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Astringent-Tasting Compounds Alter Ion Transport Across Isolated Canine Lingual Epithelia

S. A. SIMON*, W. L. HALL* AND S. S. SCHIFFMAN†[1]

**Departments of Neurobiology and Anesthesiology and †Departments of Psychiatry and Psychology, Duke University Medical Center, Durham, NC 27710*

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SIMON, S. A., W. L. HALL AND S. S. SCHIFFMAN. *Astringent-tasting compounds alter ion transport across isolated canine lingual epithelia.* PHARMACOL BIOCHEM BEHAV **43**(1) 271-283, 1992.—The effects of acid and astringent compounds on ion transport across isolated canine lingual epithelia were measured in an Ussing chamber. Lowering the pH from 7.4 to 3.2 decreases ion transport, as measured by the short-circuit current (I_{sc}), when the dorsal surface of the tongue is bathed in 0.5 M NaCl and increases I_{sc} when it is bathed in 0.05 M NaCl. In 0.5 M NaCl, tannic acid (0.1 M) inhibits I_{sc} at both pH 3.2 and 7.4. At 0.05 M NaCl, pH 7.4 tannic acid also inhibits I_{sc}. Thus, inhibition of I_{sc} by tannic acid does not depend upon the pH, meaning that the reduction in transport arises from tannic acid. In the presence of NaCl (at both 0.05 and 0.5 M NaCl), 0.1 M AlK $(SO_4)_2$ or 0.1 M $AlNH_4(SO_4)_2$ also inhibit I_{sc}. For these salts, the decrease in I_{sc} arises from the aluminum ion and not from K^+, NH_4^+, or SO_4^{--}. Other less astringent compounds (gallic and tartaric acids) had only slight effects on I_{sc}. The main findings of this study are that both tannic acid and the aluminum salts inhibited ion transport, likely Na^+ influx, via amiloride-inhibitable channels in isolated lingual epithelia. Inhibition of such Na^+ channels may contribute to astringent taste.

Astringency Tannic acid Aluminum salts Transport Amiloride

RECENT advances have been made in understanding the transduction mechanisms for sweet, sour, salty, and bitter tastes. The transduction mechanisms for these four tastes involve the interaction of ions or organic molecules with channels or receptors on the apical membranes of taste cells (1,13,19). The cellular basis of astringent taste, however, is virtually unknown as most of the proposed mechanisms of astringency involve the ability of tannic acid, and associated compounds, to precipitate water-soluble proteins (2). Astringent tastes are usually associated with drying or puckering sensations and are produced by a range of compounds including polyphenols and aluminum salts (5). A common polyphenolic compound that produces astringent sensations is tannic acid, which is found in tea and wine.

In the past, there has been controversy whether astringency is a taste sensation or a tactile sensation involving mechanoreceptors activated by the precipitation of proteins [see (20)]. However, electrophysiologic recordings in rodents indicate that astringency is a taste because compounds such as tannic acid stimulate chorda tympani nerves (which innervate taste cells) but do not stimulate general sensory lingual nerves (12,20).

The purpose of this study was to investigate the cellular mechanism by which astringent compounds transduce and modify transport in taste cells. Cellular responses to astringent compounds were evaluated here by measuring transport across isolated lingual epithelia, especially those involved with the influx of Na^+. DeSimone and colleagues (7,17,27) and Simon and colleagues (21,23,24) previously established that transport across isolated rat or dog lingual epithelia is correlated with responses from chorda tympani fibers (i.e., is related to events in taste transduction). Moreover, they showed that Na^+ enters taste cells through amiloride-inhibitable channels and exits them via Na,K-ATPase in their basolateral membranes (22). Since solutions containing astringent-tasting compounds are usually acidic, it is necessary to evaluate the effect of lowering the pH on transport across lingual epithelia. In addition, some astringent-tasting compounds are complex salts (e.g., aluminum salts), and thus it is necessary to determine which of the ionic species of these salts are responsible for the astringent sensation (20). Our major conclusion is that both tannic acid and the aluminum salts inhibit Na^+ influx pathways into lingual epithelia.

[1]To whom requests for reprints should be addressed.

METHOD

Stimuli

Five astringent compounds were employed as test stimuli to determine their effect on sodium transport: gallic acid, tannic acid, tartaric acid, $AlK(SO_4)_2$, and $AlNH_4(SO_4)_2$. The structures of these compounds are shown in Fig. 1. Two salts, K_2SO_4 and $Al_2(SO_4)_3$, were also tested to compare with results from $AlK(SO_4)_2$ and $AlNH_4(SO_4)_2$. The three organic astringent compounds and the two salts will be referred to as *test compounds*. Transport induced by test compounds was also examined in the presence of amiloride hydrochloride (an epithelial sodium transport inhibitor), ouabain (a specific inhibitor of Na^+,K^+ – ATPase), and $LaCl_3$ [an inhibitor of transport across tight junctions (9)]. HEPES was used as a buffer at pH 7.4. Tannic acid (MW 1,700) was obtained from Fluka Chemical Company (Buchs, Switzerland). Its purity was checked by differential scanning calorimetry and yielded an endothermic peak at 208°C. All other compounds were obtained from Sigma Chemical Co. (St. Louis, MO) and were regent grade.

Isolation of Lingual Epithelia

Adult dogs (both male and female) were sacrificed by an intravenous injection of pentobarbital (70 mg/kg) or KCl during anesthesia, and the anterior two thirds of their tongues was removed and placed in modified Krebs-Henseleit (KH) solution on ice. The muscle layer was then dissected away, and the remaining epithelial layer was placed in an Ussing chamber (3.1 cm^2) between symmetrical KH solutions at 35 ± 1°C, as described previously (26). The composition of the KH solution was: 118 mM NaCl, 6 mM KCl, 5.6 mM D-glucose, 1.2 mM $MgSO_4$, 2.0 mM $CaCl_2$, 25 mM $NaHCO_3$ and 1.3 mM NaH_2PO_4. The KH solution was adjusted to pH 7.4 when equilibrated with 95% O_2:5% CO_2.

Electrical Measurements

Measurements of the short-circuit current, I_{sc} and the open-circuit potential, V_{oc}, were obtained in an Ussing chamber; the details have been described previously (26). These parameters were obtained using a WPI voltage clamp apparatus (World Precision Instruments, Sarasota, FL). V_{oc} is defined with respect to the mucosal solution, and I_{sc} has a negative sign when cations flow from the mucosal to serosal solutions or when anions flow from the serosal to mucosal solutions. V_{oc} was measured with calomel electrodes in saturated KCl solutions interfaced to 1–2% agar bridges containing 0.15 M NaCl. Platinum wires were used as current-passing electrodes. These four electrodes were connected to a voltage clamp circuit that compensates for the series resistance arising from the electrodes and the KH solutions. The transepithelial resis-

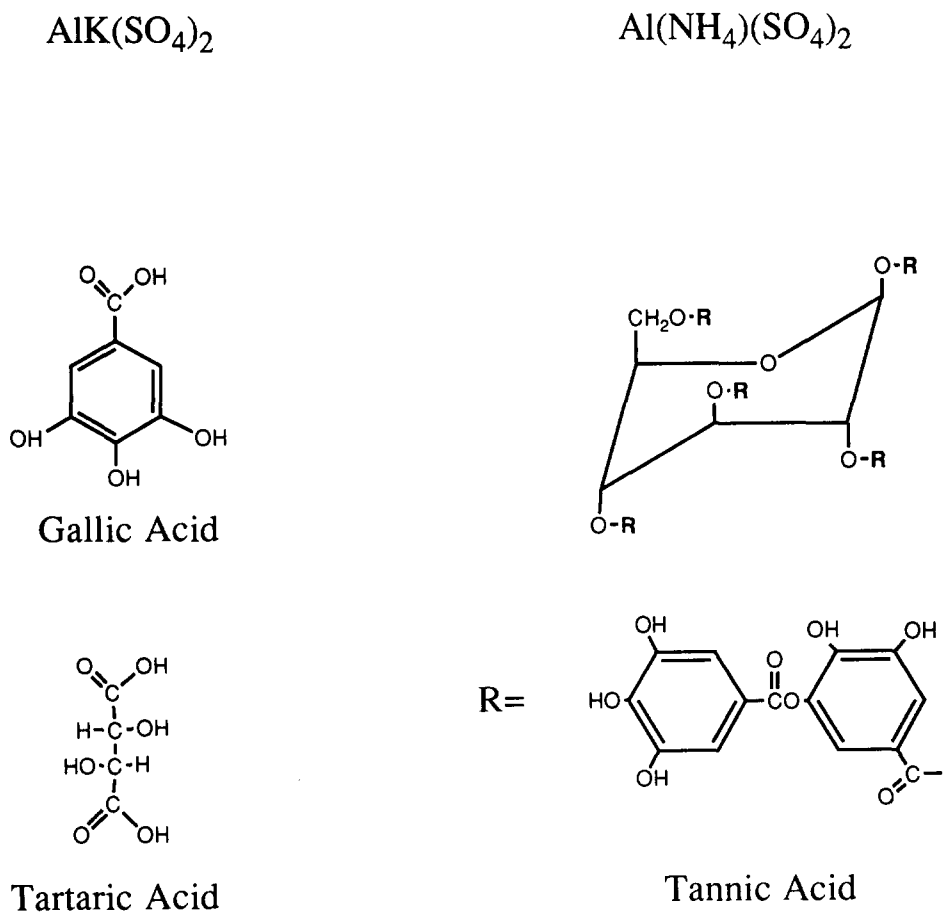

FIG. 1. Chemical structures of compounds tested in this study. Gallic, tartaric, and tannic acids are all shown in their neutral forms and the aluminum salts are shown without their associated waters.

tance, R_m, was determined either by dividing V_{oc} by I_{sc}, since the current vs. voltage relationship is linear (4), or injecting a constant-current pulse while V_{oc} was being recorded. Since all experiments with the astringent compounds commenced with different solutions on the mucosal and serosal sides, V_{oc} and I_{sc} contain contributions arising from liquid-junction potentials. To account for the component arising from liquid-junction potentials, the data are presented as differences in V_{oc}, I_{sc}, and R_m with respect to the reference solutions (described below), which are salt solutions without astringent compounds.

Experimental Procedure

Reference solutions (Table 1). Baseline measurements of V_{oc}, I_{sc}, and R_m were first obtained in symmetrical solutions of KH to assess the viability of the isolated tongue. Because KH is a complex solution containing both organic and inorganic compounds, the test compounds were next evaluated on the mucosal side (where transduction occurs) in four reference solutions. Experiments commenced by replacing the KH solution on the mucosal side with one of the reference solutions:

1. 0.05 M NaCl, 2 mM HEPES, pH 7.4
2. 0.05 M NaCl, pH 3.2
3. 0.5 M NaCl, 2 mM HEPES pH 7.4
4. 0.5M NaCl, pH 3.2 (Table 1)

The pH value of 3.2 was chosen because it is the approximate pH of unbuffered tannic acid solutions (20). The pH of 7.4 was chosen because it is close to the value of physiologic pH and also because at this pH any contribution to the I_{sc} arising from H^+ will be small (18). The reference solutions were adjusted to their respective pHs with HCl or NaOH when equilibrated with 100% O_2. We chose to increase the pH using NaOH rather then some organic buffer or ionic base (e.g., KOH) because it is established that Na^+ transport occurs, for the most part, through amiloride-inhibitable channels (26). Other organic compounds or bases (e.g., KOH) could have been chosen to adjust the pH, but these compounds exhibit their own effects on epithelial transport that are not as well understood as they are for Na^+. The solution bathing the serosal surface always contained KH equilibrated with 95% O_2:5% CO_2.

Control solutions (Table 2). To account for the additional Na^+ that was added as NaOH to adjust the pH to 3.2 and 7.4 in the test solutions, a set of control experiments was performed. Control solutions consisted of the reference solutions plus a concentration of NaCl that was equal to the concentration of Na^+ (in NaOH) present in the test solutions (see below) as a result of addition of NaOH (see Table 2). Measurements of V_{oc}, I_{sc}, and R_m induced by a test compound were obtained both directly after a reference solution or after a reference solution followed by a control solution. Amiloride and ouabain were added from concentrated stock solutions.

Test solutions. Measurements of V_{oc}, I_{sc}, and R_m were next obtained when the mucosal solution was exchanged for a test solution. A test solution consisted of the reference solutions **plus** 0.1 M of the test compound (e.g., 0.1 M tannic acid) and a concentration of NaOH necessary to adjust the pH to 3.2 or 7.4. The additional NaOH was necessary because, as previously mentioned, solutions of astringent compounds have acidic pH, and it was desired to compare the responses to astringent compounds at pH 3.2 and 7.4. Amiloride and ouabain were added from concentrated stock solutions.

Data Presentation

Measurements of I_{sc} or V_{oc} were obtained on a strip chart recorder. These tracing were photocopied and then scanned using a Hewlett-Packard scanner (Scanjet Plus). The scanned image was then digitized and the line figures generated.

RESULTS

Measurements of V_{oc}, I_{sc}, and R_m Without Astringent-Tasting Compounds in the Mucosal Solution

Viability of the lingual epithelia. With symmetrical KH solutions on both sides of the tongue, V_{oc}, I_{sc}, and R_m were 17.1 ± 3.1 mV, −22.2 ± 6.6 μA/cm², and 825 ± 239 Ω cm² (mean ± SEM; n = 40), respectively. These data are consistent with those previously published (3,4,26) and indicate that the lingual epithelia used in these experiments were viable.

Reference solutions. Experiments were performed with the four reference solutions used in experiments for the three organic compounds placed on the mucosal side of the lingual epithelium. The reference solutions contained 0.05 M NaCl and 0.5 M NaCl at pHs 3.2 and 7.4 (Table 1). A comparison of the responses to 0.05 M NaCl at both pH values reveals that decreasing the pH *increases* both V_{oc} and I_{sc} by about a factor of two but does not significantly change R_m. In contrast, at 0.5 M NaCl decreasing the pH from 7.4 to 3.2 *decreases* I_{sc} by 41% and increases R_m by this same percentage (since V_{oc} remains unchanged). These data demonstrate that the response to decreasing the pH is dependent upon the NaCl concentration.

Control solutions. The adjustment of test solutions to either pH 7.4 or 3.2 required the addition of NaOH. Since increasing the Na^+ concentration increases V_{oc} and I_{sc} (3,4), experiments were performed to determine the changes in V_{oc}, I_{sc}, and R_m that occur for a concentration of NaCl that is equivalent to the concentration of the Na^+ present in the test solutions at either pH 3.2 or 7.4 (Table 2). At pH 3.2, the

TABLE 1

V_{oc}, I_{sc}, AND R_m OF ISOLATED CANINE LINGUAL EPITHELIUM FOR REFERENCE SOLUTIONS ON THE MUCOSAL SIDE

Reference Solution	V_{oc} (mV)	$-I_{sc}$ (μA/cm²)	R_m (Ω cm2)	n
0.05 M NaCl, 2 mM HEPES, pH 7.4	5.05 ± 0.29	2.91 ± 0.18	1,768 ± 89	11
0.05 M NaCl, pH 3.2	11.0 ± 0.89	7.65 ± 0.64	1,746 ± 102	10
0.5 M NaCl, 2 mM HEPES, pH 7.4	27.1 ± 2.0	109 ± 12.9	283 ± 35	17
0.5 M NaCl, pH 3.2	27.4 ± 2.0	64.3 ± 3.0	445 ± 39.6	18

n, number of experiments performed under these conditions.

TABLE 2

RESPONSES OF V_{oc}, I_{sc}, AND R_m REPRESENTING DIFFERENCES IN THESE PARAMETERS BETWEEN CONTROL AND REFERENCE SOLUTIONS BATHING THE MUCOSAL SURFACE OF CANINE LINGUAL EPITHELIA

	1	2	3
	NaCl Added(mM)		
pH = 3.2*	15	30	55
ΔV_{oc} (mv)			
0.05 M NaCl	0.65 ± 0.45	1.55 ± 0.65	2.30 ± 0.80
0.50 M NaCl	−0.2 ± 0.4	−0.5 ± 0.4	−0.85 ± 0.5
$-\Delta I_{sc}$ (μA/cm^2)			
0.05 M NaCl	0.81 ± 0.48	2.10 ± 0.81	3.23 ± 0.97
0.50 M NaCl	0.2 ± 0.4	+0.5 ± 0.4	−0.9 ± 0.6
$-\Delta R_m$ (Ω cm^2)			
0.05 M NaCl	−63 ± 37	−151 ± 65	−216 ± 71
0.50 M NaCl	3 ± 4	−2 ± 2	−7 ± 3.5
	NaCl Added (mM)		
pH = 7.4	70	300	130
ΔV_{oc} (mv)			
0.05 M NaCl	7.05 ± 0.95	21.35 ± 4.95	12.6 ± 3.30
0.50 M NaCl	0.2 ± 2.85	−2.80 ± 5.05	−0.80 ± 4.45
$-\Delta I_{sc}$ (μA/cm^2)			
0.05 M NaCl	9.35 ± 2.58	64.35 ± 1.77	19.67 ± 1.29
0.50 M NaCl	8.55 ± 1.77	24.53 ± 6.76	13.13 ± 0.74
$-\Delta R_m$ (Ω cm^2)			
0.05 M NaCl	−467.4 ± 249.9	−955.02 ± 82.80	−626.06 ± 183.0
0.50 M NaCl	−35.72 ± 33.39	−115.54 ± 20.24	−60.68 ± 34.63

*Fifteen, 30, and 55 mM correspond to the Na^+ concentration added in the form of NaOH to gallic (1), tannic (2), and tartaric (3) acids to adjust their pH to 3.2.

†Seventy, 300, and 130 mM correspond to the Na^+ concentration added in the form of NaOH to (1) gallic, (2) tannic, and (3) tartaric acids to adjust their pH to 7.4.

following NaCl concentrations were added to 0.1 M test solutions: gallic acid, (15 mM NaCl), tannic acid (30 mM NaCl), and tartaric acid (55 mM NaCl). At pH 7.4, the following NaCl concentrations were added: gallic acid (70 mM NaCl), tannic acid (300 mM NaCl), and tartaric acid (130 mM NaCl). The changes in V_{oc}, I_{sc}, and R_m [e.g., ΔV_{oc} = V_{oc} (control) − V_{oc} (reference)] that occur after the addition of NaCl corresponding to NaCl present in the test solutions are presented in Table 2.

Addition of 15, 30, and 55 mM NaCl to 0.05 M NaCl pH 3.2 results in relatively small changes in V_{oc}, I_{sc}, and R_m (Table 2), that is, under these conditions the maximal change in I_{sc} was 3.2 μA/cm^2 induced by the addition of the Na^+ in solutions of 0.1 M tartaric acid. Similarly, for 0.5 M NaCl pH 3.2 the changes in I_{sc} introduced by the addition of up to 30 mM NaCl (the amount of Na^+ present in solutions of 0.1 M tannic acid at pH 3.2) are small, with the maximal change in I_{sc} being about 1μA/cm^2 (Table 2, Fig. 2A).

In contrast, for solutions containing 0.05 M NaCl pH 7.4 the addition of up to 300 mM NaCl (corresponding to the Na^+ concentration in tannic acid at pH 7.4) greatly increased V_{oc} and I_{sc} by about 21 mV and 64 μuA/cm^2, respectively (Table 2). The addition of 300 mM NaCl to 0.5 M NaCl pH 7.4 slightly decreased V_{oc} but increased I_{sc} about 24 μA/cm^2 (Table 2, Fig. 2B). NaCl concentrations of 70 and 130 mM when added to 0.5 M NaCl induced comparatively smaller increases in I_{sc} (Table 2).

The epithelial sodium transport inhibitor, amiloride, at 0.1 mM inhibited I_{sc} at high NaCl concentrations both at pH 3.2 (Fig. 2A) and pH 7.4 (Fig. 2B). However, the magnitude of the inhibition was smaller at pH 3.2 than at pH 7.4. At both pH's the addition of 1 mM ouabain to the serosal solution further decreased I_{sc}.

Measurements of V_{oc}, I_{sc}, and R_m With Organic Test Compounds in the Mucosal Solution

Measurements in 0.05 M NaCl. Figure 3 shows the mean ± SEM (at least four experiments were performed for each condition) of responses of lingual epithelium to gallic, tannic, and tartaric acids at 0.05 M NaCl at pHs 3.2 and 7.4. The responses of the aluminum salts are also shown but will be discussed in a later section. In these experiments, the test solutions replaced the reference solutions (examples of such types of experiments are shown in Fig. 4). At 0.05 M NaCl pH 3.2, only 0.1 M tartaric acid (Fig. 4A) gave responses in V_{oc} and I_{sc} of sufficient magnitude so that they could not be fully sufficiently accounted for by the added salt (Table 2). For example, at pH 3.2 the average I_{sc} induced by a test solution containing 0.1 M tartaric acid applied directly after a 0.05 M NaCl reference solution was about 14 μA/cm^2 (Fig. 4A). The additional Na^+ in the solution required to obtain a pH of 3.2 induced an increase in I_{sc} of 3.2 μA/cm^2 (Table 2). Under these conditions, the stimulation of I_{sc} by 0.1 M tartaric acid pH 3.2 was slightly inhibited by 0.1 mM amiloride (Fig. 4A).

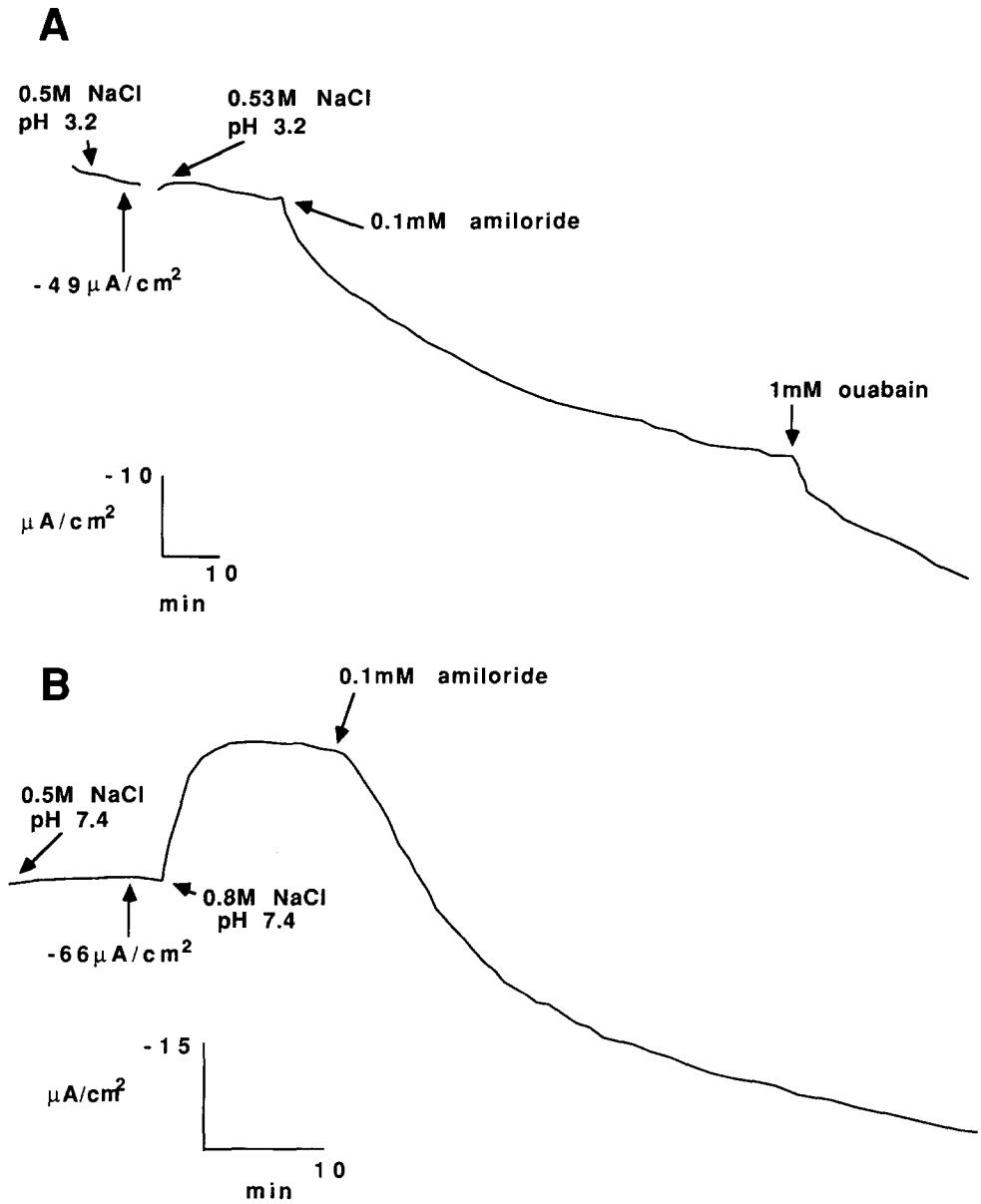

FIG. 2. Responses of short-circuit current (I_{sc}) of canine lingual epithelia to hyperosmotic concentrations of NaCl at pHs 3.2 and 7.4 (A) Trace of I_{sc} in response to the addition of 0.03 M NaCl to 0.5 M NaCl followed by the addition of 0.1 mM amiloride and 1 mM ouabain. Ouabain was added to the solution bathing the serosal solution whereas amiloride was added to the mucosal bathing solution. The 0.03 M NaCl represents the Na^+ concentration in 0.1 M tannic acid solution at pH 3.2 (Table 2). (B) Trace of I_{sc} to the addition of 0.3 M NaCl to 0.5 M NaCl to obtain a concentration of 0.8 M NaCl. Amiloride was then added to this solution. The 0.3 M NaCl represents the Na^+ concentration in 0.1 M tannic acid solution at pH 7.4 (Table 2).

At 0.05 M NaCl, pH 7.4 significant changes in I_{sc}, V_{oc}, and R_m were observed with gallic, tartaric (Fig. 4B), and tannic acids. In all three cases, I_{sc} and V_{oc} increased and R_m decreased. In contrast to the small inhibition of I_{sc} induced by tartaric acid with 0.1 mM amiloride at pH 3.2 (Fig. 4A), at pH 7.4 I_{sc} is markedly decreased by 0.1 mM amiloride (Fig. 4B). The Na^+,K^+ATPase inhibitor, ouabain, when added to the serosal solution at 1 mM caused an additional decrease in I_{sc} (Fig. 4B). For gallic and tartaric acids, the increase in I_{sc} was approximately the same as the amount expected from the Na^+ added to adjust the pH of the mucosal solution (see Table 2 and the Discussion Section). For tannic acid, there was a large inhibition in I_{sc} once the contribution of the added salt is accounted for, that is, an increase in I_{sc} of 64 $\mu A/cm^2$ was expected (Table 2) whereas I_{sc} only increased about 15 $\mu A/cm^2$.

Measurements in 0.5 M NaCl. Figure 5 shows the effects of gallic, tannic, and tartaric acids at 0.5 M NaCl pHs 3.2 and 7.4. In these experiments, the test solutions replaced the reference solutions. At pH 3.2, the test solution containing 0.1 M tannic acid significantly inhibited I_{sc} when it replaced the reference solution (Fig. 5). Figure 6A shows that the addi-

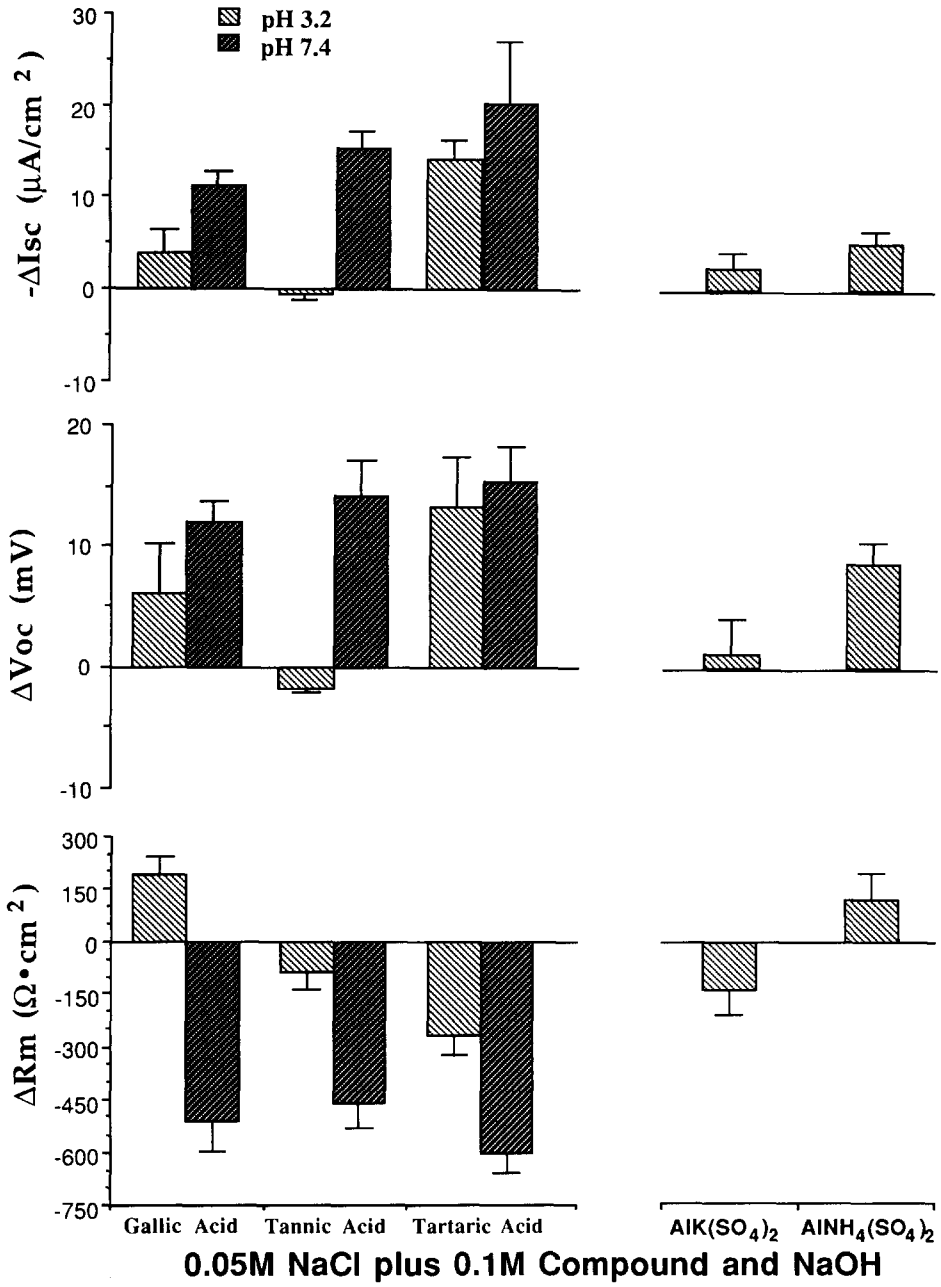

FIG. 3. Differences in the responses of I_{sc}, V_{oc}, and R_m across canine lingual epithelia originally bathed in 0.05 M NaCl. These changes in I_{sc}, V_{oc}, and R_m represent differences in the steady-state values of these three parameters between the test and reference solutions. The test solution contained 0.05 M NaCl ± 2 mM HEPES at pHs 3.2 or 7.4 plus 0.1 M astringent compound and the NaOH added to adjust the pH. The reference solutions contained 0.05 M NaCl ± 2 mM HEPES at pHs 3.2 or 7.4. Data are presented as the mean ± SE of at least four experiments.

tion of 0.03 M NaCl to 0.5 M NaCl pH 3.2 increased I_{sc} by a few μA and that the addition of 0.1 M tannic acid (in 0.53 M NaCl pH 3.2) then resulted in a marked *inhibition of* I_{sc}. Moreover, in the presence of tannic acid at pH 3.2 ouabain, but not amiloride, was effective in further inhibiting I_{sc} (Fig. 6A).

AT pH 7.4, replacement of the reference solution with a test solution containing 0.5 M NaCl and 0.1 M tannic acid increased I_{sc} by about 9.7 μA/cm^2 (Fig. 5). To obtain pH 7.4 in the test solution, it was necessary to add 0.3 M NaOH. This concentration of NaCl increased I_{sc} in solutions containing 0.5 M NaCl about 24 μA/cm^2 (Table 2). Thus, from this viewpoint tannic acid *inhibited* I_{sc} about 14 μA/cm^2 (i.e., 24 − 10 μA/cm^2). However, to clearly demonstrate that tannic acid inhibits I_{sc} at pH 7.4 we performed an experiment by first adding 0.3 M NaCl pH 7.4 to 0.5 M NaCl pH 7.4 and then

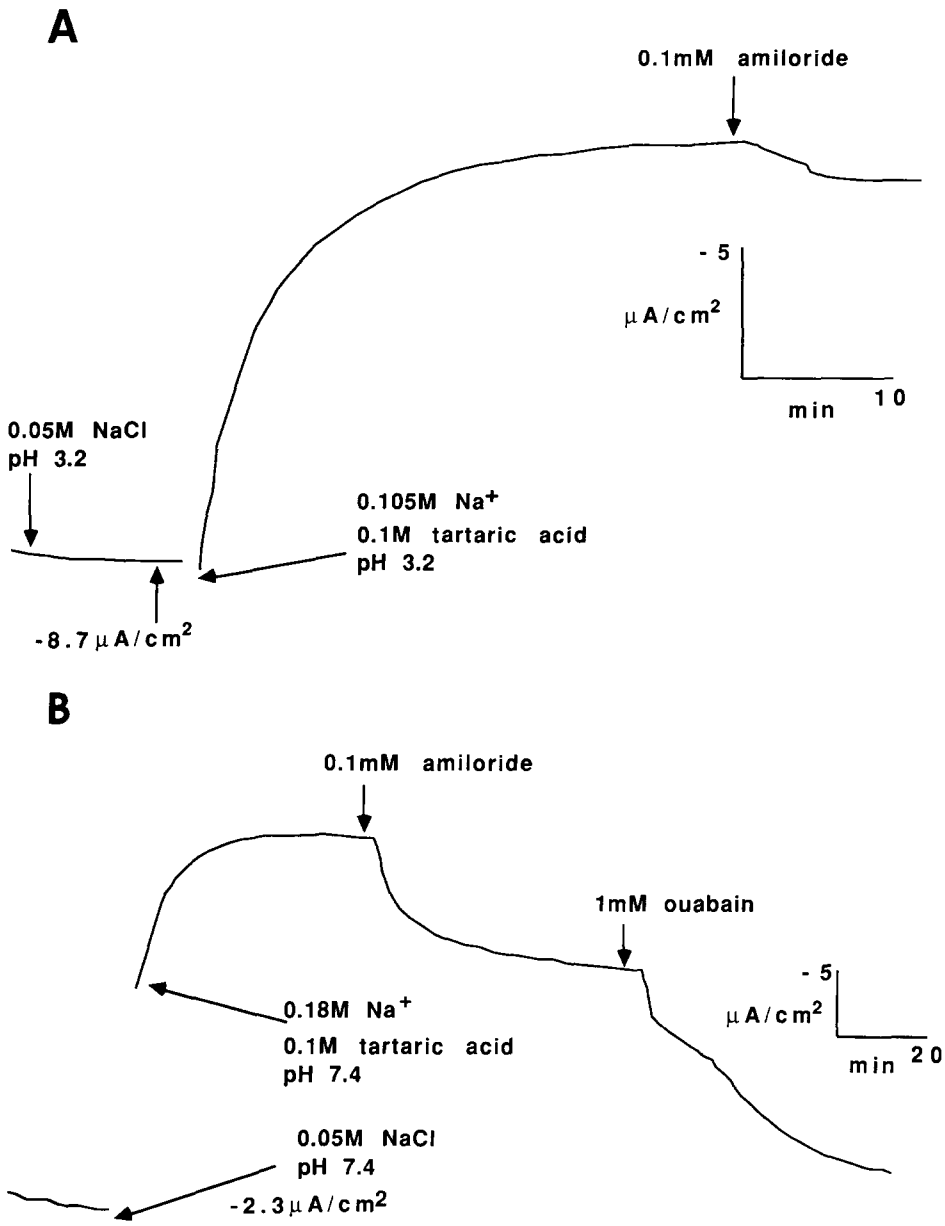

FIG. 4. Responses of I_{sc} of canine lingual epithelia to 0.1 M tartaric acid. (A) Mucosal solution originally contained 0.05 M NaCl pH 3.2. At the arrow, this solution was replaced with one having tartaric acid and an additional 0.055 M NaOH, which was necessary to adjust the pH to 3.2. The addition of amiloride produced a small inhibition. (B) Similar type of experiment conducted at pH 7.4. Here, both amiloride added to the mucosal solution and ouabain added to the serosal solution produced large decreases in I_{sc}. Breaks in the traces indicate the changing of the mucosal solution.

replacing this solution with one containing 0.8 M Na^+ plus 0.1 M tannic acid at pH 7.4 (Fig. 6B). Under these conditions, tannic acid *inhibited* I_{sc} by about 40%. Also, in the presence of 0.1 mM tannic acid amiloride did not further inhibit I_{sc} since the slope of I_{sc} did not change upon its addition (Fig. 6B). Tartaric acid at pH 7.4 increased I_{sc} by about 20 $\mu A/cm^2$ (Table 2). However, this increase was predominantly accounted for by the Na^+ present in the tartaric acid solution, which increased I_{sc} by about 13 $\mu A/cm^2$ (Table 2). Gallic acid produced small effects on I_{sc}, V_{oc}, and R_m.

Measurements in $AlK(SO_4)_2$, $AlNH_4(SO4)_2$, and $Al_2(SO_4)_3$

In 0.05 M NaCl pH 3.2, the direct addition of $AlK(SO_4)_2$ and $AlNH_4(SO4)_2$ produced only small changes in V_{oc}, I_{sc}, and R_m (Figs. 3 and 7A, note current scale in 7A). In the presence of these salts, both 0.1 mM amiloride and 5 mM $LaCl_3$ decreased I_{sc} by < 1 $\mu A/cm^2$ (Fig. 7A). To determine whether the small increases upon addition of $AlK(SO_4)_2$ reflect an underlying cancellation of opposing currents, the effect of adding K_2SO_4 by itself was investigated, that is, increasing the K^+ concentration would be expected to increase I_{sc} (21). The

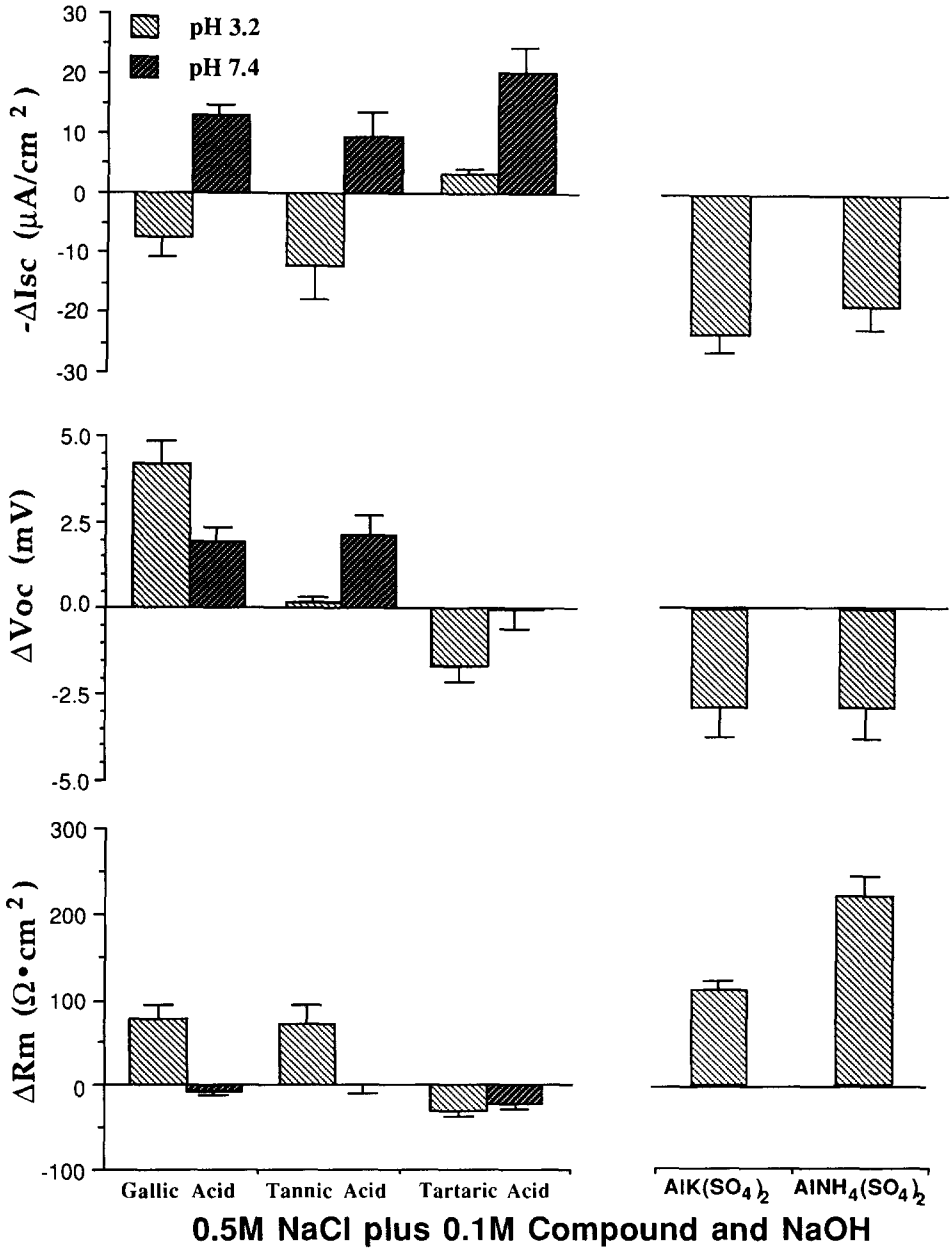

FIG. 5. Differences in the responses of I_{sc}, V_{oc}, and R_m across canine lingual epithelia originally bathed in 0.5 M NaCl. These changes in I_{sc}, V_{oc}, and R_m represent differences in the steady-state values of these three parameters between the test and reference solutions. The test solution contained 0.5 M NaCl ± 2 mM HEPES at pHs 3.2 or 7.4 plus 0.1 M astringent compound and the NaOH added to adjust the pH. The reference solutions contained 0.5 M NaCl ± 2 mM HEPES at pHs 3.2 or 7.4. Data are presented as the mean ± SE of at least four experiments.

addition of 0.1 M K_2SO_4 (in 0.05 M NaCl) to 0.05 M NaCl pH 3.2 resulted in reversible (not shown) increases in V_{oc} and I_{sc} of 8.25 ± 0.85 mV and −11.6 ± 1.0 $\mu A/cm^2$, respectively (Fig. 7B). Since the increase in I_{sc} is larger for $K_2(SO_4)_2$ than for $AlK(SO_4)_2$ and $AlNH_4(SO_4)_2$ (again note current scales), these data suggest that Al^{+++} may be inhibiting I_{sc}.

At 0.5 M NaCl pH 3.2, 0.1 M $AlK(SO_4)_2$ or 0.1 M $AlNH_4(SO_4)_2$ significantly inhibit I_{sc} (Fig. 5 and 8A) when test solutions containing these aluminum salts are applied to the lingual epithelium directly after a reference solution. Moreover, large and *irreversible* increases in R_m are measured (Fig. 5). To demonstrate that aluminum is the cation responsible for these irreversible decreases in I_{sc}, the following experiments were performed. First, 0.1 M K_2SO_4 pH 3.2 (in 0.5 M NaCl) was added to 0.5 M NaCl pH 3.2, and this operation reversibly increased V_{oc} and I_{sc} and decreased R_m 0.5 ± 0.2 mV, −7.10 ± 0.65 $\mu A/cm^2$, and −157 ± 52 Ω cm^2, respectively. When 0.1 M $AlK(SO_4)_2$ or $AlNH_4(SO_4)_2$ (in 0.5 M NaCl

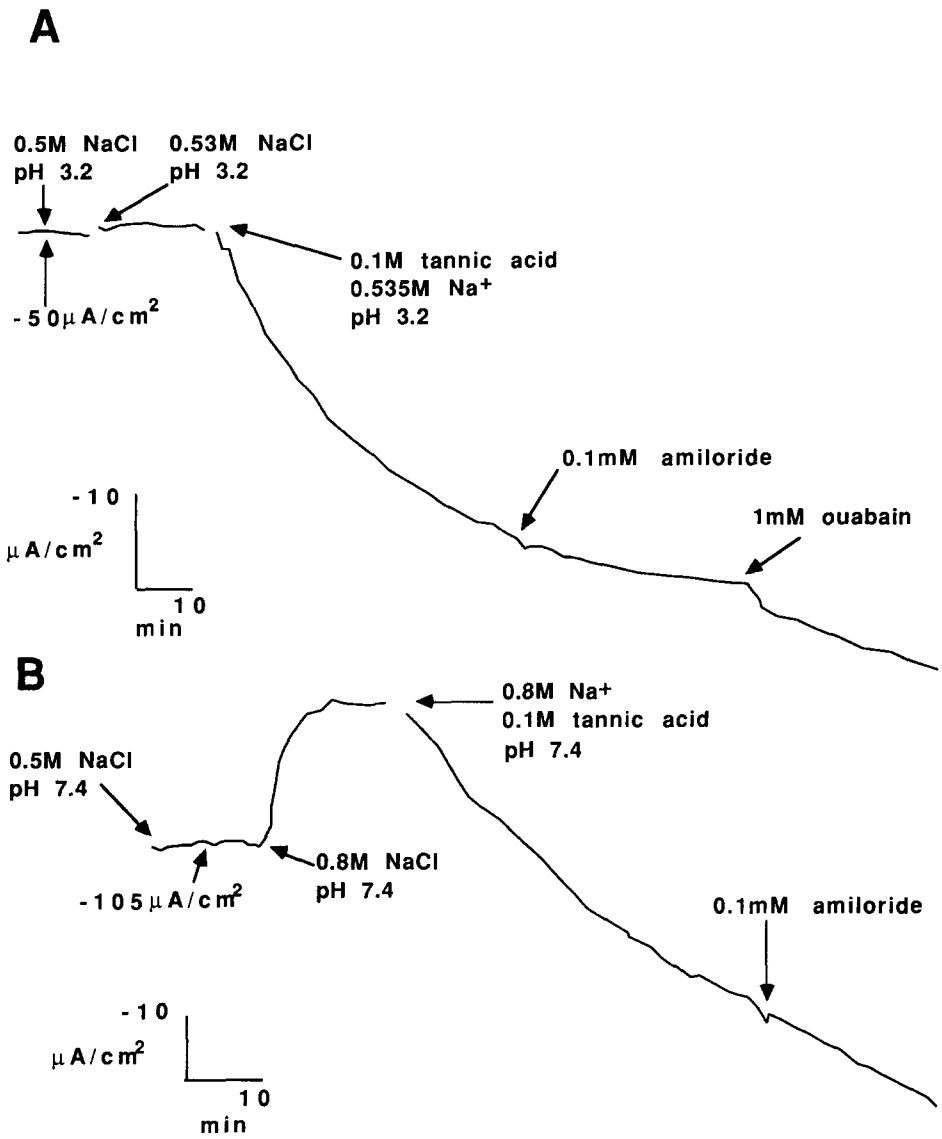

FIG. 6. Responses of I_{sc} across canine lingual epithelia to 0.1 M tannic acid. (A) Mucosal solution originally contained 0.5 M NaCl pH 3.2. At the arrow, 0.03 M NaCl was added to this solution to give a final NaCl solution of 0.53 M NaCl. This solution was then replaced (see break) with one having 0.1 M tannic acid and in addition 0.030 M NaOH, which was necessary to adjust the pH to 3.2. The addition of amiloride had no effect whereas ouabain induced a significant inhibition of I_{sc} (B) Similar type of experiment conducted at pH 7.4. Breaks in the traces indicate the changing of the mucosal solution.

were added to 0.5 M NaCl pH 3.2, I_{sc} initially increased, most likely as a consequence of the added K^+ and SO_4^{-2}, and then decreased (Fig. 8A). The initial increase in I_{sc} is also seen when 0.5 M NaCl plus $Al_2(SO_4)_3$ was added to 0.5 M NaCl (Fig. 8B) and may simply reflect the response of the tongue to rapidly changing ionic conditions upon solution replacement. When a transport inhibitor such as aluminum is present (Figs. 8A and 8B), I_{sc} declines after the initial stimulation. The addition of 0.1 M $Al_2(SO_4)_3$ caused an irreversible reduction in V_{oc} and I_{sc} and an increase in R_m of -15.6 ± 1.1 mV, 49.7 ± 1.0 $\mu A/cm^2$, and 462 ± 52 Ω cm^2, respectively. The addition of 0.1 mM amiloride did not further reduce I_{sc} in the presence of aluminum salts (not shown).

DISCUSSION

Reference and Control Solutions

To understand cellular mechanisms responsible for electrical changes that occur in lingual epithelia upon exposure to astringent-tasting compounds, it was necessary to separate the responses of lowering the pH from the responses produced by the astringent test compounds themselves. This is necessary because the standard astringent-tasting compounds tested here acidify unbuffered aqueous solutions. Of the five astringent compounds tested, tartaric acid is the least astringent (15) and is sometimes considered simply sour (11); gallic acid is bitter,

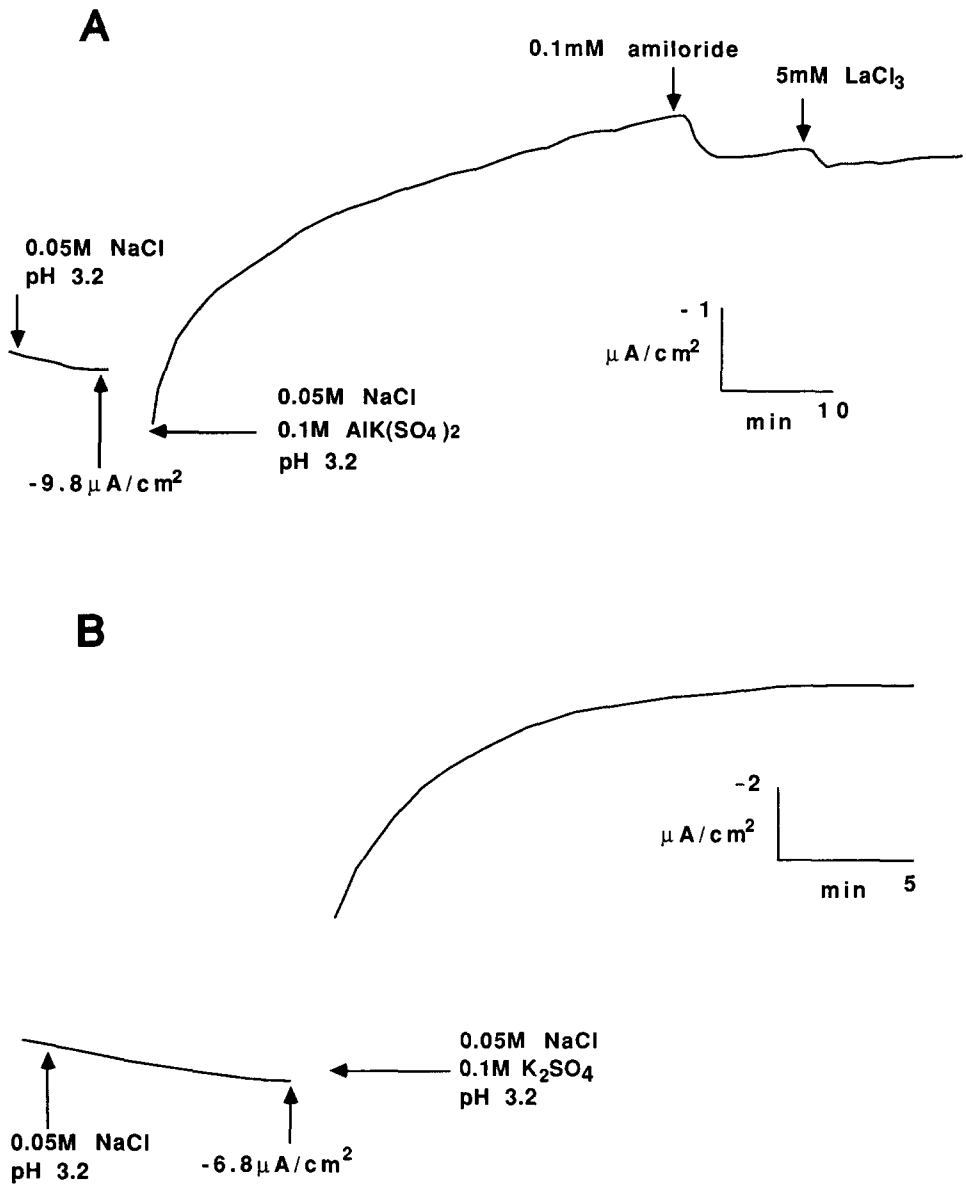

FIG. 7. Responses of I_{sc} across canine lingual epithelia to aluminum salts. (A) Response (I_{sc}) of canine lingual epithelia originally having 0.5 M NaCl pH 3.2 on its mucosal surface to the replacement of this solution with one containing $AlK(SO_4)_2$. Both amiloride and $LaCl_3$ addition produced small decreases when added to the mucosal solution. (B) Control experiment to test the response to 0.1 M K_2SO_4. Breaks in the traces indicate the changing of the mucosal solution.

sour, and weakly astringent (S. S. Schiffman, personal observation). In contrast, tannic acid is very astringent over a pH range from 2-6.3.

It has been shown previously that for hyperosmotic concentrations of NaCl at pH 7.4 I_{sc} across isolated canine lingual epithelia arises principally from an amiloride-inhibitable influx of Na^+ (7,18). It has also been shown that in isotonic KH buffer I_{sc} arises from the sum of a net influx of Na^+ and a net efflux of Cl^- (18). Some I_{sc} reflects transport across taste cells since the chorda tympani and epithelial responses are correlated (7,27). The remainder of I_{sc} reflects transport across the rest of the epithelium and may be involved in responses of lingual nerves to salts (25).

Lowering the pH of hyperosmotic concentrations of 0.5 M NaCl pH 7.4 to pH 3.2 decreases I_{sc} about 40% and increases R_m (Table 1). One explanation of these data is that amiloride-inhibitable epithelial Na^+ channels are blocked by lowering the pH, as are many other cation-selective channels (8), including a K^+ channel involved in sour taste (14). This inhibition would increase the transcellular resistance, which is normally lower than the paracellular resistance under these conditions (10), and hence increase R_m.

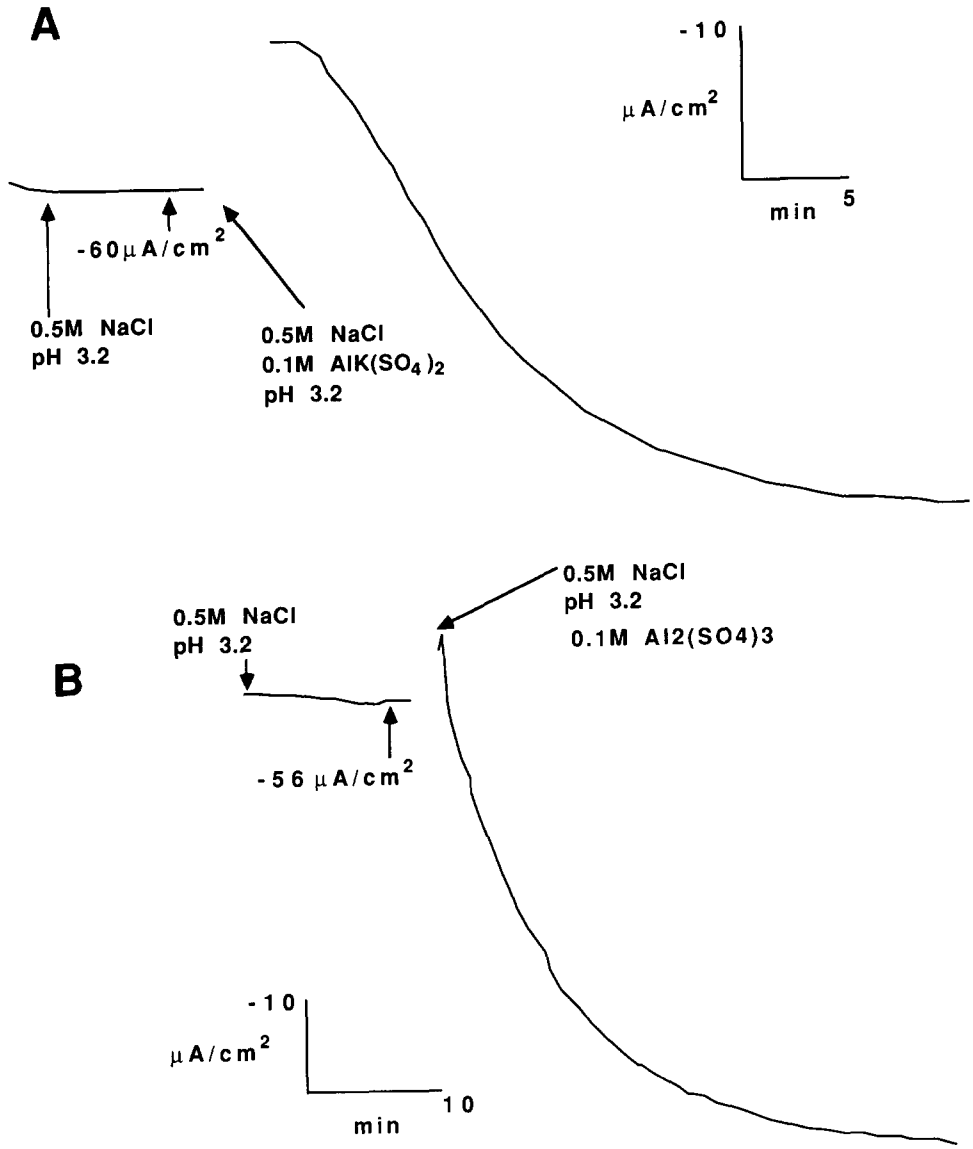

FIG. 8. Responses of I_{sc} across canine lingual epithelia to the addition of aluminum salts in 0.5 M NaCl. (A) Inhibition of I_{sc} by 0.1 M $AlK(SO_4)_2$. (B) Inhibition of I_{sc} by 0.1 M $Al_2(SO_4)_3$. Breaks indicate changes in mucosal solution.

At 0.05 M NaCl, lowering the pH from 7.4 to 3.2 increased both V_{oc} and I_{sc} (Table 1). Without additional experiments, it is not possible to know what transport pathway(s) were stimulated (or inhibited) by lowering the pH.

Responses to Tartaric, Tannic, and Gallic Acids

To obtain information on whether the responses of the organic astringent compounds influence transport themselves, the responses to acidification and to the addition of Na^+ had to be accounted for. This, in part, was accomplished by accounting for the additional Na^+ by measuring the response to an equal concentration of NaCl (Table 2). We fully understand these operations are not strictly equivalent as the Cl^- in these two solutions are different and hence this difference may be reflected in I_{sc} (27). However, once the choice was made to increase the pH to eliminate the response to acid this problem necessarily arises. Another factor to be considered in interpreting these data is that the method of replacing the reference solution with a test solution (e.g., Figs. 4 and 8) may not be strictly equivalent to the method of first adding NaCl to the reference solution to obtain the same Na^+ concentration that will be in the test solution and then replacing this solution with the test solution (e.g., Fig. 6). Differences may arise because before the test solution is substituted, the tongue is equilibrated with different solutions. It should be noted that

in most of the literature on astringent compounds these factors are not considered.

0.05 M NaCl. The increases in I_{sc} arising from the addition of tartaric and gallic acids at 7.4 arise predominantly from the addition of Na^+. For the purposes of this study, the investigation of astringency at this pH is more appropriate since the additional complexities of acidifying the solution are minimized. For example, the 20-$\mu A/cm^2$ increase in I_{sc} by 0.1 M tartaric acid pH 7.4 (Fig. 3) can be entirely accounted for by the additional Na^+ that was added in the form of NaOH to increase the pH to 7.4 (19.7 $\mu A/cm^2$ Table 2). This interpretation, namely, that the increase in I_{sc} arises from an increase in Na^+ influx, would also explain why the tartaric acid response is inhibited by amiloride and why the amiloride inhibition is less at lower pHs (compare Figs. 2A and 2B).

Tannic acid at pH 3.2 had small inhibitory effects on I_{sc} when contributions of the added Na^+ are accounted for, whereas tartaric acid had small stimulatory effects. We attribute the small stimulation or inhibition by organic astringent compounds at 0.05 M NaCl pH 3.2 to be a consequence of the blockage of transport pathways involved in cation channels by protons. At this pH and salt concentration, it is difficult to disentangle what actual changes in transport are occurring.

At pH 7.4, the addition of 0.1 M tannic acid to 0.05 M NaCl increased I_{sc} by 15 $\mu A/cm^2$ (Fig. 3), whereas an increase of 64 $\mu A/cm^2$ was expected as a consequence of the large increase in Na^+ concentration added to tannic acid solutions to bring the pH to 7.4 (Table 2). *In this respect, tannic acid inhibited Na^+ transport, most likely through epithelial amiloride-inhibitable sodium channels.* This interpretation is consistent with the observation that in the presence of tannic acid in 0.3 M NaCl amiloride does not inhibit I_{sc}.

0.5 M NaCl. Tannic acid inhibits I_{sc} at hyperosmotic concentrations of NaCl at pH 3.2 (Figs. 5 and 6A) and pH 7.4 (Fig. 6B). We attribute the inhibition of I_{sc} at pH 3.2 to arise from the further decrease in Na^+ influx through those amiloride-inhibitable channels that are not already blocked by protons. Under these conditions, amiloride does not inhibit I_{sc}, most likely because all the amiloride-inhibitable channels are already inhibited either by tannic acid or protons (Fig. 6A). The further inhibition of I_{sc} in tongues having tannic acid pH 3.2 by 1 mM ouabain (Fig. 6A) showed the tongue was indeed viable under these conditions. At pH 7.4, 0.1 M tannic acid inhibited I_{sc} after NaCl was added to the test solution (Fig. 6B). When the tannic acid solution was added directly to the reference solution (0.5 M NaCl), I_{sc} increased but not as much as expected. At pH 7.4, I_{sc} would be expected to increase by (24 $\mu A/cm^2$) from the addition of Na^+ (see Table 2). Thus, tannic acid in the mucosal solution actually reduced I_{sc} by about 14 $\mu A/cm^2$. Hence, tannic acid decreased I_{sc} over a wide range of pH and Na^+ concentrations. Under these conditions, where I_{sc} arises from a Na^+ influx, it is probable that tannic acid inhibits I_{sc} by inhibiting Na^+ influx through amiloride-inhibitable channels. The changes in transport observed with gallic and tartaric acids were difficult to distinguish from changes caused by increasing the Na^+ concentration, most likely because they are only weakly astringent.

Aluminum Salts

The addition of aluminum salts to the dorsal surface of canine lingual epithelia exhibits some similarities, as well as some differences, in responses from tannic acid. Both have two antagonistic components that contribute to I_{sc}. For the astringent aluminum salts in 0.05 M NaCl, the increase in I_{sc} (Figs. 3 and 7A) can be attributed to the presence of K_2SO_4 since its addition by itself increases I_{sc} (Fig. 7B). When this is accounted for, it is the aluminum rather than K^+, NH_4^+, or SO_4^{--} that is responsible for the inhibition of I_{sc}. At 0.5 M, the aluminum salts clearly decrease I_{sc} (Fig. 8), showing that they may also inhibit Na^+ influx. Moreover, under these conditions the addition of amiloride does not further decrease I_{sc}, which suggests that aluminum inhibits amiloride-inhibitable Na^+ channels. Interestingly, the aluminum salts do not behave in the same ways as $LaCl_3$ as the former decreases R_m (Figs. 3 and 5) and the latter increases R_m, most likely by virtue of blocking tight junctions (10). Under these conditions, aluminum salts likely block epithelial sodium channels.

GENERAL COMMENTS

The main finding of this study is that both tannic acid and the aluminum salts inhibited transport (likely Na^+ influx) across isolated lingual epithelia. For tannic acid, the inhibition does not depend upon the pH or Na^+ concentration, and hence this inhibition likely reflects the interaction of this compound with amiloride-sensitive channels present in taste cells. Since transport across isolated lingual epithelia is correlated with events underlying taste transduction, it is likely that the inhibition of I_{sc} induced by tannic acid and the aluminum salts reflects changes in the inhibition of the same transport pathways in taste cells. Both Kawamura et al. (12) and Schiffman et al. (unpublished observation) found that tannic acid inhibited responses from the rat chorda tympani to a variety of stimuli, including NaCl. Since responses of rat chorda tympani fibers to NaCl are inhibited by amiloride (19), one component of astringent taste may involve the inhibition of the amiloride-inhibitable Na^+ channel. These data directly demonstrate that astringent-tasting compounds interact with lingual epithelia and can cause changes in transport that are related to taste. Thus, it is unlikely that astringency is the consequence of a chemically induced tactile sensation (16) or of precipitating water-soluble mucus proteins (2) but rather the consequence of altering transport proteins involved in taste transduction.

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