Pharmacology Biochemistry & Behavior, Vol. 29, pp. 257–267. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Transport Pathways in Rat Lingual Epithelium

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Received 3 August 1987

SIMON, S. A., R. ROBB AND S. S. SCHIFFMAN. *Transport pathways in rat lingual epithelium.* PHARMACOL BIOCHEM BEHAV **29**(2) 257–267, 1988.—Measurements of ion transport across isolated lingual epithelium of rat were correlated with electrophysiological recordings from taste nerves. At hyperosmotic concentrations of NaCl, sodium ions enter the mucosal membrane of the isolated epithelium partially through an amiloride-inhibitable pathway and exit the serosal membrane through a Na^+–K^+-ATPase. At hyposmotic concentrations of KCl, potassium ions enter the mucosal membrane through a K^+ pathway that is inhibited by 4-aminopyridine and exit at the serosal membrane through a K^+ pathway that is inhibited by $BaCl_2$. The inhibition of sodium transport by amiloride and potassium transport by 4-aminopyridine is consistent with previously published electrophysiological recordings from the chorda tympani nerve bundle (CT) and recordings from nucleus of the solitary tract (NST) obtained here. The responses to NaCl are greater than the responses to KCl at equimolar concentrations over the entire concentration range both in epithelial and neural measurements. At hyposmotic concentrations of NaCl the epithelial responses include inward sodium and outward chloride components. Isolated rat tongue is only slightly stimulated by D-glucose or sucrose as are the CT and NTS responses. These data suggest that events in taste transduction can be understood, in part, by measuring the epithelial responses of isolated rat tongue.

Transduction Amiloride Taste Barium 4-Aminopyridine

MEASUREMENTS of ion transport across isolated lingual epithelium of dog, rabbit, and rat show that ions are both actively and passively transported across the tongue [7, 8, 11, 13, 21, 27–29]. Moreover, such epithelial responses correlate with events that underlie taste transduction [6, 13, 28]. Hyperosmotic concentrations of NaCl applied to the mucosal surface of mammalian tongue have been shown to stimulate both epithelial responses and responses in taste nerves. In rats, the response to NaCl has been partially inhibited by the diuretic, amiloride [4, 13, 26]. Inhibition by amiloride has been observed in: epithelial measurements from isolated rat tongue [13], recordings from the chorda tympani nerve bundle [5, 6, 13, 21, 33] and the nucleus of the solitary tract (NST) [25,26] of rats. In addition, amiloride has been shown to inhibit the NaCl response in isolated dog [6–8, 11, 27] and rabbit tongues [28] as well as in human psychophysical measurements, where the perceived taste intensity of NaCl is lowered by amiloride [26].

Since many of the neurophysiological studies and models of taste transduction were developed using the rat model [2, 6, 9, 10, 13, 17, 22, 24, 30, 32], the epithelial responses of rat tongue to various tastants, in addition to NaCl, were studied here and correlated with electrophysiological recordings from both the chorda tympani nerve (CT) and from the nucleus of the solitary tract (NST). The correlation of epithelial transport and neurophysiological data in rats was first reported by Heck *et al.* [13]. They showed that at hyperosmotic concentrations, Na^+ transport was partially inhibited by amiloride in a manner similar to amiloride's inhibition of the CT responses. We have extended the studies of Heck *et al.* [13] to characterize the entrance and exit pathways for K^+, Na^+ and, to a lesser extent, Cl^- at both hyperosmotic and hyposmotic concentrations. It was also found that the response of isolated rat tongue to saccharides was small compared to that of NaCl. This result is in agreement with previously published CT responses [3, 22, 24, 33] and with our measurements from the NTS. Potassium pathways in the mucosal and serosal membranes were characterized by utilizing the well known K^+ transport inhibitors, 4-aminopyridine and $BaCl_2$ [15,31]. Sodium pathways were studied using ouabain, a drug that is known to inhibit the Na^+–K^+-ATPase as well as amiloride. Our observations indicate a good correlation between epithelial responses elicited from the isolated rat tongue and the responses measured from gustatory nerves.

METHOD

Adult Sprague-Dawley rats were obtained from Charles

River Inc. For epithelial studies, the rats were sacrificed using sodium pentobarbital (150 mg/kg), and their tongues were excised. For neurophysiological studies, rats were anesthesized with sodium pentobarbital (48 mg/kg) and additional anesthesia was delivered as necessary. Salts and buffers were reagent grade. Amiloride, ouabain and bumetanide were obtained from Sigma Chemical Company and 4-aminopyridine (4-AP) from Aldrich Chemical Company.

Epithelial Studies

An excised section of rat tongue, with the muscle layer removed and that contained only filiform and fungiform papillae, was placed in an Ussing chamber (0.63 cm^2) between symmetrical solutions of a modified Krebs-Henseleit (K-H) buffer at 36°C. The composition of the K-H solution was: 129 mM NaCl, 5 mM KCl, 5 mM D-glucose, 1.5 mM $MgSO_4$, 2 mM $CaCl_2$, 5.3 mM TrisCl pH 7.4. All other solutions used in these experiments were buffered to pH 7.4 using either 5 mM HEPES or 2 mM TrisCl. The epithelial response to salts was generated by initially changing the K-H solution on the mucosal side with a solution that contained the lowest salt concentration. Then responses to higher concentrations were obtained by successive additions of more concentrated solutions. The experiments with D-glucose were always in a salt solution containing 50 mM NaCl, 2 mM TrisCl pH 7.4 (called "solution A"). This NaCl concentration is close to what is found in rat saliva (41.4 mM NaCl; [14]). Inhibition by amiloride of the NaCl responses was obtained by measuring the short circuit current, (I_{sc}) and open circuit potential (V_{oc}) with successively higher NaCl solutions containing 0.1 mM amiloride. Inhibitors of transport pathways including 5 mM $BaCl_2$, 5 mM 4-AP, 1 mM ouabain or 0.1 mM bumetanide were added from concentrated solutions to the solutions bathing the tongue. Transport was also measured when K-H in the serosal solution was replaced with a solution not containing Cl^-. The composition of this solution was: 129 mM Na Isethanate (NaIS), 2.5 mM K_2SO_4, 1.5 mM $MgSO_4$, 2.0 mM $CaSO_4$, 5 mM HEPES, 6 mM D-glucose pH 7.4.

The measurement of the short circuit current, I_{sc}, and the open circuit potential, V_{oc} has been described previously [27]. V_{oc} is defined with respect to the mucosal solution (which is designated to be 0 mV) and I_{sc} is defined to have a negative sign when cations flow from the mucosal to the serosal solution or anions flow from the serosal to the mucosal solution. Calomel electrodes in saturated KCl solutions, interfaced to agar bridges containing 0.15 mM NaCl or 0.15 M KCl, were used to measure V_{oc}. Platinum wires were used as current passing electrodes. These electrodes were connected to a voltage clamp circuit that compensated for the series resistance arising from the electrodes and the solutions under symmetric ionic conditions. The transepithelial resistance, R_m, was determined either by dividing V_{oc} by I_{sc} since the current versus voltage relationship was linear, or by injecting a known current through the tongue and measuring the deflection in V_{oc} (see Fig. 1). This latter method was exclusively used to calculate R_m when the I_{sc} was small.

The magnitude of V_{oc} and I_{sc} presented here are the directly measured values. This presentation of the data was done to facilitate comparisons with previous studies on lingual epithelia [7, 8, 27, 28]. However, since many of the measurements were performed using asymmetric solutions, where liquid junction or dilution potentials develop, corrections to the measured values are necessary to correctly interpret the data. The dilution potentials, V_{dil}, were measured by placing the calomel electrodes (in saturated KCl solutions) and agar bridges in each half of an Ussing chamber. The two half chambers were separated by a sheet of parafilm (American Can Company) to prevent direct mixing of the solutions. The two half chambers were joined through two salt-agar bridges and a Keithley 602 electrometer. The electrometer was zeroed when the two agar bridges and the solutions in the two half chambers contained identical solutions. To measure V_{dil}, one agar bridge contained the same solution that was present in the "mucosal" chamber and the other agar bridge contained the same solution that was present in the "serosal" chamber. In this manner the open circuit potential of a tongue bathed in asymmetric salt solutions is $V_{oc}-V_{dil}$. Since the current-voltage relation is linear, values for I_{sc} and R_m can be calculated from the given data. Steady state for V_{dil} ranged from values for V_{dil} from 3.1 mV for 400 mM KCl to −5.7 mV for 300 mM TrisCl. All other values were smaller.

Recordings From the NST

Recordings from the NST were done on anesthesized rats whose core temperature was maintained at 36°C. Solutions of tastants at 36°C, were flowed over the rat tongue as previously described [32]. Recordings were obtained from a microelectrode (1–3 MΩ) connected via a silver wire to a DAM-50 amplifier (WPI) and a window discriminator (Frederick-Haer). The output of the discriminator was connected to a computer and to a rate interval monitor (Frederick-Haer). The latter was connected to a strip chart recorder to obtain the analog signals. For computer analysis of the data, the number of action potentials 3 sec prior to the addition of the tastant was averaged and defined as the background activity. The total number of action potentials within 2 seconds after the first maximum (in bins of 100 msec) minus the background was taken as the response. After 2 sec the elicited response was in its tonic phase.

The procedure for generating dose-response curves to NaCl and KCl was as follows. The tongue was first adapted to unbuffered distilled water for 1 min. Then solutions of either NaCl or KCl buffered with 2 mM TrisCl to pH 7.4 were flowed over the tongue for 30 sec, whereupon the tongue was rinsed with distilled water and the procedure repeated twice more. The mean response of these three measurements was considered to be a single data point for one experiment. For responses to 4-AP, this compound was added to the KCl-TrisCl solutions and flowed over the tongue as a single solution after the control response of the KCl-TrisCl solution was obtained. Data are presented as mean±S.E.M.

RESULTS

Epithelial Studies

Symmetric solutions. Rat tongues bathed between symmetric solutions of K-H have the following epithelial parameters: $V_{oc}=9.8\pm0.7$ mV; $I_{sc}=-6.4\pm0.3$ $\mu A/cm^2$ and $R_m=1885\pm48$ $\Omega\cdot cm^2$ (n=18). These data are in good agreement with those obtained by Heck *et al.* [13]. With symmetric solutions of K-H, the addition of 1 mM ouabain to the serosal solution almost completely inhibited V_{oc} and I_{sc} after one hour without significantly changing R_m (Fig. 1). The current-voltage relation is linear to ±150 mV in the presence and absence of ouabain.

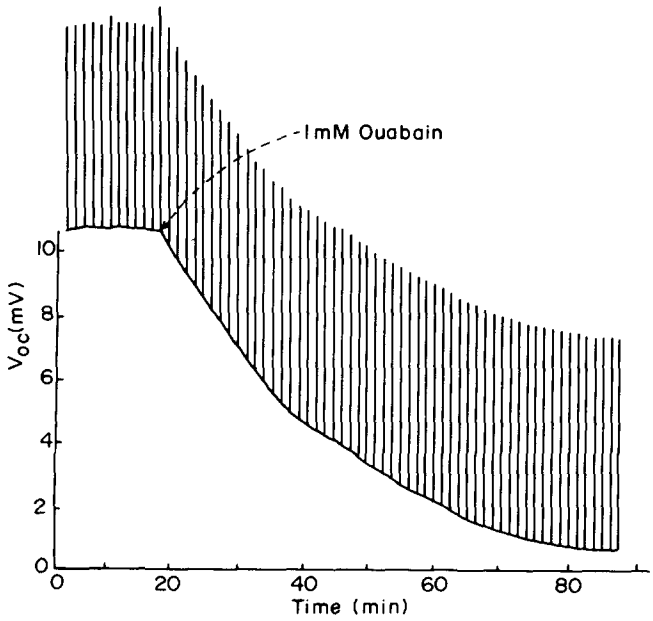

FIG. 1. Recording of open circuit potential, V_{oc} of a rat tongue bathed in symmetrical solutions of Krebs-Henseleit (K-H) buffer before and after the addition of 1 mM ouabain to the serosal solution (arrow). The upward excursions of the voltage trace are the consequence of current injections of 2 μA. The height of the excursions are proportional to the transepithelial resistance. The membrane area was 0.63 cm^2. Values of the short circuit current, I_{sc}, were -5.7 μA/cm^2 when ouabain was added (arrow) and -2.3 μA/cm^2 at 80 min.

Asymmetric solutions. The response of V_{oc} and I_{sc} to changes in NaCl (and KCl) in the mucosal solution are seen in Fig. 2A and B respectively. The magnitude of the responses of both V_{oc} and I_{sc} to NaCl is greater than for KCl between 1 mM and 1.0 M. For either NaCl or KCl, V_{oc} *decreases* with increasing concentration until $V_{oc} \approx 13$ mV for NaCl and 5 mV for KCl near isotonic concentrations. At higher concentrations V_{oc} increases significantly (for NaCl) to 16.9±0.3 mV (n=5) at 1.0 M and remains relatively constant for KCl (at 1.0 M KCl, V_{oc}=4.1±0.4 mV; n=4). I_{sc} remains constant at about -6 μA/cm^2 to 50 mM NaCl, whereupon it increases exponentially to -28 μA/cm^2 at 1.0 M NaCl (Fig. 2B). In comparison, the response of I_{sc} to KCl remains relatively constant at about -2 μA/cm^2 to 200 mM KCl, whereupon at higher concentrations it increases to about -5 μA/cm^2 at 1.0 M KCl. A dramatic decrease in the transepithelial resistance, R_m, is found with increasing NaCl or KCl concentrations. For NaCl, R_m decreased from 6082±687 Ω·cm^2 at 1 mM to 606±27 Ω·cm^2 (n=5) at 1.0 M. For KCl, R_m decreased from 6222±281 Ω·cm^2 (n=5) at 1 mM to 1167±304 Ω·cm^2 (n=5) at 1.0 M KCl. Thus, isolated rat tongue can discriminate between Na^+ and K^+ over the entire concentration range.

The responses of V_{oc} and I_{sc} to NaCl in the presence and absence of 1 mM ouabain in the serosal solution is seen in Fig. 3A and B respectively. From hyposmotic to isotonic concentrations V_{oc} remains positive and has about half the value as in the absence of ouabain. For hyperosmotic concentrations of NaCl, V_{oc} is inhibited about 15 mV by ouabain and thus attains a relatively constant potential close to 0 mV. The presence of 1 mM ouabain inhibits I_{sc} over the entire concentration range with the reduction most pronounced at hyperosmotic concentrations of NaCl (93% at 1.0 M). At 1.0 M NaCl, R_m is increased by ouabain to 1510±146 Ω·cm^2. The addition of 1 mM ouabain also decreased the responses of V_{oc} and I_{sc} to KCl (not shown). At 1.0 M KCl in the mucosal solution, the addition of 1 mM ouabain to the serosal solution reduced V_{oc} to 0.7±0.7 mV (n=3) and I_{sc} to -2.8±1.1 μA/cm^2 (n=3). To determine if a stable potential can be maintained in the absence of either mucosal Na^+ or K^+, V_{oc} and I_{sc} were also measured in the presence of 300 mM TrisCl pH 7.4. Under these conditions the steady state values of V_{oc} and I_{sc} were 12.9 mV and -11.6 μA/cm^2 respectively.

Effect of amiloride on epithelial responses. To explore further the transport pathways through rat tongue, responses to NaCl and KCl were studied in the presence of 0.1 mM amiloride. The response of V_{oc} to NaCl was inhibited about 5 mV over the entire concentration range (Fig. 2A). On the other hand, I_{sc} was not significantly affected by 0.1 mM amiloride for NaCl concentrations between 1 and 50 mM. However, at 500 mM NaCl, 0.1 mM amiloride inhibited I_{sc} to its maximal extent of about 30% (Fig. 2B). Amiloride did not significantly affect the response to KCl at hyperosmotic concentrations.

Effect of $BaCl_2$ on epithelial responses. When the mucosal solution contained 300 mM KCl and 2 mM TrisCl pH 7.4 and the serosal contained K-H, addition of $BaCl_2$ to the serosal solution inhibited both V_{oc} and I_{sc} in a concentration dependent manner (Fig. 4). The response of I_{sc} to $BaCl_2$ shows the presence of two discrete inhibitory processes. The first, which occurs between 30 μM and 100 μM $BaCl_2$, shows a slight but significant decrease in I_{sc}, whereas the second, which commences at about 0.5 mM $BaCl_2$, shows a linear decrease until complete (and irreversible) inhibition is attained. The response of V_{oc} is unchanged until a $BaCl_2$ concentration of 0.5 mM is reached, whereupon at higher $BaCl_2$ concentrations it decreases linearly.

Effect of 4-aminopyridine (4-AP) on epithelial response. The K^+-transport inhibitor 4-aminopyridine (4-AP) was applied to rat tongue to test for the presence of K^+ selective pathways [15]. In the presence of 50 mM KCl and 2 mM TrisCl pH 7.4 in the mucosal solution, the addition of 5 mM 4-AP to the mucosal solution completely inhibited both V_{oc} and I_{sc} after about 1 hour (Fig. 5). However, with 300 mM KCl and 2 mM TrisCl pH 7.4 in the mucosal solution, the addition of 5 mM 4-AP to either mucosal or serosal solutions did not significantly inhibit either V_{oc} or I_{sc}.

Effect of sodium isethanate (NaIS) on epithelial response. To determine the effect of Cl^- replacement on the I_{sc} and V_{oc} at different NaCl concentrations, dose-response curves with sodium isethanate (NaIS) on both the mucosal and serosal sides were obtained. Isethanate was selected since it is a large organic anion that is not transported through most chloride selective pathways [15]. The response of V_{oc} and I_{sc} with NaIS in the *mucosal* solution and with K-H in the serosal solution was similar to that with NaCl in the mucosal solution when corrections for the dilution potential were subtracted from V_{oc}. However, when the Cl^- in the serosal solution was replaced by IS^-, the dose-response curve to NaCl changed dramatically as shown in Fig. 3A and B. The largest difference between the response of V_{oc} and I_{sc} with and without Cl^- in the serosal solution is seen with 1 mM NaCl where V_{oc} is about 38 mV more negative and I_{sc} about 6 μA/cm^2 more positive. However, increasing the mucosal NaCl concentration, monotonically increases both V_{oc} and I_{sc} until they reverse sign (at about 300 mM NaCl) to

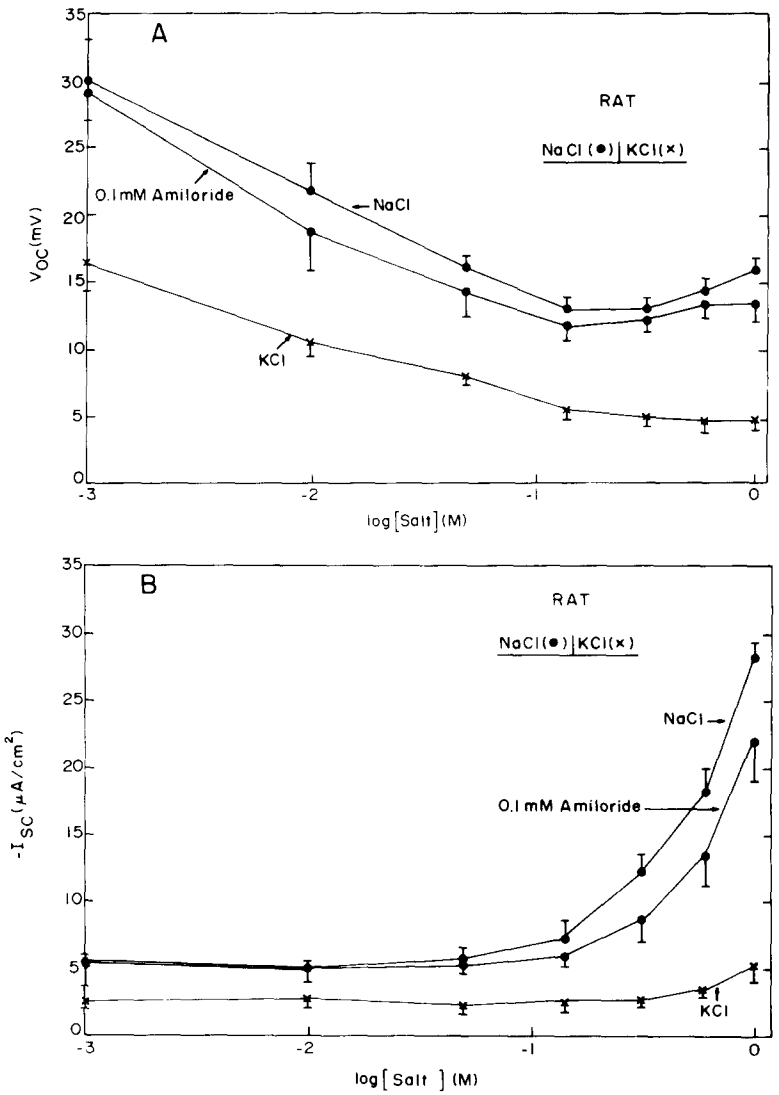

FIG. 2. (A) Graph of V_{oc} versus: NaCl (●); NaCl plus 0.1 mM amiloride; and KCl (×). The serosal solution in all three experiments contained Krebs-Henseleit buffer. All the mucosal solutions were buffered to pH 7.4 with 2 mM TrisCl. (B) Graph of the short circuit current, I_{sc} for same conditions given in A.

have the same sign as when the serosal solution contained K-H. Addition of 1 mM ouabain to the serosal solution containing NaIS slightly inhibited V_{oc} and I_{sc} (<1 mV and 1 μA/cm^2) at 1.0 M NaCl. These data suggest that the transport occurs via an ouabain-insensitive pathway [20].

Finally, with 300 mM KCl and 2 mM TrisCl pH 7.4 in the mucosal solution and K-H in the serosal solution, the addition of 0.1 mM bumetanide to the serosal solution did not significantly affect either V_{oc} or I_{sc}.

Effect of D-glucose and sucrose on epithelial response. Addition of D-glucose to a solution containing 50 mM NaCl and 2 mM TrisCl pH 7.4 (solution A) on the mucosal side of *dog* tongue greatly stimulates V_{oc} (22 mV) and I_{sc} ($\approx$15 μA/cm^2), and this stimulation is inhibited 80–90% by 0.1 mM amiloride [8,11]. In contrast, the addition of D-glucose (to 1.0 M) in solution A to rat tongue stimulates I_{sc} only about 1 μA/cm^2 and V_{oc} only about 2 mV (Fig. 6). The response of V_{oc} and I_{sc} to sucrose was not significantly different than the response to D-glucose. Also, in two experiments, 0.1 mM amiloride inhibited V_{oc} and I_{sc} by 20% at 1.0 M D-glucose. However, addition of 1 mM ouabain to the serosal solution completely inhibited transport (V_{oc}=0.7±1.2 mV; I_{sc}=−1.6± 1.4 μA/cm^2, n=3), when the mucosal solution contained solution A plus 1.0 M D-glucose.

Neurophysiological Recordings From the NST

Responses to NaCl and KCl. To correlate the epithelial responses from isolated rat tongue with those elicited in more distal regions, multiunit recordings from the NST were obtained for various concentrations of NaCl and KCl. The NST responses to 50 mM NaCl and 2 mM TrisCl pH 7.4 and to 50 mM KCl and 2 mM TrisCl pH 7.4 are shown in Fig. 7A and B respectively. The data are presented as a histogram whose ordinate represents the number of action potentials elicited per 100 msec and the abscissia consists of segments of 100 msec in width. These data represent the results of computer analysis of the analog signals seen in the inset of

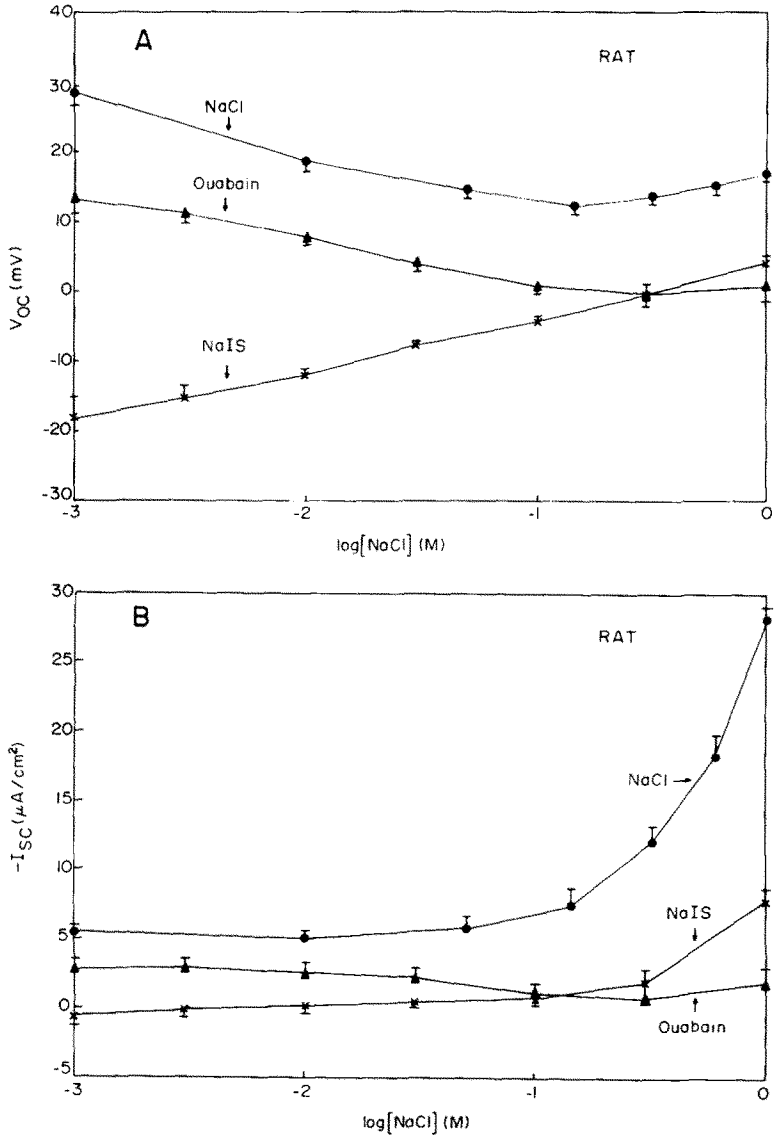

FIG. 3. Plots of V_{oc} (panel A) and I_{sc} (panel B) versus NaCl in the mucosal solution. The plot labeled "NaCl" (●) refers to tongues having NaCl in the mucosal solution and K-H in the serosal solution. The curves labeled "ouabain" (▲) show the response to NaCl in the presence of 1 mM ouabain in the serosal K-H solution. The curves labeled "NaIS" (×) refer to experiments where the NaCl in the serosal solution was replaced by the Na isethanate (NaIS) (see the Method section for additional details). In all experiments the mucosal solution contained a NaCl solution buffered to pH 7.4 by 2 mM TrisCl. The number of experiments for the "NaCl," "ouabain" and "NaIS" were 5, 3 and 3 respectively.

these figures. They are used to quantitate the response to salts. Both formats of the NST responses consist of two phases: a fast rising phase and a steady state phase. The NST responses, R, to different NaCl and KCl concentrations are presented in Fig. 8. The responses are about twice as great for NaCl than for KCl, a result in agreement with the epithelial responses (Fig. 2). The NST responses to NaCl and KCl can be described by a Langmuir isotherm that has the form: $R/R_{max}=Salt/(K_D+Salt)$ where R_{max} represents the maximum NST response in impulses/100 msec and K_D represents the apparent dissociation constant (in mM). When the above equation is linearized, a least squares fit to the mean values yields: $R_{max}=130.1$ impulses/100 msec and $K_D=17.3$ mM; $r=0.99$ for NaCl: $R_{max}=70.0$ impulses/100 msec and $K_D=24.4$ mM; $r=0.94$ for KCl.

Inhibition by 4-AP. The NST response to 50 mM KCl and 2 mM TrisCl pH7.4 was 98.6±8.5 impulses/100 msec (n=6) which decreases to 47.8±5.4 (n=6) in the presence of 5 mM 4-AP. In the presence of 300 mM KCl and 2 mM TrisCl pH 7.4 the NST response was 167.3±7.9 (n=6). This value is not significantly different than the NST activity of 160.8±9.5 (n=6) in the presence of 300 mM KCl and 2 mM TrisCl pH 7.4 plus 5 mM 4-AP.

Response to D-glucose and sucrose. The NST response to

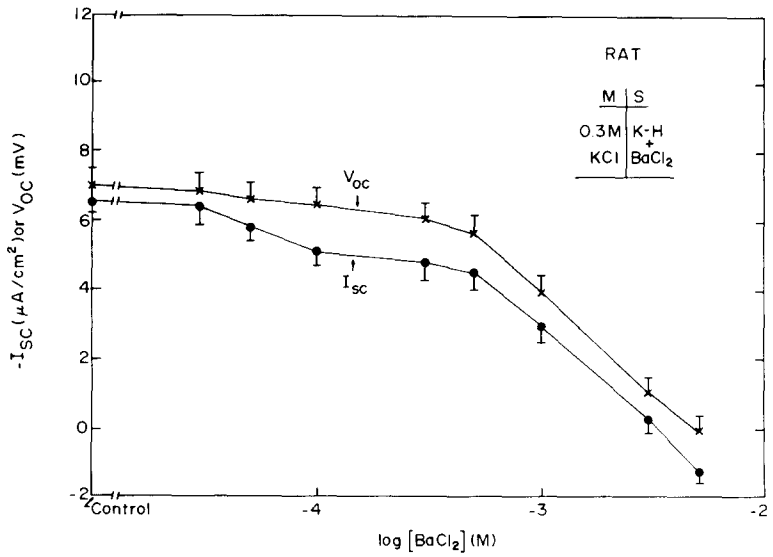

FIG. 4. Response of V_{oc} and I_{sc} to $BaCl_2$ added to K-H buffer in the serosal solution. The mucosal solution contained 300 mM KCl and 2 mM TrisCl pH 7.4. These results represent the mean±S.E.M. of 4 experiments.

solutions of 0.5 M D-glucose (25.6±18.4; n=4) or 0.5 M sucrose (20.4±15.1; n=4) was not significantly different from the background (15.6±36.2; n=4) responses.

DISCUSSION

The results of this study permit the identification of several of the entry and exit pathways for Na^+, K^+ and Cl^- through isolated rat tongue. A figure illustrating these pathways is shown in Fig. 9. Epithelial transport in isolated rat tongue together with recordings from the NST and previously published electrophysiological measurements from the CT suggest that ion transport through the rat lingual epithelium is correlated with events in taste transduction. An important finding of this study is that V_{oc} across rat tongue at low concentrations (<50 mM NaCl) is much larger (more positive) than found in dog or rabbit tongues. This result may explain the comparatively poor inhibition by amiloride in rat tongue for NaCl and saccharides.

Symmetric Solutions of K-H Buffer

In symmetric solutions of K-H, the transepithelial resistance, R_m, remains unchanged upon the addition of ouabain even though V_{oc} and I_{sc} are completely inhibited (Fig. 1). The complete inhibition by ouabain demonstrates that V_{oc} has its origin in the ion gradients generated by the Na^+–K^+-ATPase located in the serosal membrane (Fig. 9). The fact that R_m is not significantly changed by ouabain suggests that the transcellular resistance is significantly greater than the ouabain-insensitive resistances that are frequently associated with paracellular pathways (at least when Na^+ is the ion being transported) [20]. The linear current-voltage relationship in the presence of ouabain is also consistent with the notion that ouabain insensitive pathway is a paracellular pathway [20]. The above argument can be more readily understood by modeling the tongue as a circuit having two resistances in series, representing the resistance of the mucosal, R_a and serosal R_s membranes respectively in parallel with an

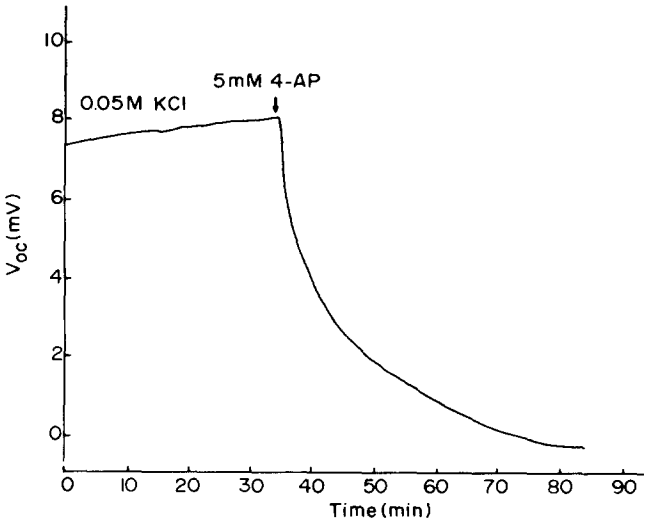

FIG. 5. Response of the open circuit potential, V_{oc} to the addition of 5 mM 4-aminopyridine (4-AP) (arrow) to a 50 mM KCl and 2 mM TrisCl pH 7.4 solution bathing the mucosal surface. The serosal solution contained K-H. At the time when 5 mM 4-AP was added (arrow) the I_{sc} was -4.1 $\mu A/cm^2$ and at 80 min was 0.16 $\mu A/cm^2$.

ouabain-insensitive resistance that is commonly associated with the paracellular pathway R_p. For such a circuit:

$$R_m=(R_a + R_s)\cdot R_p/(R_a + R_s + R_p) \quad (1)$$

If the cellular resistance, $(R_a + R_s)$, is $>>R_p$ then $R_m \approx R_p$. Therefore R_m would not be expected to change upon the addition of ouabain since ouabain would increase $(R_a + R_s)$.

With symmetric K-H solutions the epithelial characteristics of adult rat tongue have a higher R_m than either dog or rabbit tongue (Table 1) and hence can be readily distinguished from these two other epithelia on this basis. Active transport across dog tongue, like adult rat tongue, is com-

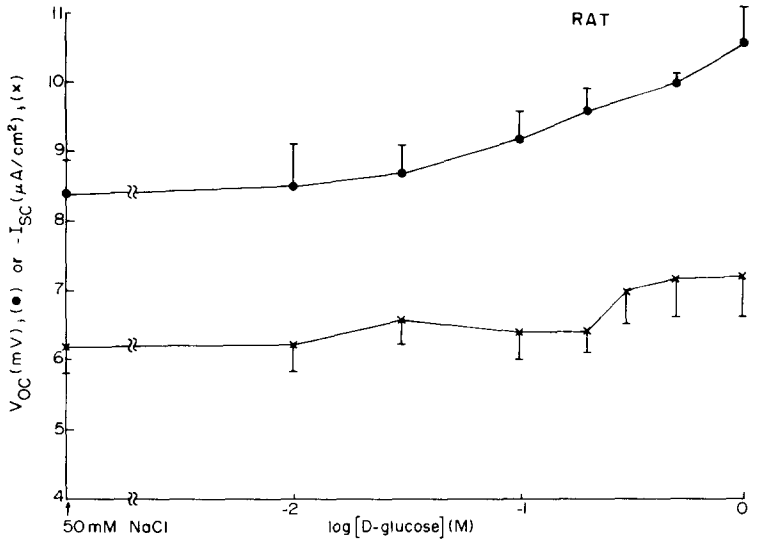

FIG. 6. Response of V_{oc} and I_{sc} to D-glucose. The mucosal solution contained 50 mM NaCl and 2 mM TrisCl pH 7.4 for all D-glucose concentrations. The serosal solution contained K-H. These data represent the mean±S.E.M. of 4 experiments.

pletely inhibited by ouabain but this is not the case for the rabbit tongue [27,28]. As shown previously, isolated lingual epithelia from different mammals respond differently to the same stimuli which indicates differences in their transport pathways [28].

Hyperosmotic Responses

The response of rat tongue to NaCl (Fig. 2) exhibits two regions that can be readily distinguished by the mucosal concentration. Hyperosmotic concentrations of NaCl will be considered first. This response is characterized by both a significant decrease in R_m and a large inhibition of I_{sc} by ouabain and amiloride (Figs. 2 and 3). These results also suggest that the hyperosmotic response arises from the inward flux of Na^+ via a cellular pathway. The inhibition by amiloride implies that there is a Na^+ entry pathway in the mucosal membrane and the inhibition by ouabain suggests Na^+ exits the tongue through the Na^+-K^+ATPase (Fig. 9). The entry of Na^+ into the taste cells will depolarize them and hence elicit a response to the CT and NST [2, 30, 32]. The partial inhibition by amiloride of hyperosmotic NaCl responses was also observed by numerous investigators in electrophysiological recordings from CT [5, 6, 9, 13, 21, 30, 33] or NST [26] in rats and by Jakinovich in recordings from the CT of gerbils [14]. These results, taken together, suggest that at least part of the Na^+ transport measured through isolated rat tongue is occurring through cells in taste buds. This assertion is consistent with the histochemical study of Zalewski [34] that demonstrated that the Na^+–K^+-ATPase is located primarily in the taste buds. The response to hyperosmotic concentrations of NaCl and its partial inhibition by amiloride is also seen in isolated dog [7, 8, 27] and rabbit tongues [28] and in psychophysical measurements in humans [26].

The comparatively small inhibition of the hyperosmotic KCl response by amiloride suggests that at hyperosmotic concentrations, K^+ and Na^+ enter (and also leave) rat tongue through different pathways (Fig. 9). This behavior is also observed in isolated dog [7, 8, 27] and rabbit tongues [28]. Measurements from the CT [13] and NST ([26], (Fig. 8)) in rats also suggest separate entry and/or exit pathways for Na^+ and K^+.

At hyperosmotic concentrations the epithelial responses are greater for NaCl than KCl, suggesting rat tongue is more permeant to Na^+ than K^+, perhaps as a result of the greater electrochemical gradient for Na^+ entry (see below). A larger response to NaCl than with KCl was also seen in recordings from the CT [2, 10, 17] and NST (Fig. 8). These data corroborate the correlation between epithelial responses from isolated tongue and electrical measurements from nerves associated with taste.

Hyposmotic Responses

At hyposmotic concentrations of NaCl and KCl, transport studies across isolated adult rat tongue show that: (1) the response of NaCl is greater than the response to KCl in the presence and absence of ouabain, and (2) for all concentrations of NaCl or KCl (with K-H on the serosal side), the algebraic signs of V_{oc} and I_{sc} do not change (Fig. 2). This latter behavior is not observed in dog [6, 7, 27] or rabbit [28] tongue where V_{oc} and I_{sc} change signs at 30–50 mM NaCl (or KCl) or in neonatal rats (unpublished observation).

Consider first that V_{oc} and I_{sc} are greater in the presence of NaCl than KCl. At the same chloride concentration the difference between the epithelial responses of these two salts must arise from the transport characteristics of Na^+ and K^+ *into* (and out of) rat tongue. Given that the intracellular potential of rat taste cells is −36 mV in 41.4 mM NaCl [24] and that the intracellular K^+/Na^+ ratio is about 10:1 [1, 15, 30], then the electrochemical gradient for K^+ entry will be less than that for Na^+ entry, a fact which may explain some of the differences between the responses of NaCl and KCl.

That ouabain inhibits about half of the hyposmotic response to NaCl [without changing the sign of V_{oc} or I_{sc} (Fig. 3)] suggests there is a net flux of Cl^- from the serosal to mucosal solution through ouabain insensitive pathways. The

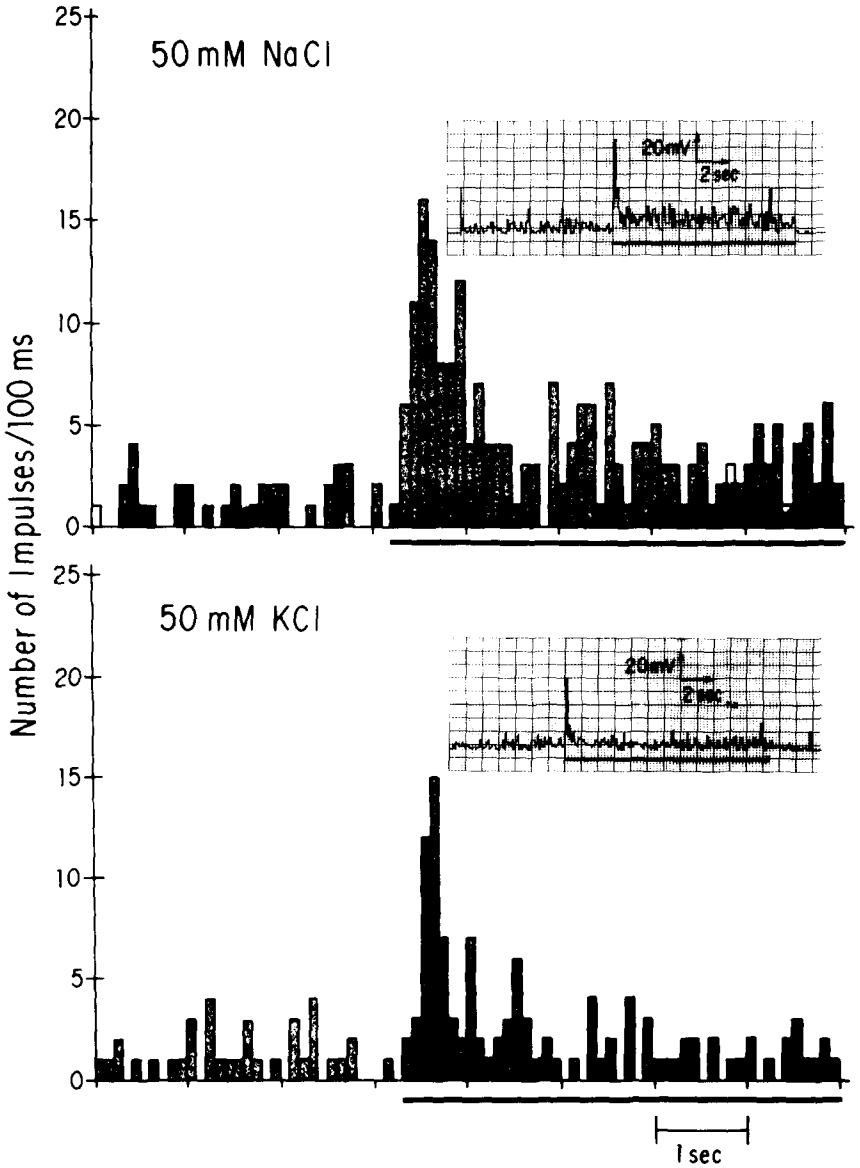

FIG. 7. Recordings from NST of rat. The ordinate represents the number of impulses (action potentials) per 100 msec. Each box is 100 msec in width. The solid line, located below the abscissa, represents the duration of the application of the taste stimuli. Upper panel: 50 mM NaCl and 2 mM TrisCl pH 7.4, 36°C. Lower panel: 50 mM KCl and 2 mM TrisCl pH 7.4, 36°C. The inserts represent the analog signals obtained on a strip chart recorder. The data in the inset was analyzed by the computer to yield the histograms.

presence of such an efflux is supported by the results of the experiment where the chloride ion was removed from the serosal solution and replaced with the relatively impermeant isethanate ion. If Cl^- were indeed contributing to the sign and magnitude of V_{oc} then it would be expected that its removal and replacement with IS^- in the serosal (but not mucosal) solution would decrease V_{oc} and increase R_m. This is indeed what was found to occur here. What is not known, at present, is if the Cl^- pathways which appear to predominate at hyposmotic concentrations, are through the cells or through a paracellular pathway that may be permeable to Cl^- but not to IS^-. If the paracellular pathway were relatively impermeable to IS^-, then the negative value of V_{oc} with hyposmotic NaCl concentrations in the mucosal solution and NaIS in the serosal solution arises from the cations in the serosal solution passively diffusing down their electrochemical gradients. From these considerations, as well as from the behavioral studies of Formaker *et al.* [9] that showed that the NaCl response contains a Cl^- component, chloride pathways were tentatively placed in both the mucosal and serosal membranes (Fig. 9).

A Na^+ pathway was also placed in the serosal membrane

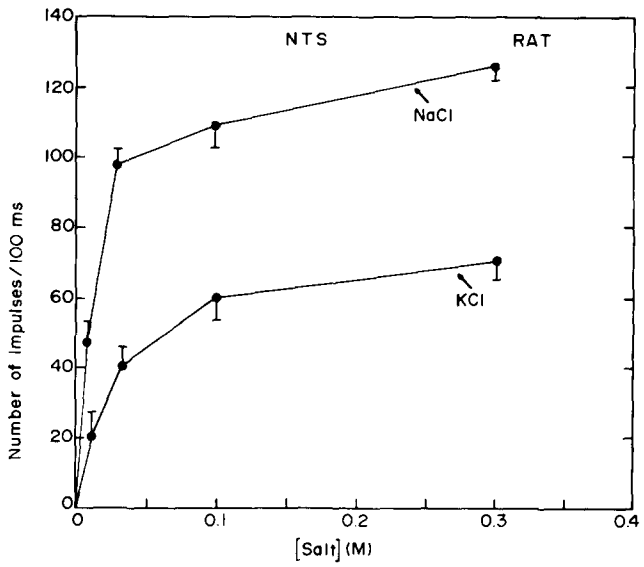

FIG. 8. Response of NST with NaCl and KCl solutions flowed over rat tongue. The responses are defined as the total number of impulses (action potentials) taken 2 sec after the first maximum (see Fig. 7) minus the average of the background responses 3 seconds prior to the addition of the stimuli. This graph was constructed from data such as those presented in Fig. 7.

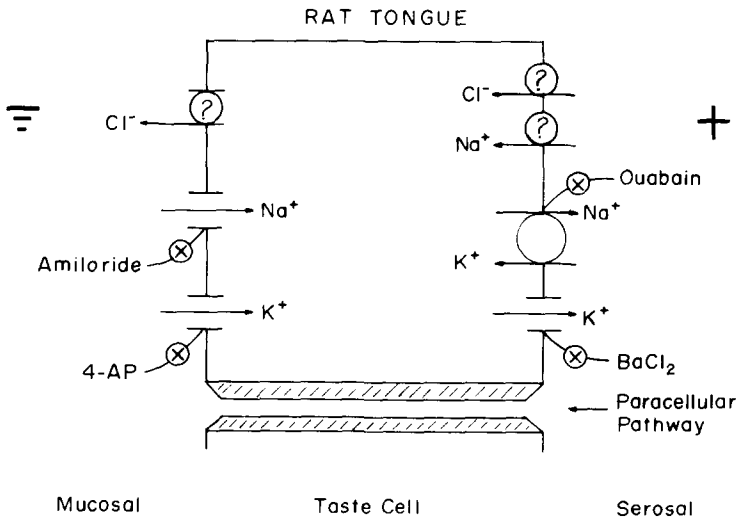

FIG. 9. Schematic diagram of transport pathways of Na^+, K^+ and Cl^- through rat tongue. The mucosal membrane is shown to contain three pathways: an amiloride inhibitable Na^+ entry pathway, a (4-aminopyridine) inhibitable K^+ entry pathway, and a possible Cl^- efflux pathway. The serosal membrane contains an ouabain inhibitable Na^+–K^+-ATPase, a Ba^{++} inhibitable K^+ exit pathway, and necessary Cl^- and Na^+ pathways that were placed in the serosal to complete the transport loops for Na^+ and Cl^- (see the Discussion section). The paracellular pathway is indicated. The + sign in the serosal solution indicates the sign of the potential relative to the mucosal side in symmetric solution of K-H buffer. The arrows indicate the usual direction of ion flow.

TABLE 1

EPITHELIAL RESPONSES OF DOG, RABBIT AND RAT TONGUES IN KREBS-HENSELEIT BUFFER (T=36°)

	Dog*	Rabbit†	Rat‡
V_{oc} (mV)	11.8	6.6	9.8
$-I_{sc}$ (μA/cm^2)	16.6	26.7	6.4
R_m (Ω·cm^2)	782	247	1885

*[23]; [24]†; ‡this work.

to explain the stability of V_{oc} and I_{sc} in the absence of mucosal Na^+ or K^+ (i.e., when the mucosal solution contained 0.3 M TrisCl). If such a Na^+ pathway were not present in the serosal membrane, the Na^+–K^+-ATPase would "pump" out all the intracellular Na^+, and the I_{sc} would decline with time. Hence, a pathway that recycles Na^+ back into the cell from the serosal solution is required. The fact that the I_{sc} can be inhibited by ouabain when KCl is on the mucosal side also suggests the presence of such a Na^+ recycling pathway. It is not certain if the serosal Na^+ and Cl^- pathways are separate or part of a self-exchange or co-transport protein. The inability of serosal bumetanide to alter the transport characteristics in the presence of 0.3 M KCl in the mucosal solution suggests that a Na^+–K^+–$2Cl^-$ co-transporter is not present on the serosal membrane [12]. We are aware that the diagram of the rat tongue presented in Fig. 9 represents only a partial description of the transport pathways present and that further work is necessary to describe fully the transport responses of rat tongues.

The maximum value of V_{oc} was found at 1 mM NaCl and hence was lower for all higher NaCl concentrations. However, Heck *et al.* [13] reported in their experiments on isolated rat tongue that V_{oc} *increased* when the mucosal NaCl concentration was increased from 1 mM NaCl to 1.5 M NaCl. The reason for the discrepancy between their data and ours is not evident especially in light of the good agreement of V_{oc}, I_{sc} and R_m obtained in symmetric solutions of K-H.

A comparison of epithelial responses and the neurophysiological recordings from the NST and CT at hyposomotic concentrations of NaCl and KCl show that they are in general agreement. At 0.1 M concentrations of NaCl and KCl, several investigators [3, 5, 17, 22] found that the CT responses were at least three times larger for NaCl than KCl. The NST measurements obtained in this work (Fig. 8) show that the response to NaCl is about twice that for KCl for all concentrations. The differences in the magnitude of epithelial responses (at hyperosmotic concentrations) to NaCl and KCl are also in a ratio of 2–3 (Fig. 2). However, despite the agreement in the relative magnitudes of the responses, the CT [3, 5, 10] and NST ([25], Fig. 8) responses to NaCl do not have the same functional form as the epithelial responses (Fig. 2). Moreover, the differences are most pronounced in the hyposmotic concentration range. Although the reasons for these differences are not well understood, it appears that the NST and CT responses reflect primarily the influx of Na^+ (perhaps through more than one Na^+ pathway [10]), whereas the epithelial responses contain contributions from both anions and cations. Since more than one Na^+ pathway may be involved in the NST response, the values of K_D and R_{max} for NaCl and KCl obtained from the NTS data in Fig. 8, which assumes the cations are interacting with a single transport pathway, may not yield a precise physical interpretation regarding the nature of transport pathways. However, this analysis may be useful in cataloging and comparing different data sets.

Inhibitors of KCl Transport

$BaCl_2$. To elucidate the entry and exit pathways for K^+, two K^+-transport inhibitors, $BaCl_2$ and 4-AP were tested on rat tongue. The inhibitory effect of $BaCl_2$ of the transport in the presence of 300 mM KCl suggests that K^+ is exiting rat tongue through a K^+ selective pathway in the serosal membrane (Figs. 4,9). Such Ba^{++} inhibitable K^+ pathways in serosal membranes have been observed in numerous epithelia [30]. This K^+ pathway was not inhibited by the addition of 5 mM 4-AP to the serosal K-H buffer and thus is distinct from a 4-AP inhibitable pathway.

4-Aminopyridine (4-AP). The response of the rat tongue in the presence of 50 mM KCl in the mucosal solution was completely inhibited by the addition of 4-AP to the mucosal solution (Fig. 5). Kim and Mistretta [18] reported that 4-AP inhibited the CT response to 50 mM KCl and the results reported here from NST are consistent with their findings on the epithelial studies. These data suggest the presence of a K^+-pathway [15,31] in the mucosal membrane (Fig. 9) which is directly involved in the response to KCl. Further agreement between the epithelial and neural responses was found when 5 mM 4-AP only minimally inhibited the epithelial, CT [18] and NTS responses in the presence of 300 mM KCl. It is possible that 4-AP and K^+ are competitive with each other or that the K^+ pathway at hyperosmotic concentrations is distinct from the one at hyposmotic concentrations as previously suggested [7,10]. It is well established that cells can have more than one K^+ pathway [19,30].

Response to D-Glucose and Sucrose

The addition of D-glucose or sucrose to isolated rat tongue only slightly stimulates V_{oc} and I_{sc} (Fig. 6) and moreover the small stimulation is only slightly inhibited by amiloride. This small stimulation by saccharides of the CT responses in rats is well established [3, 22, 24] and the NST results presented here are entirely consistent with them as are the epithelial studies. Interestingly, amiloride also does not inhibit the CT responses to saccharides in rats [33] or gerbils [16].

In comparing the magnitude of I_{sc} and the amiloride inhibition for saccharides and NaCl in rat tongue with those of dog tongue, it was found that the amiloride inhibition in dog tongue is much greater than in rat tongue under identical conditions. For example, in dog tongue bathed in 1.0 M NaCl I_{sc} is about 80 μA/cm^2 [27], and it is inhibited about 60% by 0.1 mM amiloride [7,11]. In contrast, in rat tongue at 1.0 M NaCl Isc$\approx$20 μA/cm^2, and it is inhibited only about 20% by amiloride. About the same ratios of I_{sc} and amiloride inhibition are seen in the responses to D-glucose [7,27]. The difference in the magnitude of I_{sc} can be rationalized if either the number or type of transport pathways were different. However, simply having a different number of transport pathways will not explain the difference between the amiloride inhibition in dog and rat tongue if they were the same pathway. Although different hyperosmotic Na^+ entry pathways may indeed explain the differences, amiloride inhibition could also arise if the membrane potential of dog and rat taste cells were not the same. Although the membrane potential of taste cells in rats have been measured to be -36 mV [24] the intracellular potential in dog taste buds has not,

to our knowledge, been measured. However, given that: (1) V_{oc} is about 20–30 mV greater in rat tongue than dog tongue with 50 mM NaCl in the mucosal solution; (2) V_{oc} is proportional to the potential across the mucosal membrane; and (3) that the measured potential in rat taste cells of −36 mV is 20–30 mV more positive (depolarized) than it is in other taste cells [1, 23, 30] and many other epithelial cells [31], it is reasonable to assume that the intracellular potential is also more negative (hyperpolarized) in the taste cells in dog tongue than in rat tongue. This difference in membrane potential could explain the smaller inhibition by amiloride in rats since amiloride inhibition has been shown to be dependent on the membrane potential [31]. Since amiloride is a cation, the greater the membrane potential (inside negative), the greater will be its binding to the receptor given that the amiloride binding site is in the electric field.

In summary, several of the entry and exit pathways for Na^+, K^+ and Cl^- through rat tongue have been described. The number, type and selectivity of these entry (and exit) pathways determine the epithelial response to a given tastant. The entry and/or exit of these ions from the taste cells will in turn elicit responses from the CT nerves and also neurons in the NST. The drugs that inhibit responses in isolated epithelium also inhibit recordings from taste nerves.

ACKNOWLEDGEMENTS

We thank Drs. R. Anholt, J. M. Gill and W. Jacobs for numerous suggestions and Ms. Ellen Eatmon for excellent secretarial assistance. This work was supported by grants NS20669, AG-00443 and a grant from the Campbell Soup Company.

REFERENCES

1. Avenet, P. and B. Lindemann. Action potentials in epithelial taste receptor cells induced by mucosal calcium. *J Membr Biol* **95:** 265–269, 1987.
2. Beidler, L. M. A theory of taste stimulation. *J Gen Physiol* **38:** 133–139, 1954.
3. Beidler, L. M., I. Y. Fishman and R. W. Hardiman. Species differences in taste responses. *Am J Physiol* **181:** 235–239, 1955.
4. Benos, D. Amiloride: A molecular probe of sodium transport in tissues and cells. *Am J Physiol* **242:** C131–C145, 1982.
5. Brand, J. G., J. E. Teeter and W. L. Silver. Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Res* **384:** 207–214, 1985.
6. DeSimone, J. A. and F. Ferrell. Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. *Am J Physiol* **249:** R52–R61, 1985.
7. DeSimone, J. A., G. L. Heck and S. K. DeSimone. Active transport in dog tongue: a possible role in taste. *Science* **214:** 1039–1041, 1981.
8. DeSimone, J. A., G. L. Heck, S. Mierson and S. K. DeSimone. The active ion transport properties of canine lingual epithelial in vitro. *J Gen Physiol* **83:** 633–656, 1984.
9. Formaker, B. K., D. L. Hill and P. S. Lassiter. An analysis of the suppressed NaCl taste responses following amiloride. *Soc Neurosci Abstr* **12:** 1351, 1986.
10. Frank, M. E., R. J. Contreras and T. P. Hettinger. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. *J Neurol Physiol* **50:** 941–996, 1983.
11. Garvin, J. L., R. Robb and S. A. Simon. Spatial map of salts and saccharides on dog tongue. *Am J Physiol*, in press, 1988.
12. Geck, P. and E. Heinz. The Na-K-2Cl co-transport system. *J Membr Biol* **91:** 97–105, 1986.
13. Heck, G. I., S. Mierson and J. A. DeSimone. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science* **223:** 403–405, 1984.
14. Hiji, Y. Gustatory response and preference behavior in alloxan diabetic rats. *Kumamoto Med J* **22:** 109–118, 1969.
15. Hille, B. *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Assoc., 1984.
16. Jakinovich, W. Stimulation of the gerbil's gustatory receptors by methyl cyclopyranosides. *Chem Sense* **10:** 591–604, 1985.
17. Katida, Y., R. M. Bradley and C. M. Mistretta. Maintenance of chorda tympani salt taste responses after nerve transection in rats. *Brain Res* **302:** 163–170, 1984.
18. Kim, M. and C. M. Mistretta. Effects of potassium channel blockers on rat chorda tympani nerve responses. *Chem Senses* **12:** 671, 1987.
19. Kocsis, J. D., T. R. Gordon and S. G. Waxman. Functional differences between 4-aminopyridine and tetraethylammonium-sensitive potassium channels in myelinated axons. *Neurosci Lett* **75:** 193–198, 1987.
20. Moreno, J. H. and J. M. Diamond. Discrimination of monovalent inorganic cations by "tight" junctions of gallbladder epithelium. *J Membr Biol* **15:** 277–318, 1974.
21. Ninomiya, Y., T. Mizukoshi, T. Higashi and M. Funakoshi. Differential responsiveness of two groups of rat chorda tympani fibers to ionic and electrical tongue stimulations. Proceedings of the 18th Japanese Symposium for the Study of Taste and Smell, edited by K. Ueda, 1984, pp. 145–148.
22. Ogawa, H., M. Sato and S. Yamashita. Multiple sensitivity of chorda tympani fibers of the rat and hamster to gustatory and thermal stimuli. *J Physiol (Lond)* **199:** 223–240, 1968.
23. Roper, S. D. Regenerative inpulses in taste cells. *Science* **220:** 1311–1312, 1983.
24. Sato, T. and L. M. Beidler. The response characteristics of rat taste cells to four basic stimuli. *Comp Biochem Physiol* **73A:** 1–10, 1982.
25. Schiffman, S. S., S. A. Simon, J. Gill and T. G. Beeker. Bretylium tosylate enhances salt taste. *Physiol Behav* **36:** 129–137, 1986.
26. Schiffman, S. S., E. Lockhead and F. W. Maes. Amiloride reduces the taste intensity of Na^+ and Li^+ salts and sweeteners. *Proc Natl Acad Sci USA* **80:** 6136–6140, 1983.
27. Simon, S. A. and J. L. Garvin. Salt and acid studies on canine lingual epithelium. *Am J Physiol* **249:** C398–C408, 1985.
28. Simon, S. A., R. Robb and J. L. Garvin. Epithelial response of rabbit tongue and their involvement in taste transduction. *Am J Physiol* **251:** R598–R608, 1986.
29. Soeda, H. and F. Sakudo. Electrical responses to taste chemicals across the dorsal epithelium of bullfrog tongue. *Experientia* **41:** 50–51, 1985.
30. Teeter, J., M. Funakoshi, K. Kurihara, S. Roper, T. Sato and K. Tonosaki. Generation of the taste cell potential. *Chem Senses* **12:** 217–234, 1987.
31. Wills, N. K. and A. Zweifach. Recent advances in the characterization of epithelial ionic channels. *Biochim Biophys Acta* **906:** 1–31, 1987.
32. Woolston, D. C. and R. R. Erickson. Concept of neuron types in gustation in the rat. *J Neurophysiol* **42:** 1390–1409, 1979.
33. Yoshi, K., Y. Kobatake and K. Kurihara. Selective enhancement and supression of frog gustatory responses to amino acids. *J Gen Physiol* **77:** 373–385, 1981.
34. Zalewski, A. A. Role of nerve and epithelium in the regulation of alkaline phosphatase activity in gustatory papillae. *Exp Neurol* **23:** 18–78, 1969.