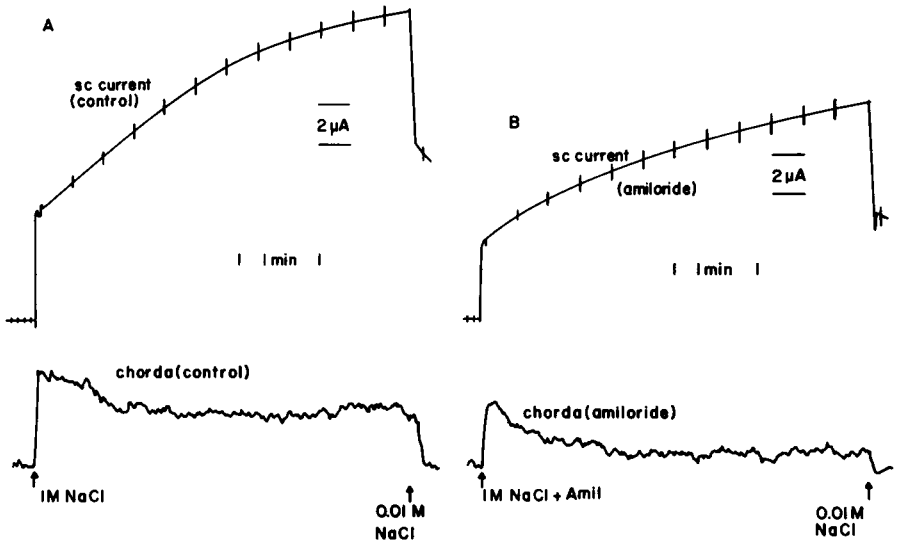

FIGURE 6 Effect of amiloride on the current and chorda tympani response to NaCl. (*A*) Control responses. (*B*) After a 1-min treatment of the tongue with 0.1 mM amiloride in the adapting solution of 0.01 M NaCl and subsequent stimulation with 1 M NaCl containing 0.1 mM amiloride. Both the current and neural response are reduced significantly.

the stimulus (cf Fig. 7) amiloride had no effect on either the short-circuit current or the neural response. Whereas there is a significant KCl-evoked current, none of it passes via amiloride-sensitive pathways. As in the case with NaCl, KCl stimulation produced two currents, each well-correlated with the phasic and tonic parts of the neural response. However, the rate of adaptation of the neural response was significantly faster than the relaxation of the KCl second current. In neither the NaCl nor the KCl case was the transepithelial resistance affected by amiloride treatment.

Response at various voltages

Recording the NaCl-evoked current and the neural response over a range of transepithelial clamping voltages

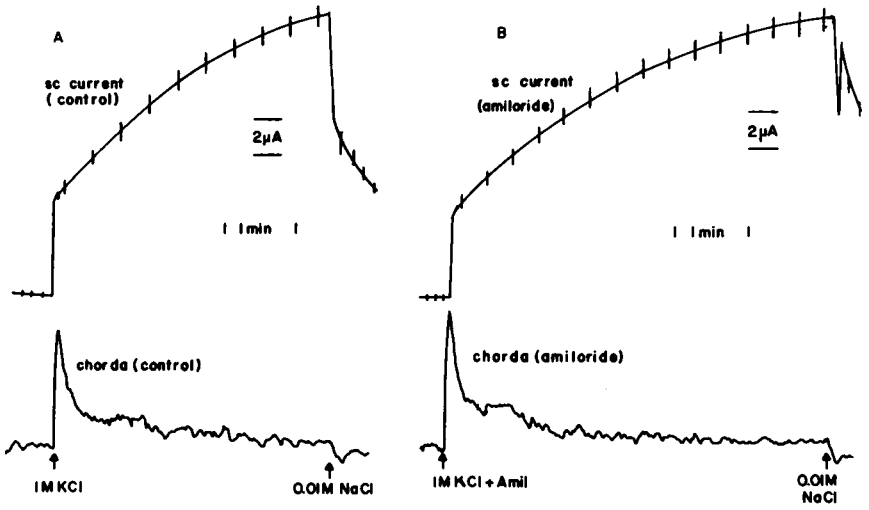

FIGURE 7 Effect of amiloride on the current and chorda tympani response to KCl. (*A*) Control responses. (*B*) After a 1-min treatment of the tongue with 0.1 mM amiloride in 0.01 M NaCl and subsequent stimulation with 1 M KCl containing 0.1 mM amiloride. Both the current and the chorda tympani response are amiloride insensitive.

shows clearly that the more electronegative the submucosal side of the tissue, the greater the taste response. In these experiments 0.3 M NaCl was used as the stimulus. The tongue was adapted in 0.01 M NaCl at short-circuit and then stimulated with NaCl. After a normal stimulation paradigm the tongue was first clamped at a given submucosal electropositive potential and then stimulated. It was then restimulated at short circuit to insure recovery, and finally clamped at a submucosal electronegative potential of the same magnitude and stimulated. Alternating between electropositive and electronegative potentials, we covered a range of potentials without appreciable loss of responsiveness. Care was taken to restrict the range of voltages to those which did not result in currents sufficiently high to evoke neural responses to current directly, i.e., electric taste (Herness, 1987). Fig. 8 *A* shows a preparation initially in the 0.01 M NaCl adapting solution short-circuited. The potential was then changed to 75 mV (submucosa electropositive). At this potential there was an increase in outward current (just equal to the clamping current which was oppositely directed). Upon reaching a new steady current, the tongue was then stimulated with 0.03 M NaCl. There was a conductance increase and an increase in net outward current. The chorda tympani response was characterized by a broad phasic component followed by a decline in activity to just above baseline. Thus rather than the steady decline or adaptation usually seen, the neural response remained nearly constant at a low level. This trend became more evident as clamping potentials were made increasingly electropositive. In contrast Fig. 8 *B* shows the response to 0.3 M NaCl when the potential was held at −75 mV. Here the response resembles the short-circuit response but with enhanced magnitude.

Fig. 9 shows the steady-state current-voltage relation for the adapting NaCl concentration (0.01 M) and the stimulating concentration (0.3 M). The applied or clamping current, I_a, has been plotted, so the slopes of the curves can be displayed as positive (positive conductances). The case of 0.01 M shows deviations from linearity at both the high and low potential extremes, whereas in the case of 0.3 M NaCl deviations from linearity appear only at negative potentials with the conductance decreasing. At any fixed potential the change in current occurring upon stimulation is represented by a vertical jump from the 0.01 M curve to the 0.3 M curve. For increasingly negative potentials the current difference represents increasing inward current and therefore increasing neural response. For increasing positive potentials the current difference reverses in sign and is correlated with decreasing neural response.

The voltage dependence of the chorda tympani response to 0.3 M NaCl is shown in Fig. 10 (*squares*). To obtain a stable measure of the response, we selected the integral over the first minute of stimulation. For any voltage the measured integral was normalized to that for the corresponding short-circuit trial in the stimulation paradigm. This reduced variations between successive trials and between preparations. Fig. 10 shows that the response grew rapidly as the potential became negative. On the other hand as the potential became increasingly positive, the neural response decreased and finally tended

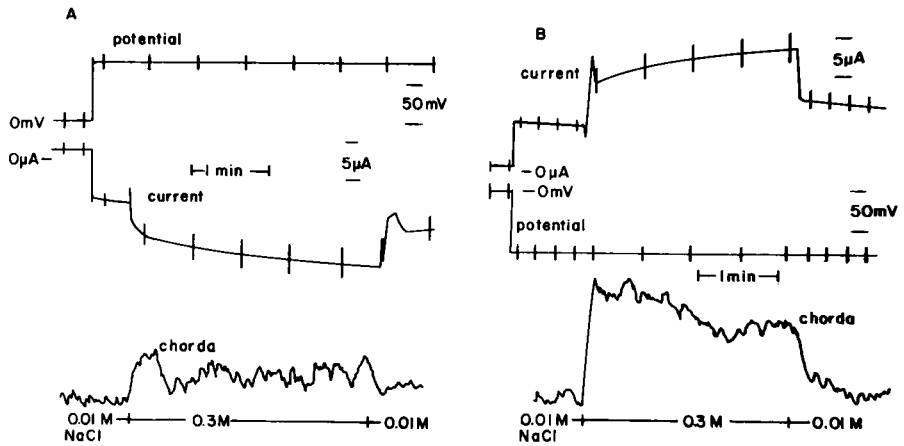

FIGURE 8 Responses to 0.3 M NaCl at: (*A*) clamping voltage of 75 mV (submucosa positive). Stimulation evokes an increased outward current. There is a weak initial chorda tympani response that returns nearly to base line. This is followed by a slow increase in response, (*B*) clamping voltage of −75 mV (submucosa negative). Stimulation evokes an increased inward current and enhanced neural response. Both current and neural response behave qualitatively like responses at short-circuit. Neural responses show a marked voltage dependence.

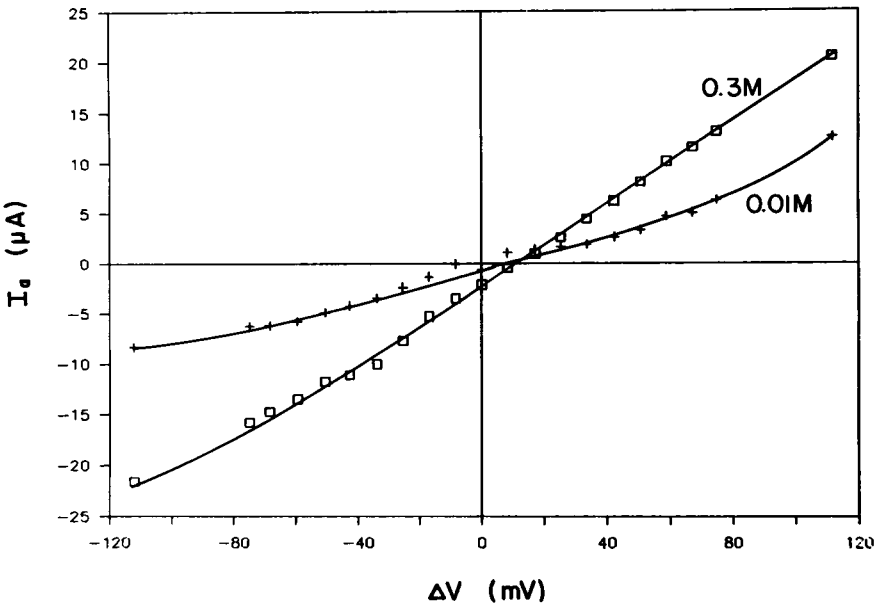

FIGURE 9 The transepithelial current-voltage relations in vivo for 0.01 M NaCl and 0.3 M NaCl. I_a refers to the applied current. There are deviations from linearity in both curves for negative voltages. The 0.01 M curve also shows deviations from linearity at high positive voltages. The difference in the curves at a given voltage represents the current upon stimulation.

toward a low level independent of applied voltage. The neural data can be represented by the following function of potential over the range of potentials investigated.

$$R = C + De^{-nV}, \tag{3}$$

where R is the neural response, V, the potential in mV, and $C = 0.32$, $D = 0.61$, and $n = 0.0092$ mV^{-1}. Thus the neural response to NaCl is composed of both voltage-dependent and voltage-independent parts. The voltage-independent part is exposed at increasingly more positive voltages.

The correlation between the neural response, R, at a given voltage and the current difference between the adapting (0.01 M) and stimulated states (0.3 M), $\Delta I(V)$, can best be seen by rescaling $\Delta I(V)$ as follows:

$$R = h + m\Delta I(V), \tag{4}$$

where $h = 0.77$, and $m = 0.066$ μA^{-1} are scale factors, and $\Delta I(V)$ is in microamperes. This correlation is shown as the curve in Fig. 10.

Open-circuit response

Chorda tympani responses are ordinarily measured under open-circuit conditions. Fig. 11 shows that under these conditions the neural response is accompanied by a potential increase and resistance decrease. Fig. 12 shows that both the peak initial normalized potential at zero current clamp and the chorda tympani response increase with NaCl concentration in a saturable fashion. However, the potential approaches saturation at lower NaCl concentrations relative to the neural response. Normalizing the potential difference at a given concentration to that at 1 M NaCl, in a manner analogous to the procedure used to plot the current, gives the open squares in Fig. 12. Like the short-circuit current (Eq. 1) the potential can also be described by a three-parameter saturable isotherm. In the case of the potential A, n, and K have the values 1.37, 0.58, and 0.18, respectively. The normalized chorda tympani response is shown as closed diamonds in Fig. 12. In this case the parameters A, n, and K are 1.37, 1.35, and 0.45, respectively. Comparing Figs. 3 and 12 shows that the short-circuit current correlates with the chorda tympani response better than the transepithelial potential per se.

Similar to the current changes measured under short-circuit, the potential at zero current clamp increases in time in two stages. The kinetics of the second potential change are usually more complex than single exponential decay. However, the relaxation properties of an implied equivalent current can be calculated from the measured potential and resistance at any time. In this case the equivalent current matches the adaptation properties of the neural response in the same manner as the directly measured inward current under voltage clamp. This is

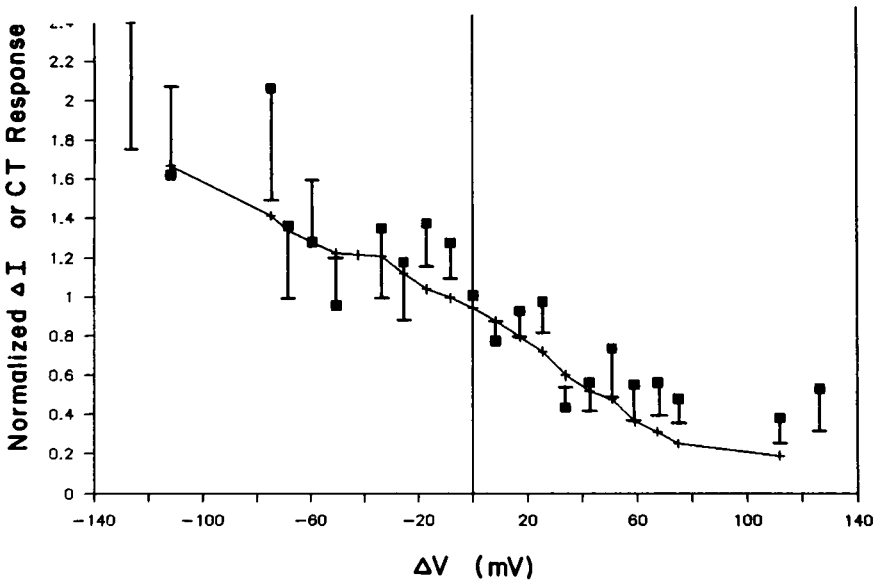

FIGURE 10 The voltage dependence of the neural response (*squares*, mean ± SEM, $n = 3$) and the normalized current (Eq. 1). The neural response is taken as the area under curves over the first minute of stimulation for a given voltage relative to the response under short-circuit conditions. The results show that the neural response correlates with NaCl-evoked current over a range of voltages. The small neural responses at the higher positive potentials become increasingly insensitive to more positive changes in potential, i.e., a voltage-insensitive neural response emerges.

further indication that the NaCl-evoked current is a more direct correlate of the neural response than the transepithelial potential. Moreover, it suggests that stimulation under normal open-circuit conditions, also involves the development of a transcellular current.

DISCUSSION

The data show that the presentation of a NaCl stimulus to the lingual mucosa results in the generation of two transepithelial currents. These correlate with the neural response in time course, NaCl concentration dependence, amiloride sensitivity, and voltage dependence. The ouabain-sensitivity of the second current suggests the involvement of an electrogenic sodium pump in the regulation of taste cell potential. This has been directly demonstrated in the case of frog taste cells where it is possible to perfuse the submucosa via the lingual artery. In this case perfusing the frog tongue with ouabain resulted in a 30% decrease in cell resting potential (Okada et al., 1986). Given that the voltage sensitivity of the sodium pump seems to be conserved in wide varieties of cells from various species (De Weer et al., 1988), it seems reasonable to conclude that the second current is pump dependent and at the cellular level represents a repolarizing current.

The simultaneous inhibition of both the NaCl-evoked current and the neural response by amiloride suggests that the Na ion initiates the salt taste response by flowing into taste cells via amiloride-blockable sodium channels (Lindemann, 1984; Saraban-Sohraby and Benos, 1986). The existence of such channels has been recently established by Avenet and Lindemann (1988) in the case of frog taste receptor cells. In the present case the parallel voltage sensitivity of both the NaCl-evoked current and the neural response further indicates the presence of such

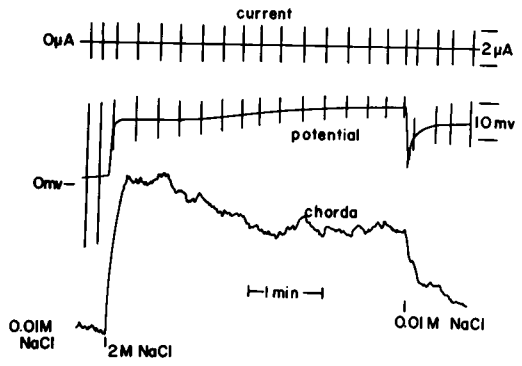

FIGURE 11 Response to NaCl under zero current clamp. This simulates the normal mode of stimulation. Neural responses resemble those under short-circuit conditions. Stimulation causes the submucosa to become electropositive. The correlates of the first and second currents are seen in the time course of the potential.

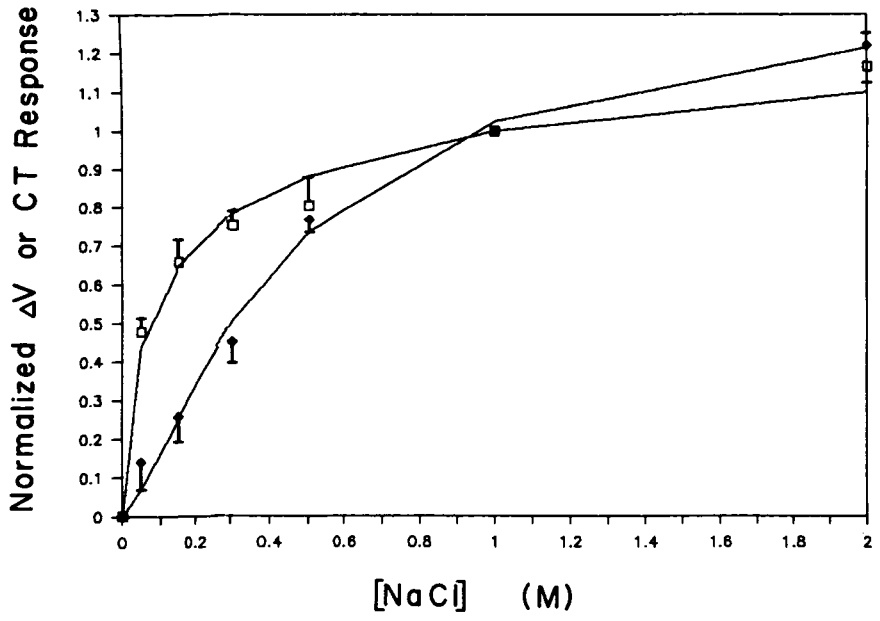

FIGURE 12 The concentration response curve of the normalized transepithelial potential at zero current clamp and the chorda tympani response. The normalized potential is displayed as open squares (mean ± SEM, $n = 4$). The chorda tympani response is displayed as closed diamonds (mean ± SEM, $n = 4$). The solid curves are least squares fits to the data (see text). The potential approaches saturation faster than the neural response.

channels in mammalian preparations. However, these studies also revealed a voltage-independent component to the NaCl-evoked neural response.

On the basis of inhibition studies with amiloride, it is now recognized that there are two distinct NaCl taste transduction mechanisms. In the case of the rat the two systems can be observed separately. The amiloride-insensitive component is present at birth while the amiloride-sensitive component develops postnatally (Hill and Bour, 1985). From the data presented here it is clear that two NaCl taste mechanisms can also be classified on the basis of voltage sensitivity, namely, as voltage dependent and voltage independent. This suggests that in addition to a voltage-sensitive channel mechanism, NaCl taste may also be mediated by an electroneutral mechanism. How these mechanisms relate to the amiloride-sensitive and amiloride-insensitive mechanisms previously described is not fully established as yet. The present study shows that under short-circuit conditions amiloride inhibits 60–70% of the neural response. Additionally Fig. 10 shows that in the limit of high positive voltages the neural response is inhibited by about the same amount relative to short-circuit responses. The recent work of Avenet and Lindemann (1988) establishes an amiloride-blockable Na-conductance in taste cells. On this basis one might expect that if an apical membrane channel is important in the taste response that the taste response could be inhibited by either reducing the channel permeability with amiloride or by creating an unfavorable driving force for Na entry by imposing electropositive potentials. The fact that both procedures inhibit the taste response suggests that a channel is involved and that furthermore, the amiloride-sensitive component is also the voltage-dependent component. On this basis one might further expect the voltage-independent mechanism to be also amiloride-insensitive. However, this remains to be shown experimentally.

With regard to the KCl response this work confirms earlier findings that in the rat NaCl and KCl taste are mediated through separate peripheral mechanisms (Schiffman et al., 1983; Heck et al., 1984; DeSimone et al., 1984; Hill and Bour, 1985). This appears to be the case also in hamsters (Herness, 1987) and monkeys (Hellekant et al., 1988). However, in the dog, where KCl responses exceed those to similar concentrations of NaCl, Mierson et al. (1988*b*) have presented evidence that the amiloride-sensitive pathway allows some K uptake and is, therefore, not strictly Na selective. However, in the rat the Na and K systems are distinct. Recently Kim and Mistretta (1987) and Simon et al. (1988) have shown that 4-amino-pyridine blocks neural responses in the rat to KCl, suggesting the presence of apical K channels, Kinnemon and Roper (1988) have obtained evidence for the presence of apical K channels in the taste cells of *Necturus*. Barium-blockable K channels have been identified in

frog taste cells (Avenet and Lindemann, 1987), and there is evidence that such channels may also exist in the basolateral membranes of rat lingual epithelial cells (Simon et al., 1988). In vitro studies also show that the permeability of the rat lingual epithelium to KCl after ouabain treatment is significantly higher than its permeability to NaCl (Mierson et al., 1988*a*). This indicates the presence of Na-pump independent transepithelial K pathways. These may include paracellular shunts. The high K permeability of the rat lingual epithelium suggests one possible explanation for the rapid adaptation of the KCl neural response. As seen in Fig. 7 there is a robust phasic neural response coinciding with the first current. However, the response adapts much faster than a NaCl response. It is possible that in the face of high apical concentrations of KCl, there is sufficient transcellular and paracellular K flux to raise the K ion concentration in the taste cell intersticies. This may interfere with repolarization of some of the afferent nerve fibers, and hence the attenuated response.

The results of this study indicate that NaCl taste transduction is initiated by the direct entry of sodium ions into the taste receptor cells. As we have shown, under voltage clamp a NaCl-evoked inward current can be measured. This would be expected to be a cell-depolarizing process, and the observation of a neural response temporally correlated with the first current indicates that events proceed in this manner. Under open-circuit conditions the inward current would be balanced by an equal but oppositely directed current presumably driven across paracellular shunts by the transepithelial potential. The magnitude of this potential would be strongly limited by shunt permeability. For this reason one expects the correlation between the transepithelial potential and neural response to be poorer than that between the current and the neural response. The results of this study confirm this.

After initial cell depolarization the processes that subsequently result in the release of neurotransmitter are still unclear. However, there is good indication that these events are mediated through voltage-gated sodium and calcium channels residing on the basolateral membranes of taste cells (Roper, 1983; Kashiwayanagi et al., 1983; Tonasaki and Funikoshi, 1984; Avenet and Lindemann, 1987, 1988). Adaptation would be expected to involve cell repolarization. In a few cases where the intracellular potential of the rat taste cell has been directly measured, a constant NaCl stimulation does produce an initial depolarization followed by a slower repolarization. (Ozeki, 1971). The similar kinetics of the second current relaxation and the neural response demonstrated herein point to the former as the source of repolarizing current. The complete suppression of the second current by ouabain indicates that it is strongly Na-pump dependent. On this basis we conclude that the cellular basis for adaptation of the NaCl taste response of the rat is the activation of basolateral electrogenic sodium pumps. Smith et al. (1978) postulated that the cellular process causing adaptation of the NaCl taste response should have the same exponential time course as neural adaptation. This study identifies such a process and offers evidence for its cellular origin. It may be further noted that recent work on spontaneously excitable cells indicates that receptor-mediated events may stimulate the cell sodium pump leading to hyperpolarization and decreases in spontaneous firing rate (Desilets and Baumgarten, 1986; Shah et al., 1988). This suggests the possibility that taste cell Na pumps could also play a role in the adaptation of receptor-mediated taste responses to sweet and bitter stimuli.

APPENDIX

Channel-pump model

The dynamics of changing cell sodium ion concentration and cell repolarization are best illustrated by a simple model. We assume that among the various ion-recognizing entities on the apical membranes of rat fungiform papilla taste-bud cells are sodium channels. Referring to the inset in Fig. 13 *A*, the presence of an apical membrane NaCl concentration, c_a, results in an inflow of Na ions, J_a. This initially depolarizes the cell. However, as the cell Na concentration, c, increases, the Na-pump is activated and Na is pumped out the basolateral side at the rate, J_b. This leads to cell repolarization at the pump-mediated rate. If we assume that neural firing rates decrease in proportion to the extent of repolarization, then the causal chain from pump to neural response is established. To compute the time dependence of both c and the cell potential is difficult even for the simplest of realistic constitutive relations for J_a and J_b. Therefore, we adopt a perturbation approach for simplicity and clarity.

We assume the short-circuited system has already achieved a steady state after stimulation of the apical side. We perturb the steady-state concentration, c_s, by lowering the internal concentration by a small amount and compute the consequent perturbation in the steady-state potential. We then follow the time-course of relaxation of both the concentration and the potential. Lowering the internal concentration increases the electrochemical potential difference for Na across the apical membrane. This increases the rate of Na entry across the apical membrane as would have been the case if the external Na concentration had been suddenly raised, thereby stimulating stimulation.

We take the flux across the apical membrane to be

$$J_a = -P(ce^{\phi} - c_a), \qquad \text{(A1)}$$

where P is the apical membrane permeability, c is the internal Na concentration, and ϕ is the normalized potential. $\phi = F\psi/RT$, where ψ is the membrane potential relative to either external compartment (short-circuited), and F, R, and T have their usual meanings. We have assumed P to be constant. In general P will vary depending on the potential profile across the apical membrane. However, if we assume a skewed potential profile similar to that of a collapsed electrical double layer where the potential remains close to zero over most of the membrane thickness and only deviates from zero close to the inner membrane boundary, then P approaches a constant. This is a reasonable approxi-

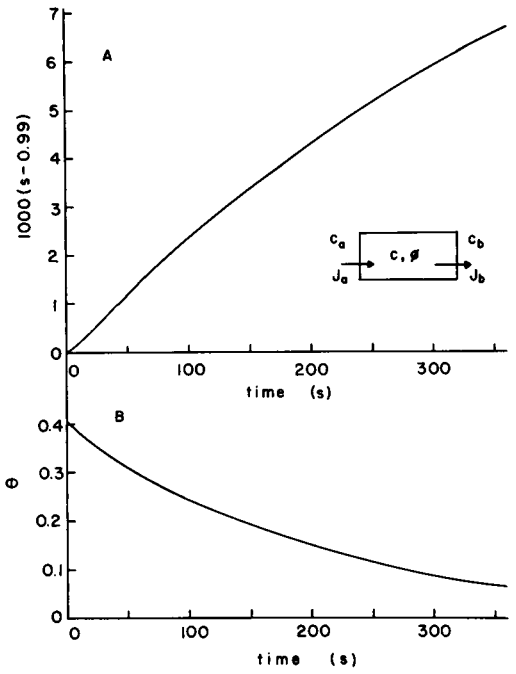

FIGURE 13 The relaxation in the normalized cell sodium concentration (A) and normalized potential (B). At time zero the internal normalized Na concentration was lowered from unity to $s_0 = 0.99$. To display the relaxation in concentration more clearly, the scale has been expanded as shown. At time zero there was a simultaneous depolarization in potential to $\theta_0 = 0.4$, i.e., about 10 mV as a consequence of the increased apical membrane Na electrochemical driving force. As Na enters across the apical membrane, it is pumped out the basolateral side and the cell repolarizes. The model shows how the second current acts as a repolarizing mechanism and therefore a precursor to neural adaptation (cf Fig. 2). It also shows how increasing the electrochemical driving force across the apical membrane by increasing the apical membrane Na concentration can initially depolarize the cell leading to neural excitation.

mation considering the high ionic strengths of the stimulating solutions, which, on the basis of electrostatic screening, would be expected to collapse the potential profile further.

On the basolateral side we take the pump rate to be

$$J_b = kc. \tag{A2}$$

This is, of course, only an approximation to an electrogenic Na pump. However, recent studies have shown the voltage-dependent step to be that of Na translocation (De Weer et al., 1988). Eq. A2 allows sodium always to be driven out of the cell as an ion, and in that respect Na translocation is the electrogenic step. Similarly, in that the outflux is independent of the basolateral Na concentration, c_b, it may occur against the basolateral Na electrochemical potential gradient, i.e., it may be active. However, we have ignored K dependence of the pump, coupling to metabolic energy sources, and basolateral K channels. This can be justified to some extent on the assumptions that metabolic substrates are abundant and that K ions will be circulated across the basolateral membrane in the actual system, and thus do not contribute a net flux during NaCl stimulation. This is essentially the approach of Huf and Howell (1976), who have used a similar constitutive relation to model the Na pump in frog skin.

The Na mass balance can be written as:

$$dc/dt = f[Pc_a - (Pe^{\phi} + k)c], \tag{A3}$$

where f is the area-to-volume ratio (assumed to be constant and the same at each membrane), and t is time. The steady-state solution is

$$c_s = c_a e^{-\phi}/(1 + ge^{-\phi}), \tag{A4}$$

where c_s and ϕ_s are the steady-state values of c and ϕ respectively, and $g = k/P$. The greater the pump rate constant k, in general, the lower will be the steady-state Na concentration within the cell. In principle, c_s can be estimated from the relation

$$c_s = I_{sc}/Fk, \tag{A5}$$

and with Eq. A4 ϕ_s can be obtained. For example in one experimental series 0.05 M NaCl gave $I_{sc} = 1.16\ \mu A/cm^2$ and 2 M NaCl gave $I_{sc} = 47.60\ \mu A/cm^2$. Assuming $P = 2.5 \times 10^{-7}$ cm/s and $g = 10$, $\phi_s(0.05) = -0.92$ (~ -23 mV), and $\phi_s(2) = -1.99$ (~ -50 mV). The P value used here is consistent with values used to model the Na permeability of tight epithelia (Lew et al., 1979) and the calculated potentials are consistent with available measured values (Ozeki, 1971).

To obtain a perturbation solution of Eq. A3, we write:

$$c = c_s + x \tag{A6}$$

$$\phi = \phi_s + \theta \tag{A7}$$

where x and θ are regarded as small perturbations from their respective steady state values. Substituting Eqs. A6 and A7 into Eq. A3 and discarding terms of higher order than one in x and θ, we can write

$$dx/dt + f(Pe^{\phi_s} + k)x = -fPe^{\phi_s}c_s\theta. \tag{A8}$$

The perturbations x and 0 are not independent of one another. In general,

$$x = x(\phi) = x(\phi_s + \theta). \tag{A9}$$

Expanding x in a power series in θ and truncating terms of order greater than unity gives

$$x = x(\phi_s) + y(t)\theta, \tag{A10}$$

where $x(\phi_s) = 0$, and

$$y = \left[\frac{\partial x}{\partial \phi}\right]_{\phi_s} \tag{A11}$$

Substituting Eq. A10 into Eq. A8, and collecting terms in y and θ gives

$$(y' + w)\theta + (\theta' + p\theta)y = 0, \tag{A12}$$

where $w = fPe^{\phi_s}c_s$, $p = f(Pe^{\phi_s} + k)$ and primes denote first time derivatives. Because θ and y are independent of one another, the coefficients of y and θ in Eq. A12 must be identically zero. This gives

$$\theta = \theta_0 e^{-pt} \tag{A13}$$

$$y = y_0 - wt, \tag{A14}$$

where θ_0 and y_0 are the initial values of θ and y, respectively. With Eqs. A6, A7, A10, A13, and A14, the solutions are

$$\phi(t) = \phi_s + \theta_0 e^{-pt} \tag{A15}$$

$$c(t) = c_s + (c_o - c_s - w\theta_o t)e^{-pt}, \quad \text{(A16)}$$

where c_0 is the initial value of c.

Both c and ϕ relax exponentially with the same rate constant $p = f(Pe^{\phi_s} + k)$. The dynamics of the system depend on the cell size through f, the apical Na permeability through P, the pump constant k, and both the apical Na concentration and the cell Na concentration through ϕ_s. It is noteworthy that Eq. A16 permits some deviation from simple exponential relaxation in the concentration in the form of an inflection point. Indeed inflection points were sometimes observed in the relaxation of I_{sc} as can be seen in Fig. 2 *D*. The analysis indicates that they arise because repolarization acts to accelerate sodium entry, which in turn further stimulates the pump.

We now demonstrate that a sudden lowering of the cell Na concentration from its steady-state value results in cell depolarization, i.e., increasing the apical membrane Na electrochemical potential difference initially depolarizes the cell much as would be expected were the apical Na concentration instead increased. This is most easily done by specific example. First we divide Eq. A16 through by c_s.

$$s = 1 + (s_o - 1 - at)e^{-pt}, \quad \text{(A17)}$$

where $s = c/c_s$ and s_o is its initial value, $a = fPe^{\phi_s}\theta_o$. Setting the second derivative of s to zero gives the inflection time, t_{in},

$$t_{in} = (s_o - 1)/a + 2/p. \quad \text{(A18)}$$

We pattern the example after the 2 M NaCl case of Fig. 2 *D*. Assuming $p = 0.005\ s^{-1}$, $t_{in} = 30$ s, and $s_o = 0.99$, then $a = 0.000027\ s^{-1}$. From the definitions of a and p it follows that

$$a/p = \theta_o/(1 + ge^{-\phi_s}). \quad \text{(A19)}$$

Using $\phi_s = -1.99$ and $g = 10$, we obtain $\theta_o = 0.4$, i.e., a lowering of the cell concentration by 1% from the steady-state value results in about a 10-mV depolarization. The relaxation back to steady state of the concentration and the potential is shown in Fig. 13. Since the relaxation in I_{sc} is proportional to the cell Na concentration, the model demonstrates that the second current can be a repolarizing current, and hence can account for neural adaptation. The model also offers some further insight into the dependence of the relaxation rate constant on concentration as shown in Fig. 4. For Na concentrations above ~0.3 M the rate constant is independent of concentration. However, at Na concentrations below 0.3 M, it is significantly greater in value than the high concentration limit. The model gives the rate constant as

$$p = f(Pe^{\phi_s} + k). \quad \text{(A20)}$$

With the aid of Eq. A4 this can be recast as

$$p = fPc_s/c_s. \quad \text{(A21)}$$

The controlling factor is the ratio of the apical Na concentration to the steady-state concentration. Evidently at low apical Na concentrations this tends to be higher than at high apical Na concentrations where it approaches a constant value. This is what might be expected if the pump constitutive relation were saturable (as it in fact appears to be [e.g., Garay and Garrahan, 1973]). In that event the pump rate would vary with load at low concentration, but at high apical Na concentration, the pump would saturate. In terms of the model we can think of a larger value of k in Eq. A20 at low apical Na concentrations and a smaller value of k at high apical Na concentrations. Effects such as these have recently been incorporated into a more detailed network thermodynamic simulation of the NaCl taste response by Fidelman and Mierson (1989). It can be further noted that although we chose to perturb the system by decreasing the steady-state concentration at fixed short-circuit conditions, an alternative approach would have been to vary the clamping potential as was done in some of the experiments presented earlier.

The channel-pump model, therefore, accounts for the specificity and kinetics of both the excitation and adaptation phases of the taste receptor response to NaCl. Excitation of the KCl response, on the other hand, depends on separate apical current pathways. KCl-evoked current probably exits the cells via Ba-blockable basolateral K channels, and may not, therefore, directly depend on pump activity.

The authors are grateful to Cheryl Williams and Mary Welter for their able technical assistance and to our colleagues S. DeSimone and S. Mierson for helpful suggestions.

This work is supported by National Institutes of Health grant NS-13767, the Campbell Institute for Research and Technology, and the Virginia Center for Innovative Technology.

Received for publication 24 August 1988 and in final form 9 January 1989.

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