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The identity of the current carriers in canine lingual epithelium in vitro

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Ion transport across the lingual epithelium has been implicated as an early event in gustatory transduction. The fluxes of isotopically labelled Na^+ and Cl^- were measured across isolated canine dorsal lingual epithelium under short-circuit conditions. The epithelium actively absorbs Na^+ and to a lesser extent actively secretes Cl^-. Under symmetrical conditions with Krebs-Henseleit buffer on both sides, (1) Na^+ absorption accounts for 46% of the short-circuit current (I_{sc}); (2) there are two transcellular Na^+ pathways, one amiloride-sensitive and one amiloride-insensitive; (3) ouabain, added to the serosal solution, inhibits both I_{sc} and active Na^+ absorption. When hyperosmotic (0.25 M) NaCl is placed in the mucosal bath, both I_{sc} and Na^+ absorption increase; net Na^+ absorption is at least as much as I_{sc}. Ion substitution studies indicate that the tissue may transport a variety of larger ions, though not as effectively as Na^+ and Cl^-. Thus we have shown that the lingual epithelium, like other epithelia of the gastrointestinal tract, actively transports ions. However, it is unusual both in its response to hyperosmotic solutions and in the variety of ions that support a transepithelial short-circuit current. Since sodium ion transport under hyperosmotic conditions has been shown to correlate well with the gustatory neural response, the variety of ions transported may likewise indicate a wider role for transport in taste transduction.

Introduction

Ion transport across the lingual epithelium has been proposed as an early event in gustatory transduction [1]. Amiloride, an inhibitor of Na^+ transport in many epithelial tissues, reduces the short-circuit current (I_{sc}) in the presence of mucosal hyperosmotic NaCl in an in vitro preparation of rat or canine dorsal lingual epithelium; it also specifically blocks the chorda tympani response to NaCl, but not to KCl, in rats [2,3]; it inhibits single unit responses in the rat nucleus tractus solitarius and it selectively attenuates NaCl perception in humans [4]. The inhibitory effect of amiloride and preliminary ion substitution experiments in vitro [1] suggested the presence of a Na^+ transport pathway. However, the identity of the current carriers could not be conclusively identified without directly measuring ion fluxes using radioactively labelled isotopes. That is the purpose of this paper.

Hyperosmotic NaCl solutions, up to 1.0 M, increase I_{sc} and transepithelial potential difference (PD) and decrease resistance (R) in the canine dorsal lingual epithelium [3]. In the hyperosmotic range, amiloride at 10^{-4} M in the mucosal solution reduces I_{sc} by approx. 70%. However, in symmetrical configuration with Krebs-Henseleit buffer on both sides, amiloride reduces the I_{sc} by less than 50%. This finding suggested two possibilities for the symmetrical configuration: (1) the I_{sc} consists of 50% Na^+ absorption and 50% absorp-

tion of another cation or secretion of an anion whose transport is not affected by amiloride, or (2) the I_{sc} is equal to the Na^+ flux, as it is in some other epithelial tissues, but there are two separate Na^+ pathways, one amiloride-sensitive and one amiloride-insensitive. To distinguish between these two possibilities, we performed ion flux measurements under symmetrical conditions with the tissue short-circuited. We report here that neither of the above possibilities turned out to be correct. In fact, in symmetrical Krebs-Henseleit buffer the amount of net Na^+ absorption is equal to less than half the value of I_{sc}; and furthermore, the net Na^+ absorption is only partially inhibited by amiloride, indicating the presence of two transcellular Na^+ transport pathways. (For a preliminary report of this work, see Ref. 5).

We report in this paper that the dorsal lingual epithelium actively absorbs Na^+ and to a lesser extent actively secretes Cl^-. Sodium absorption is inhibited by amiloride in the mucosal solution or by ouabain in the serosal side.

In an attempt to identify the remaining current carriers, we conducted ion substitution experiments, in which various ions were substituted for the constituents of the bathing solutions. We show here that the epithelium is capable of sustaining a short-circuit current under symmetrical conditions with a wide variety of larger cations and anions, though not as effectively as with Na^+ and Cl^-.

Methods

Animals and bathing solutions

Mongrel dogs, 15–25 kg in weight, were anesthetized with sodium pentobarbital and killed by exsanguination. The preparation of the dorsal lingual epithelium, stripped of its connective tissue and adherent muscle layers, has been described previously [3]. Unless specified otherwise, the tissue was bathed on both sides in Krebs-Henseleit buffer consisting of 118 mM NaCl/5.6 mM KCl/1.9 mM $CaCl_2$/1.2 mM $MgSO_4$/1.3 mM NaH_2PO_4/25 mM $NaHCO_3$/5.6 mM glucose; pH was 7.4 when the solution was bubbled with 95% O_2/5% CO_2. Bicarbonate-free buffer consisted of 142 mM NaCl/5.6 mM KCl/1.9 mM $CaCl_2$/1.2 mM $MgSO_4$/0.18 mM NaH_2PO_4/1.12 mM Na_2HPO_4/5.6 mM glucose, and was bubbled with 100% O_2. Chambers were kept at 34°C.

Chemicals and drugs

All chemicals were reagent grade. Ouabain octahydrate was obtained from Sigma Chemical Co. (0-3125) and SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid) was obtained from U.S. Biochemicals. The following were gifts: amiloride from Dr. E.G. Cragoe of Merck, Sharp and Dohme; bumetanide from Dr. P. Sorter of Hoffman-LaRoche, Inc.; Diamox (acetazolamide) from Dr. R. Caspari of Lederle Laboratories; and methazolamide from Dr. T.H. Maren, University of Florida (Gainesville). ^{22}Na and ^{36}Cl were from New England Nuclear; test solutions were 0.2–0.3 μCi/ml.

The compounds used in the ion substitution experiments were sodium acetate, sodium gluconate, tetramethylammonium chloride, tetramethylammonium methylsulfate, choline chloride, Tris chloride, Tris acetate, Tris nitrate, Tris benzoate, and arginine hydrochloride. With the exception of the Tris salts, all solutions were 0.15 M with no adjustment for pH or osmolarity. The Tris compounds were adjusted to pH 7.4 as follows: the solution was made up as 0.126 M Tris nitrate, as an example. Tris base was added to bring the solution up to pH 7.4. This resulted in a final Tris concentration of 0.149 M. Mannitol was added to make the total osmolarity 0.3 osM. For Tris acetate, Tris nitrate and Tris benzoate, the concentration of anion was 0.126 M, Tris was 0.14–0.15 M, and mannitol was 0.025–0.030 M. For Tris chloride, Cl^- was 0.126 M and Tris was 0.174 M, with no mannitol added.

Flux measurements and voltage-clamp system

Two tissues from one tongue were mounted in lucite chambers specially designed to minimize chamber volume and to permit rapid changes in both bathing solutions (via a vacuum system). The epithelium was mounted as a flat sheet between two lucite half-chambers having exposed areas of 1.77 cm^2 and volumes of 0.9 ml each. The chamber design was modified from that of Biber and Mullen [6]. It was not possible to minimize edge damage; chamber designs with a Silastic gasket leaked, presumably due to the stiffness of the filiform papillae in this epithelium. Hence the tissue was clamped directly between the lucite surfaces and the half-chambers held tightly in

place with wing-nuts. This produced somewhat lower transepithelial resistance than previously reported. When the value for resistance given by DeSimone et al. [3] is corrected for solution resistance, the value in this study represents a 16% decrease in the value of the tissue resistance.

The potential difference across the tissue was measured by calomel electrodes connected to the solution by 3% agar-saline bridges. A second pair of bridges connected to sintered Ag-AgCl pellets was used for passing current through the tissue. The potential difference (PD), short-circuit current (I_{sc}), and resistance (R) were determined by using a dual-channel automatic voltage clamp, built by one of us (G.L.H.), that was adjustable for changes in fluid resistance between the PD bridges.

The I_{sc} was monitored continuously on a two-channel strip-chart recorder (Watanabe). Resistance was determined by pulsing current for 1 s (± 10 mV) and voltage was calculated from the values of I_{sc} and R. The V–I curves had previously been determined to be linear [3]; periodic checks showed the measured value of PD to be always within ± 0.5 mV of the calculated value.

For all experiments other than flux measurements, the chamber design was the same as that used previously [3], i.e., chamber volume of 7 ml, silicone rubber gaskets, and the two halves held together with a turn-screw brace. Stirring was provided by a magnetic stirrer on each side as well as by bubbling gas. Electrical parameters were determined by using a single channel voltage clamp (Custom Control VCC 600) and monitoring I_{sc} on a strip chart recorder (Gould/Brush model 105).

Statistics

Results are expressed as means $\pm$ S.E.; n is given in parentheses. Differences were considered significant if the P value, calculated from the paired Student's t-test, was less than 0.05.

Results

Flux studies in symmetrical configuration

Table I lists the electrical parameters and Na^+ and Cl^- fluxes in symmetrical Krebs-Henseleit buffer. The unidirectional mucosal to serosal Na^+ and Cl^- fluxes were measured simultaneously across one tissue and the opposite unidirectional fluxes of these ions were measured across a second tissue from the same tongue. The electrical resistances did not vary more than 15% for each tissue pair; short-circuit currents and open-circuit PD's for paired tissues were not significantly different. Steady-state rates of accumulation of ^{22}Na and ^{36}Cl were reached within 45 min of the addition of the isotopes and were maintained for at least 30 min (two collection periods). Labelled solutions were changed at least once an hour. ^{22}Na radioactivity was determined by gamma counter (Beckman γ 3000). Subsequently, total ^{22}Na and ^{36}Cl radioactivity was determined by liquid scintillation counting (Beckman LS335). Specific activities of all test solutions in a given series were identical.

TABLE I

ION TRANSPORT AND ELECTRICAL PARAMETERS IN SYMMETRICAL KREBS-HENSELEIT BUFFER

Values are means $\pm$ S.E.; $N = 19$ (pairs of tissues). The values were obtained 30 min after the equilibration period. $J_{M \to S}$, unidirectional mucosal to serosal flux; $J_{S \to M}$, unidirectional serosal to mucosal flux. J_{net}, net flux. Positive value for J_{net} indicates absorption. J_{net}^R, residual, or unmeasured, ion flux, $I_{sc}/F - (J_{net}^{Na} - J_{net}^{Cl})$. Unidirectional Na^+ and Cl^- fluxes are simultaneous measurements for paired tissues; electrical parameters for each tissue pair were averaged and counted as single value.

I_{sc} =	36.5 ± 2.7	$\mu A \cdot cm^{-2}$	$J_{M \to S}^{Cl}$ =	0.68 ± 0.06 $\mu equiv. \cdot cm^{-2} \cdot h^{-1}$
=	1.36 ± 0.10	$\mu equiv. \cdot cm^{-2} \cdot h^{-1}$	$J_{S \to M}^{Cl}$ =	0.83 ± 0.06 $\mu equiv. \cdot cm^{-2} \cdot h^{-1}$
R =	396 ± 26	$\Omega \cdot cm^2$	J_{net}^{Cl} =	−0.15 ± 0.07 $\mu equiv. \cdot cm^{-2} \cdot h^{-1}$
PD =	13.4 ± 0.5	mV		
$J_{M \to S}^{Na}$ =	1.15 ± 0.09	$\mu equiv. \cdot cm^{-2} \cdot h^{-1}$	J_{net}^R =	0.56 ± 0.05 $\mu equiv. \cdot cm^{-2} \cdot h^{-1}$
$J_{S \to M}^{Na}$ =	0.51 ± 0.05	$\mu equiv. \cdot cm^{-2} \cdot h^{-1}$	J^{Na}/I_{sc} =	0.46 ± 0.06
J_{net}^{Na} =	0.64 ± 0.10	$\mu equiv. \cdot cm^{-2} \cdot h^{-1}$		

Net Na^+ absorption accounted for 46% ± 6% of the I_{sc}. Net Cl^- secretion was small but statistically significant ($P < 0.05$, paired Student's t test). Approx. 41% of the I_{sc} was due to transport of an ion (or ions) other than Na^+ or Cl^-.

Hyperosmotic NaCl

Fig. 1 illustrates the effects of varying the mucosal NaCl concentration on Na^+ fluxes, Cl^- fluxes, and electrical parameters in isolated canine dorsal lingual epithelium. Krebs-Henseleit buffer was maintained on the serosal side throughout. To balance junction potentials so that the epithelium was clamped at zero throughout, the voltage clamp was set to 0.0 mV for buffer, 0.5 mV for 0.15 M NaCl, and 3.3 mV for 0.25 M NaCl. These values were calculated from Eqn. 1 of Barry and Diamond [7] using activities, and from the Henderson equation [8]. When the mucosal solution was changed from buffer to 0.15 M NaCl, electrical parameters and net Na^+ flux stayed relatively constant; net Cl^- secretion disappeared, presumably due to a Cl^- gradient across the tissue which counter balanced the small amount of Cl^- secretion present. (Cl^- concentration in buffer 1 is 126.9 mequiv./l). When the mucosal solution was replaced by hyperosmotic (0.25 M) NaCl solution, I_{sc} and *PD* increased, *R* decreased, and net Na^+ absorption and net Cl^- absorption increased due in both cases to an increase in mucosal (M) to serosal (S) unidirectional flux. The proportion of I_{sc} carried by Na^+ ions was 54 ± 14% for symmetrical buffer, 62 ± 12% for 0.15 M NaCl, and 105 ± 11% for 0.25 M NaCl. The residual ion flux (for definition of this term, see Table I) was 0.34 ± 0.06 μequiv./cm^2 per h in buffer, 0.43 ± 0.05 μequiv./cm^2 per h for 0.15 M NaCl, and 0.22 ± 0.12 μequiv./cm^2 per h for 0.25 M NaCl. The last value was not significantly different from zero (Student's t-test).

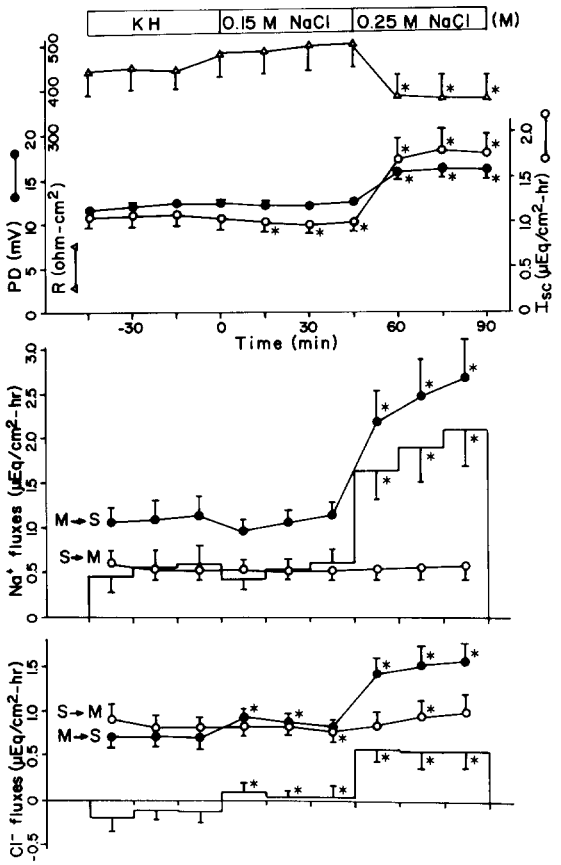

Fig. 1. Effects of varying the mucosal solution on electrical parameters (*PD*, potential difference; I_{sc}, short-circuit current; *R*, resistance), Na^+ fluxes, and Cl^- fluxes in isolated canine dorsal lingual epithelium ($n = 7$). M → S, unidirectional flux from mucosal to serosal side. S → M, unidirectional flux from serosal to mucosal side. The histogram represents net flux. *, significant difference from values at zero time ($P < 0.05$, paired Student's t-test). K-H, Krebs-Henseleit buffer.

Effects of ouabain

Ouabain in the serosal solution decreased the I_{sc} and *PD*, as shown in Fig. 2. Net Na^+ absorption decreased, due both to a decrease in M to S unidirectional flux and to an increase in S to M unidirectional flux. After 1½ h of exposure to ouabain, I_{sc} had decreased by 94 ± 3%, and net Na^+ flux had decreased by 77 ± 8%. The decrease in I_{sc} was 1.45 ± 0.18 μequiv./cm^2 per h; the decrease in Na^+ flux was 0.50 ± 0.11 μequiv./cm^2 per h. Ouabain in the mucosal solution had no effect on electrical parameters.

Effects of amiloride

Fig. 3 illustrates the effects of amiloride on electrical parameters and ion fluxes. I_{sc} and PD decreased; net Na^+ absorption decreased due both to a decrease in M to S unidirectional flux and an increase in S to M unidirectional flux. After 1 h of exposure to amiloride, I_{sc} had decreased by 37 ±

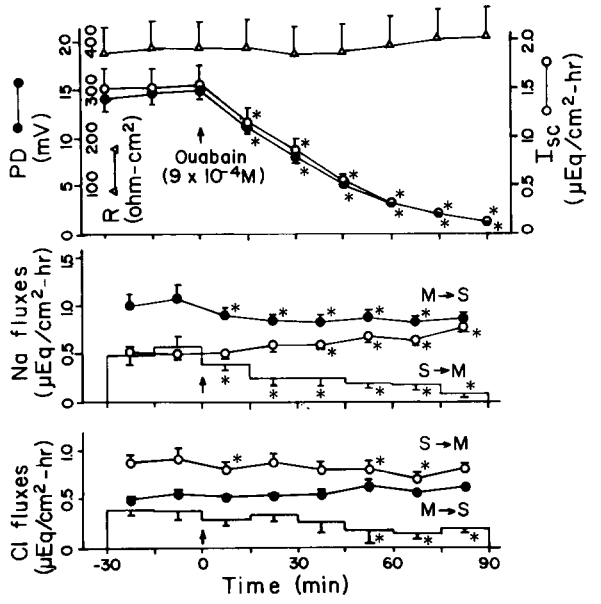

Fig. 2. Effects of ouabain, $9 \cdot 10^{-4}$ M, on electrical parameters, Na^+ and Cl^- fluxes in isolated canine dorsal lingual epithelium ($n = 6$). Krebs-Henseleit solution was on both sides of tissue. At zero time, ouabain was added and maintained in the serosal solution. All symbols represent the same as in Fig. 1.

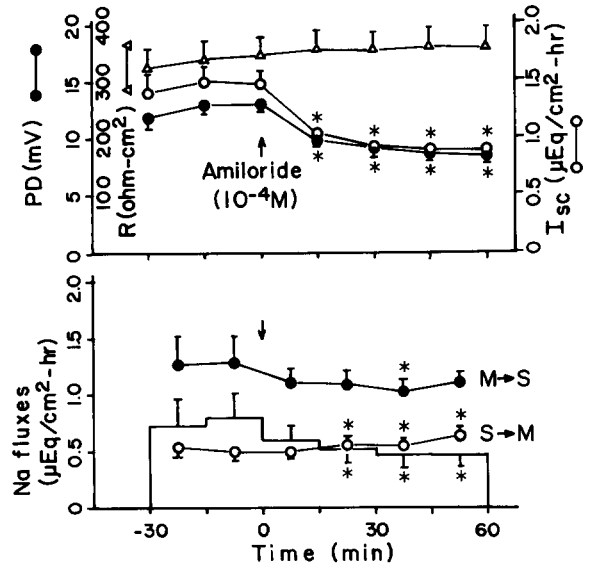

Fig. 3. Effects of amiloride, $1 \cdot 10^{-4}$ M, on electrical parameters and Na^+ fluxes in isolated canine dorsal lingual epithelium ($n = 6$). Krebs-Henseleit solution was on both sides of the tissue. At zero time, amiloride was added and maintained in the mucosal solution. All symbols represent the same as in Fig. 1.

5%; net Na^+ flux had decreased by 38 ± 8%. The decrease in I_{sc} was 0.58 ± 0.12 μequiv./cm^2 per h; the decrease in net Na^+ flux was 0.33 ± 0.13 μequiv./cm^2 per h. Residual ion flux decreased from 0.64 ± 0.08 μequiv./cm^2 per h to 0.35 ± 0.02 μequiv./cm^2 per h.

The percent decrease in I_{sc} due to amiloride varied considerably from one tissue to another. For six tissues, the effect ranged from 22% to 53% inhibition of I_{sc}. The percent decrease in net Na^+ absorption correlated well with the percent decrease in I_{sc}. There was also variation in the percent of I_{sc} attributable to Na^+ absorption from one pair of tissues to another; however there did not appear to be any relationship between the percent of I_{sc} attributable to Na^+ absorption and the percent inhibition of I_{sc} due to amiloride.

Effects of other inhibitors

It was apparent from preliminary ion substitution studies (Ref. 1, and unpublished results) that ion coupling influences the ion fluxes and I_{sc} across this tissue. Hence, we tried using a variety of transport inhibitors based on ion coupling schemes thought to occur in other epithelia. A typical protocol to measure effects of inhibitors was (1) to measure the electrical parameters in symmetrical buffer; (2) to record a hyperosmotic response by measuring I_{sc} when the mucosal solution was changed from 0.03 M NaCl (which produces approximately a null potential across the tissue [3]) to 0.50 M NaCl; (3) to return to symmetrical buffer until I_{sc} reached a steady state; (4) to repeat (2) and (3) to make sure the hyperosmotic response was reproducible; (5) to add inhibitor to one or both sides of the chamber for some suitable period of time (usually 20–60 min) to watch for a change in I_{sc}; (6) to repeat the hyperosmotic response to see if the magnitude of the change in I_{sc} was affected. Hence, we looked both for changes in electrical parameters in symmetrical buffer configuration, and for effects on the hyperosmotic response. The inhibitors examined were (1) SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid), which blocks anion exchange in many cells including epithelia [9], $1 \cdot 10^{-4}$ M; (2) bumetanide, which blocks Na,Cl co-transport in a variety of tissues [10,11,12], 10^{-4}–10^{-3} M; (3) acetazolamide (Di-

amox) and (4) methazolamide, both carbonic anhydrase inhibitors [13], 800 μM. Acetazolamide and methazolamide were used both in normal buffer and in HCO_3^--free buffer. Each of these inhibitors produced little or no effect.

Ion substitution experiments

We performed a variety of ion substitution experiments to determine (a) the contribution of individual ions to the I_{sc}, *PD*, and *R*, and (b) the extent of ion coupling in the transport mechanisms for this tissue. In all experiments for which single cation and anion species were substituted for buffer on both sides, the solutions were bubbled with 100% O_2 instead of the 95% O_2/5% CO_2 mixture. Whenever the solutions on the mucosal and serosal sides were not identical in electrolyte content (asymmetrical conditions), saturated KCl agar bridges were substituted for the agar-saline bridges to minimize junction potentials; solutions were changed frequently to avoid K^+ accumulation. All experiments were begun with a symmetrical buffer configuration. As an initial test of tissue ion sensitivity, the hyperosmotic response was recorded by replacing the mucosal solution with 0.03 M NaCl, which gave a steady-state baseline, followed by a 0.5 M NaCl stimulus. Sensitivity was verified by a biphasic response function of increased I_{sc} with time (see Fig. 8 in Ref. 3). All tissues were short-circuited.

A typical protocol for an ion substitution experiment was as follows. (1) Determine I_{sc} and *R* in symmetrical buffer bubbled with 95% O_2/5% CO_2. Open-circuit *PD* was usually calculated from I_{sc} and *R*; however, in some cases the *PD* was measured directly and that was the value used. (2) Replace the buffer on both sides with 0.15 M NaCl and bubble with 100% O_2. (3) Replace the 0.15 M NaCl on one or both sides with, for example, 0.15 M Tris benzoate. Observe the changes of the three electrical parameters with time. (4) Return to symmetrical 0.15 M NaCl. This sequence could be repeated a number of times with different solutions for the same tissue. The tissue was returned periodically to symmetrical buffer to replenish the substrate supply and to check for viability. Each time a solution was changed the chamber compartment was rinsed several times to insure a thorough exchange of solution in the extracellular spaces.

Replacing buffer with 0.15 M NaCl on both sides of the tissue produced no significant changes in electrical parameters. If left in symmetrical 0.15 M NaCl over a period of hours, I_{sc} would decay, presumably due to lack of metabolic substrate. However, this problem could easily be avoided by returning the tissue to buffer 1 periodically to allow I_{sc} to recover.

These experiments yielded a number of surprising results. Fig. 4 represents a typical response to symmetrical Tris benzoate. I_{sc} initially decreased until it reached a minimum value at point A; subsequently I_{sc} rebounded and reached a maximum at point B; it then decreased asymptotically to a final value close to that at point C. From one experiment to another the time-courses were slightly different; however, in every case for symmetrical Tris benzoate it was possible to identify a minimum, a maximum, and a final reading for I_{sc}. It was convenient to compare each of these three values to the initial value of i_{sc} in 0.15 M NaCl for that experiment, so that each tissue served as its own control. If the tissue was allowed to stand too long in the new solution, eventually I_{sc} decreased further and did not recover, suggesting damage to the tissue. To avoid this, the tissue was generally returned to NaCl within 15 min. Hence the time

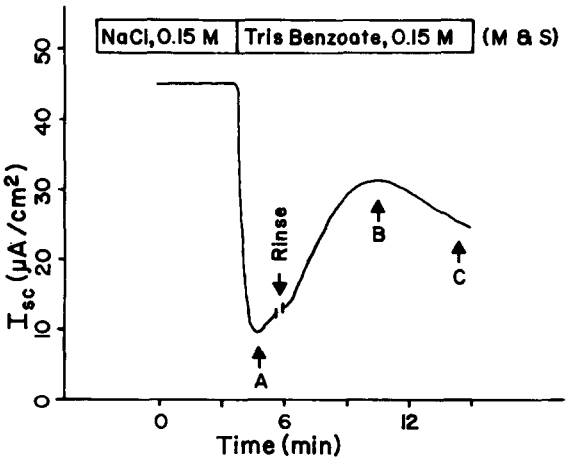

Fig. 4. Tracing of representative response of canine dorsal lingual epithelium to 0.15 M Tris benzoate placed on both sides of the tissue. At the indicated time 0.15 M NaCl was replaced by Tris benzoate. A indicates a 'minimum' in the I_{sc}, B a 'maximum' in the I_{sc}, and C the 'final' reading of the I_{sc}.

point at which the 'final' reading (see Fig. 4 and Table II) was taken was somewhat arbitrary. It was taken at a point at which I_{sc} was relatively steady. Nevertheless, there was consistency in the percent decrease of I_{sc} at point C among tissues for a given compound.

For six experiments with Tris benzoate, I_{sc} at point A decreased an average value of 67% compared to control, at point B it decreased an average value of 34% compared to control, and at point C it decreased an average value of 44% compared to control. *R* and *PD* were determined at the same time-points. These data are tabulated for ten different salts in Table II. The salts are arranged in order of increasing ionic weight of the cation, and, for each cation, in order of increasing ionic weight of the anion. Though the specifics vary for each salt, some general characteristics stand out. First, the minimum value (value at A) for I_{sc} for each salt was always less than in NaCl, yet was never zero or even close to zero. Since these experiments were performed in symmetrical solutions, the results indicate that the epithelium may be capable of transporting these large (i.e., larger than Na^+ and Cl^-) cations and anions, though not as effectively as Na^+ and Cl^-. Second, in each case I_{sc} rebounded to some extent from its minimum value. This suggests adaptation phenomena occurring in the epithelium. We have presented these data because the results were so unexpected, even though for some compounds no more than one or two experiments were performed.

In a second series of experiments, the solution was changed on the mucosal side or the serosal side only, usually done in succession on the same tissue separated in time by a period in symmetrical NaCl. After an initial increase or decrease, depending on the experiment, there was evidence of adaptation similar to that seen in the symmetrical substitutions. Of six salts used, only Tris chloride reduced the I_{sc} by more than 50% at any of the time points, and even then the I_{sc} rebounded after an initial minimum. On the mucosal side, four salts (sodium acetate, tetramethylammonium chloride, tetramethylammonium methylsulfate, and Tris chloride) caused an initial decrease in the I_{sc}; two salts (sodium benzoate and sodium gluconate) caused a small increase in I_{sc}. On the serosal side, four salts (sodium acetate, sodium benzoate, sodium gluconate, and tetramethylammonium methylsulfate) caused an initial decrease in I_{sc}, while two (tetramethylammonium chloride and Tris chloride) caused an initial increase in I_{sc}. Two compounds (sodium acetate and tetramethylammonium methylsulfate) produced a decrease in I_{sc} whether applied from the mucosal or the serosal side of the epithelium. All salts produced an increase in *R* when applied to the mucosal side; when applied to the serosal side the changes in *R* were much smaller.

In another set of experiments, we compared the electrical properties of the epithelium in bicarbonate-free buffer to those in Krebs-Henseleit buffer. Both the electrical properties in symmetrical configuration and the hyperosmotic response were essentially the same with and without HCO_3^- when compared in the same tissue.

Discussion

The canine dorsal lingual epithelium actively absorbs Na^+, comprising approx. half the short-circuit current when the epithelium is bathed with buffer on both sides. A lesser amount of Cl^- is actively secreted; however, the remaining current carrier(s) remains unidentified. (Simon and Garvin [14] have confirmed that in the portion of the canine dorsal lingual epithelium posterior to the portion we have used, there is both Na^+ and Cl^- active transport.) 30–40% of the current is due to an ion other than Na^+ or Cl^-, both under control conditions and after inhibition with amiloride. The reduction in net Na^+ absorption due to either amiloride or ouabain is less than the corresponding reduction in I_{sc}. For each inhibitor the percentage decrease in I_{sc}, the percentage decrease in net Na^+ absorption, and the percentage decrease in net residual flux are of comparable value. It is possible that transport of the other ion(s) is dependent on Na^+ transport.

Bicarbonate ion is a likely candidate for a current carrier. Circumstantial evidence suggests that HCO_3^- is transported by the human lingual epithelium. The possibility that a carbonic anhydrase is present in the lingual epithelium was indicated when patients being treated with the carbonic anhydrase inhibitors acetazolamide or methazolamide reported a curious taste of carbonated

beverages [15]. Swenson and Maren [16] hypothesized that this was due to the taste of CO_2 which would normally be rapidly dissipated by catalysis due to carbonic anhydrase. This catalysis could occur in the lingual epithelium; or after the CO_2 diffuses through to the red blood cells, which are known to contain carbonic anhydrase; or in both the epithelium and the red blood cells. If bicarbonate is transported by the epithelium, the transport could be via either a conductive or a nonconductive pathway. Our results show that neither acetazolamide nor methazolamide affects a conductive pathway in the in vitro canine epithelium. A possibility to account for the tasting of CO_2 in the presence of an inhibitor is that carbonic anhydrase in the epithelium contributes to a nonconductive pathway, in which case a carbonic anhydrase inhibitor would not affect the I_{sc}. Our results also indicate that if HCO_3^- is transported by this epithelium, the canine epithelium does not appear to require an exogenous supply of HCO_3^-.

The canine lingual epithelium has a tissue resistance which is intermediate between 'tight' and 'leaky' epithelia. The tissue may in fact be considerably tighter electrically than indicated by the specific resistance reported here. We have calculated the specific resistance using the cross-sectional area of the chamber opening. However, this does not take the filiform and fungiform papillary structure into account. Doing so would undoubtedly increase the area, and hence the specific resistance, several-fold.

This time required for the effect of amiloride on the tongue depends on experimental conditions. In vivo, the effect on taste occurs rapidly. Heck et. al. [2] observed inhibition of the response to NaCl solutions in the rat following a 5 min pretreatment with amiloride in deionized water; Teeter et al. [17], working in the gerbil, used a 2 min pretreatment period. DeSimone and Ferrell [34] have observed that the rat chorda tympani response to 0.5 M NaCl is reduced by 50% after only a 2 s pretreatment with amiloride in deionized water. If the vitro lingual epithelium is exposed to 0.5 M NaCl, a 20 s pretreatment with amiloride in 0.001 M NaCl is sufficient to elicit 50% of the decrease in I_{sc} of the hyperosmotic response. When amiloride is added directly in isosmotic buffer in vitro (this paper), the effect takes longer, probably because the presence of Na^+ in the bathing medium competes for the inhibitory site.

This work represents the first evidence that there are two independent transcellular pathways in the lingual epithelium for Na^+, one amiloride-sensitive and one amiloride-insensitive. More than half the active Na^+ absorption in symmetrical buffer is not sensitive to amiloride. The amiloride-insensitive Na^+ flux corresponds to the amiloide-insensitive component of I_{sc} in vitro. It probably corresponds as well to the amiloride-insensitive component of the neural response in vivo [2] and to the amiloride-insensitive component of the response to NaCl in human taste experiments [4]. An analysis of the kinetics of inhibition by amiloride of the rat integrated chorda tympani suggests that there are two types of lingual Na-transport system, one amiloride-insensitive and one amiloride-sensitive, and that the latter is the major transport route under hyperosmotic conditions [34]. It is the amiloride-sensitive component of I_{sc} in vitro that is activated at high NaCl concentrations [3].

Other mammalian epithelia have also displayed sizable amiloride-insensitive sodium-transport pathways. Among these are the dog tracheal epithelium [18] and the rat colon [19]. In the rat colon there are two transcellular routes for Na^+ absorption. Amiloride-insensitive pathways predominate in Na-replete normal rats. Amiloride-sensitive pathways predominate in Na-depleted animals, and are a consequence of prolonged aldosterone stimulation [19]. Future studies on the in vitro lingual epithelium from the rat will determine the effect of adrenalectomy on the responses to NaCl and KCl. The nature of the amiloride-insensitive Na^+ pathway in the lingual epithelium is unknown. It does not respond to several inhibitors which are effective in other epithelia for various types of coupled transport. Edmonds and MacKenzie [19] hypothesized that in the colon the amiloride-insensitive pathways involve some form of complex ionic interaction, while the amiloride-sensitive pathways are probably conductive channels.

The lingual epithelium is unlike most epithelia in its response to hyperosmotic salt solutions; NaCl above 0.15 M causes an increase in PD and I_{sc} and a decrease in R that is reversible [3]. Hence, it

was of interest to identify the current carriers in hyperosmotic salt solutions, since the tissue may use different pathways than in symmetrical configuration. In hyperosmotic (0.25 M) NaCl solution, the net residual current was not significantly different from zero, and net Na^+ absorption was greater than or equal to I_{sc}. Even though amiloride reduced the I_{sc} by an average value of 37% in symmetrical buffer (this work), in the 0.5 M–1.0 M range the inhibition due to amiloride is approx. 70% [3]. Hence it appears that in hyperosmotic salt solution a much greater proportion of the Na^+ absorption occurs via an amiloride-sensitive pathway than in symmetrical buffer.

When we removed K^+ from the serosal bathing medium of the lingual epithelium, the electrical properties were barely affected. It is possible that the Na^+ pump is functioning in some mode other than Na^+-K^+ exchange [20,21]. An alternative explanation is that the submucosal layer constitutes a diffusion boundary and the K^+ is recycled across the basolateral membrane [22]. It is known that frog skin ceases to absorb Na^+ in the absence of serosal K^+ if the submucosal layer is first removed with collagenase and the tissue is depleted of K^+ [23]. In the lingual epithelium on the other hand, we know that the serosal border is permeable to K^+: if high K^+ is placed in the serosal medium, the epithelium is rapidly depolarized (unpublished results).

We initially planned the ion substitution experiments to identify other current carriers and to test for the presence of ion coupling at the apical or basolateral border, assuming that large cations and anions would not be transported by the Na^+ and Cl^- transport systems. However, the ability of the system to sustain a transepithelial short-circuit current under symmetrical conditions suggests that those ions may be current carriers, i.e., are transported. If the system were in a steady state with respect to all species, then we could certainly conclude that the large ions are being actively transported across the epithelium. (For this purpose a steady state exists when the flux across the apical border equals the flux across the basolateral border for each ion species; in other words, the cells are neither accumulating nor being depleted of ions.) At this point we have no way of knowing if a true steady state has been reached in 15 min. It is possible that large cations diffuse passively into the cells across the apical border and K^+ carries the current across the basolateral border. Macey and Koblick [24] observed that the frog skin is permeable to choline and tetramethylammonium and issued a note of caution in interpreting experiments in which these cations are used as inert substitutes for Na^+. (There was no evidence for active transport, however.) Nevertheless, the lingual epithelium is unusual in that it exhibits a short-circuit current when ions are substituted on both sides.

A note of caution must be issued in interpreting these results. In order to make comparisons, the salt concentration (or, in the case of the Tris compounds, the cation concentration) was held constant. This means that the solution pH and osmolarity may have varied. However, it is unlikely that the small changes in these variables can account for the results obtained.

Both the symmetrical and the asymmetrical substitution experiments strongly suggest the presence of ion coupling for transport across both the mucosal and the serosal border of the transporting cell layer. There is no simple scheme to account for all the data. We initially expected that the larger the cation or anion, the more inert it would be with respect to the Na^+ or Cl^- transport system, respectively. However, the percentage decreases in I_{sc} for point A in Table II cannot be ranked in order of ionic weight. It is not possible to explain the data merely on the basis of changes in passive diffusion across the tissue, with no ion coupling effects. For example, sodium acetate on the mucosal side causes a decrease in I_{sc}. Yet a Cl^- gradient from serosal to mucosal side would be expected to increase I_{sc}. Hence, it appears that the anion at the apical side influences the Na^+ transport step at the apical border. As a second example, symmetrical sodium acetate causes an initial decrease in I_{sc} compared to control (Table II). Yet, the decrease is more than can be accounted for by a loss of Cl^- secretion, implying again that the anion influences sodium absorption. In the frog skin, anions other than Cl^- in the mucosal bathing solution stimulate unidirectional sodium influx; the percent stimulation is correlated with the square of the crystal ion radius. This suggests that dimensional properties of the anions are im-

TABLE II

ELECTRICAL PARAMETERS IN SYMMETRICAL SALT SOLUTIONS – PERCENT CHANGE COMPARED TO CONTROL

Values are means ± S.E. N is the number of experiments. Values represent percent decrease (−) or increase (+), compared to control value in symmetrical NaCl. See text for explanation of A, B and C.

Solution [a]	N	A (minimum)			B (maximum)			C (final)		
		I_{sc}	R	PD	I_{sc}	R	PD	I_{sc}	R	PD
Sodium acetate	1	−31	21	−16	−15	46	24	−31	46	4
Sodium gluconate	2	−33.6 ± 0.4	12 ± 9	−26 ± 7	–	–	–	−22 ± 6	30 ± 2	–
Tetramethyl-ammonium·Cl	2	−40 ± 5	11.6 ± 0.3	−33 ± 6	−22 ± 4	7.8 ± 2.1	−14.4 ± 0.2	−36 ± 2	8.0 ± 2.4	−31 ± 4
Tetramethyl-ammonium methylsulfate	4	−35 ± 6	26 ($n=1$)	−30 ($n=1$)	−16 ± 3 ($n=3$)	24 ± 8 ($n=2$)	−20 ± 23 ($n=2$)	−65 ± 7	24 ± 2	−54 ± 10
Choline chloride	2	−32 ± 2	5.9 ± 0.7	−27 ± 4	−16 ± 4	14 ± 3	−4 ± 2	−34 ± 4	26 ± 13	−22 ± 9
Tris chloride	6	−44 ± 3	24 ± 5 ($n=4$)	−32 ± 6 ($n=4$)	−29 ± 1 ($n=4$)	35 ± 4 ($n=3$)	−4 ± 4 ($n=3$)	−36 ± 7 ($n=3$)	32 ± 4 ($n=3$)	−13 ± 8 ($n=3$)
Tris acetate	6	−63 ± 6	62 ± 5 ($n=5$)	−45 ± 11 ($n=5$)	−49 ± 4 ($n=4$)	83 ± 18 ($n=3$)	−14 ± 5 ($n=3$)	–	–	–
Tris nitrate	4	−57 ± 4	19 ± 2	−47 ± 6	–	–	–	−30 ± 5	21 ± 3	−14 ± 7
Tris benzoate	6	−67 ± 3	49 ± 7 ($n=3$)	−58 ± 5 ($n=3$)	−34 ± 9	70 ± 8 ($n=4$)	0 ± 11 ($n=4$)	−44 ± 3	71 ± 6	1 ± 8
Arginine-HCl	1	−36	27	−20	−26	43	5	−32	45	1

[a] All solutions are 0.15 M, except for Tris compounds (see Methods).

portant in the interaction with Na^+ transport. However, the nature of this interaction is not known [25].

There is in vivo evidence that is compatible with both the idea that the lingual epithelium is at least permeable to larger ions though not to the same extent as Na^+ and Cl^-, and that ion coupling at the apical border plays a role in this transport. Hallbäck et al. [26] observed a marked osmolal gradient from tip to base of cat large filiform papillae that depended on the content of the solution bathing the intact tongue. Tissue osmolality was decreased if either the sodium was replaced with choline or the chloride was replaced with sulfate. When choline was substituted for Na^+, the tissue osmolality was lowered; however, there was still a small gradient remaining from tip to base [26, Fig. 5], possibly indicating that some choline may have been transported.

Active sodium absorption in epithelial tissues is thought to occur via the ubiquitous Na^+-K^+ pump located at the basolateral membrane [27]. What might be the nature of a pump mechanism capable of transporting such a wide variety of cations? One possibility, already mentioned, is that large cations diffuse passively through the apical border, and a smaller cation carries the current across the basolateral border. A second possibility is that there are multiple types of pumps at the serosal membrane. A third possibility is that the Na^+ pump in this tissue is capable of transporting other cations. In that case the coupling between ATPase and transport protein may be electrical rather than material, as proposed by Bunow [28,29].

The observations from our ion substitution experiments have interesting implications for gustatory transduction. Many of these compounds are saporous to humans. Sodium acetate and sodium gluconate both have a salty component (Schiffman, personal communication; see also Ref. 30 and 31). Furthermore, the anion contributes significantly to the overall taste quality of sodium salts [32]. Choline chloride and arginine hydrochloride both have a bitter to salty taste (Schiffman, personal communication; see also Ref. 32). In another study in quite a different system, Yoshii and Kurihara [33] examined the ion dependence of the eel taste response to amino acids. They found

that the taste response as measured in the palatine nerve to amino acids depended on the presence of ions in the solution, and that not only NaCl but a wide variety of larger cations (including choline and Tris) and anions could sustain a response. These data indicate that, whatever the transduction mechnism, large ions can support a taste response, perhaps by carrying current into the cells as suggested by our experiments.

We have shown that the lingual epithelium, like other epithelia of the gastrointestinal tract, actively transports ions. However, it is unusual both in its response to hyperosmotic solutions and in the variety of ions that support a transepithelial short-circuit current. Amiloride-sensitive and insensitive sodium fluxes in the lingual epithelium correlate well with the drug's effect on the NaCl taste response as measured both neurophysiologically and psychophysically. The apparent existence of additional pathways for various saporous salts suggests that the transport paradigm of taste transduction may extend beyond NaCl.

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References

1 DeSimone, J.A., Heck, G.L. and DeSimone, S.K. (1981) Science 214, 1039–1041
2 Heck, G.L., Mierson, S. and DeSimone, J.A. (1984) Science 223, 403–405
3 DeSimone, J.A., Heck, G.L., Mierson, S. and DeSimone, S.K. (1984) J. Gen. Phys. 83, 633–656
4 Schiffman, S.S., Lockhead, E. and Maes, F.W. (1983) Proc. Natl. Acad. Sci. USA 80, 6136–6140
5 Mierson, S., Heck, G.L., DeSimone, S.K. and DeSimone, J.A. (1984) Assoc. Chemoreception Sci. Abstr. No. 93
6 Biber, T.U.L. and Mullen, T.L. (1977) Am. J. Physiol. 232, C67–C75
7 Barry, P.H. and Diamond, J.M. (1970) J. Membrane Biol. 3, 93–122
8 MacInnes, D.A. (1961) The Principles of Electrochemistry, p. 232, Dover, New York
9 Cabantchik, Z.I., Knauf, P.A. and Rothstein, A. (1978) Biochim. Biophys. Acta 515, 239–302
10 Cohen, M.R., Hinsch, E., Vergona, R., Ryan, J., Kolis, S.J. and Schwartz, M.A. (1976) J. Pharmacol. Exp. Ther. 197, 697–702
11 Widdicombe, J.H., Nathanson, I.T. and Highland, E. (1983) Am. J. Physiol. 245, C388–C396
12 Larson, M. and Spring, K.R. (1983) J. Membrane Biol. (1983) 74, 123–129
13 Maren, T.H. (1967) Physiol. Rev. 47, 595–781
14 Simon, S.A. and Garvin, J.L. (1984) Assoc. Chemoreception Sci. Absts., No. 118
15 Maren, T.H., Haywood, J.R., Chapman, S.K. and Zimmerman, T.J. (1977) Invest. Ophthalmol. Visual Sci. 16, 730–742
16 Swenson, E.R. and Maren, T.H. (1978) Resp. Physiol. 35, 129–159
17 Teeter, J.H., Silver, W.L. and Brand, J.G. (1983) Neurosci. Abstr. 9, 1022
18 Widdicombe, J.H. and Welsh, M.J. (1980) Fed. Proc. 39, 3062–3066
19 Edmonds, C.J. and Mackenzie, J. (1984) J. Physiol. 346, 61–71
20 Robinson, J.D. and Flasher, M.S. (1979) Biochim. Biophys. Acta 549, 145–176
21 Essig, A. and Leaf, A. (1963) J. Gen. Phys. 46, 505–515
22 Leaf, A. (1965) Ergeb. Physiol. 56, 216–263
23 Aceves, J. (1977) Pflügers Arch. 371, 201–209
24 Macey, R.I. and Koblick, D.C. (1963) Am. J. Physiol. 205, 1063–1066
25 Biber, T.U.L. and Mullen, T.L. (1980) Biochem. Pharmacol. 29, 2265–2268
26 Hallbäck, D.A., Jodal, M. and Lundgren, O. (1979) Acta Physiol. Scand. 105, 469–480
27 Ussing, H.H. and K. Zerahn (1951) Acta Physiol. Scand. 23, 110–127
28 Bunow, B. (1978) J. Theor. Biol. 75, 51–78
29 Bunow, B. (1978) J. Theor. Biol. 75, 79–96
30 Schiffman, S.S., McElroy, A.E. and Ericson, R.P. (1980) Physiol. Behav. 24, 217–224
31 Schiffman, S.S. and Ericson, R.P. (1971) Physiol. Behav. 7, 617–633
32 Schiffman, S.S. and Sennewald, K. and Gagnon, J. (1981) Physiol. Behav. 27, 51–59
33 Yoshii, K. and Kurihara, K. (1983) Brain Res. 280, 63–67
34 DeSimone, J.A. and Ferrell, F. (1985) Am. J. Physiol., in the press