Sugar-activated Ion Transport in Canine Lingual Epithelium

Implications for Sugar Taste Transduction

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ABSTRACT There is good evidence indicating that ion-transport pathways in the apical regions of lingual epithelial cells, including taste bud cells, may play a role in salt taste reception. In this article, we present evidence that, in the case of the dog, there also exists a sugar-activated ion-transport pathway that is linked to sugar taste transduction. Evidence was drawn from two parallel lines of experiments: (*a*) ion-transport studies on the isolated canine lingual epithelium, and (*b*) recordings from the canine chorda tympani. The results in vitro showed that both mono- and disaccharides in the mucosal bath stimulate a dose-dependent increase in the short-circuit current over the concentration range coincident with mammalian sugar taste responses. Transepithelial current evoked by glucose, fructose, or sucrose in either 30 mM NaCl or in Krebs-Henseleit buffer (K-H) was partially blocked by amiloride. Among current carriers activated by saccharides, the current response was greater with Na than with K. Ion flux measurements in K-H during stimulation with 3-*O*-methylglucose showed that the sugar-evoked current was due to an increase in the Na influx. Ouabain or amiloride reduced the sugar-evoked Na influx without effect on sugar transport as measured with tritiated 3-*O*-methylglucose. Amiloride inhibited the canine chorda tympani response to 0.5 M NaCl by 70–80% and the response to 0.5 M KCl by ~40%. This agreed with the percent inhibition by amiloride of the short-circuit current supported in vitro by NaCl and KCl. Amiloride also partially inhibited the chorda tympani responses to sucrose and to fructose. The results indicate that in the dog: (*a*) the ion transporter subserving Na taste also subserves part of the response to K, and (*b*) a sugar-activated, Na-preferring ion-transport system is one mechanism mediating sugar taste transduction. Results in the literature indicate a similar sweet taste mechanism for humans.

INTRODUCTION

In earlier studies of the ion-transport properties of the canine lingual epithelium in vitro, it was noted that sugars stimulate an amiloride-sensitive mucosal-to-submuco-

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sal transepithelial short-circuit current (DeSimone et al., 1982, 1984; Mierson et al., 1982; Simon et al., 1986). The observation raised the possibility that a sugar-activated ion-transport pathway might be a factor in saccharide taste transduction. The possibility was strengthened by the report that the taste of sugars in humans could be reduced by lingual applications of amiloride (Schiffman et al., 1983). On the basis of these observations, it was assumed that amiloride would produce a significant reduction in the chorda tympani response to sugars in any of a variety of standard animal preparations. However, studies have failed to demonstrate this in the rat (Brand et al., 1985; Blochaviak and Jakinovich, 1985), gerbil (Jakinovich, 1985), and hamster (Herness, 1987). Consistent with these findings is the failure to demonstrate a sugar-activated ion-transport system in vitro using a rat lingual epithelium (DeSimone, J. A., unpublished observation). The sugar-activated system was also absent from macaque (*Macaca fascicularis;* DeSimone, J. A., unpublished observation) and frog (Soeda and Sakudo, 1985) tongues. In the rabbit tongue, sugars evoked a much slower and lower-magnitude response relative to the dog tongue (Simon et al., 1986). It is well documented that sweet taste sensitivity varies markedly among mammals (Hellekant et al., 1985; Sato, 1985), and evidence exists for multiple receptors for sugars even within a species (Schiffman et al., 1981; Tonosaki and Funakoshi, 1984*a*, *b*; Vlahopoulos and Jakinovich, 1986). Thus, a neurophysiological correlate of sugar-activated ion transport must be sought in the dog itself.

In this article, we characterize the sugar-activated canine lingual transport system in vitro, focusing on the ions transported, the relative specificity of the response among cations, and the possibility of coupling between ion and sugar influxes. A parallel line of experiments on responses from the canine chorda tympani shows for the first time that the dog's neural response to NaCl is partially blocked by amiloride and that recovery is a first-order process. Thus, the dog has an amiloride-sensitive NaCl taste system similar to that already described in rodents (Schiffman et al., 1983; Heck et al., 1984; De Simone et al., 1984; Ninomiya et al., 1984; Brand et al., 1985; DeSimone and Ferrell, 1985; Jakinovich, 1985; Herness, 1987). However, unlike in the rodent, the response to KCl is also in part amiloride sensitive, and the responses to fructose and sucrose are partially amiloride sensitive. The results show that the canine has more than one type of saccharide-detecting taste system. One of these appears to be a sugar-activated, Na-preferring, apical membrane ion pathway. Given the outcome of earlier human studies (Schiffman et al., 1983), our results indicate that the dog may be a good neurophysiological model for human sugar taste. Some of the data presented here have appeared in published abstracts (Mierson et al., 1987*a*, *b*).

METHODS

Lingual Epithelium In Vitro

Dissection. Dorsal lingual epithelia from dogs were prepared in the manner described in previous publications (DeSimone et al., 1984; Mierson et al., 1985). Briefly, mongrel dogs, 15–25 kg in weight, were anesthetized with sodium pentobarbital and killed by exsanguination or by surgical removal of the heart. The tongue was removed anterior to the circumvallate papillae and placed dorsum down on a dissecting board. The lingual mucosa was stripped

of its adherent muscle layers and mounted in modified Ussing chambers (cf. DeSimone et al., 1984).

Voltage-clamp system. The potential difference (PD) across the tissue was measured by calomel electrodes connected to the solution by 3% agar/saline bridges. Current was passed with sintered Ag/AgCl electrodes in 0.15 M NaCl and delivered to the tissue via a second set of salt bridges. The short-circuit current (I_{sc}) and the resistance (R) were measured with single-channel voltage clamps (Physiologic Instruments, Houston, TX) and monitored on a two-channel strip-chart recorder. Resistance was determined by pulsing current for 0.6 s (±10 mV) and PD was calculated from the values of I_{sc} and R. Series fluid resistance between the PD bridges was automatically compensated in all experiments with Krebs-Henseleit buffer (K-H) on both sides of the tissue; addition of nonelectrolyte sugars did not affect the solution resistance. In experiments under asymmetrical electrolyte conditions, the effect of sugar was always expressed as the difference between I_{sc} in the presence of sugar and the baseline I_{sc} without sugar. Hence, the effects of liquid junction potentials canceled. Various means of correcting for liquid junction potentials when using hyperosmotic NaCl solutions have been discussed previously (DeSimone et al., 1984; Mierson et al., 1985).

Flux measurements. For flux measurement, two tissues from one tongue were selected according to the criterion that resistances matched to within 15%. The flux chambers and basic protocols have been previously described (Mierson et al., 1985). The chambers, modified from those of Biber and Mullen (1977), were conical in shape and were designed to minimize the volume while permitting rapid change of solutions. The exposed area of the tissue was 1.77 cm^2 and the volume of each chamber was 0.9 ml. Solutions were maintained at 34°C and mixed by bubbling gas. All flux measurements were made under short-circuit conditions. Unidirectional mucosal-to-submucosal fluxes of Na, Cl, and 3-*O*-methylglucose were measured simultaneously across one of the tissues and undirectional submucosal-to-mucosal fluxes were measured across the paired tissue. 15-min collection periods were begun after a 45-min equilibration period. To establish baseline conditions, the first two collection periods were carried out under symmetrical conditions with K-H in all chambers. Thereafter, K-H with unlabeled 0.5 M 3-*O*-methyglucose was placed in the mucosal side of each chamber pair. The effect of the added sugar on the fluxes of the labeled isotopes of Na, Cl, and 3-*O*-methylglucose was then obtained. Labeled solutions were changed at least once an hour. ^{22}Na radioactivity was determined by gamma counter (γ 3000, Beckman Instruments, Inc., Fullerton, CA). Subsequently, [^{3}H]3-*O*-methylglucose radioactivity and total ^{22}Na and ^{36}Cl radioactivity were determined by liquid scintillation counting (LS335, Beckman Instruments, Inc.). All test solutions in a given series had identical specific activities.

For all experiments other than the flux measurements, a larger chamber (7 ml vol) was used. A few experiments were performed on tissues treated with collagenase, to eliminate the connective tissue and thus diffusion barriers due to connective tissue. Our method was modified from those of Aceves and Erlij (1971) on frog skin and of Mistretta (1971) on rat tongue. The tissue was treated on the submucosal side for 2 h with a 4% solution of bacterial collagenase in oxygenated K-H; subsequently, the connective tissue could be gently pulled away from the epithelial cell layers. The remaining tissue sustains its characteristic papillary structure and lateral connectivity. It is translucent but sufficiently strong to be easily mounted in an Ussing chamber. We found that removing the connective tissue made no significant difference in the steady state electrical parameters in response to sugars.

Solutions. The basic bathing solution was K-H, 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 $NaHCO_3$, and 5.6 mM glucose; the pH was 7.4 when the solution was bubbled with 95% O_2/5% CO_2. In some experiments, K-H was removed from the mucosal chamber and replaced with an electrolyte solution according to a specific protocol (cf. Results). The electrolyte solutions used were: 30 mM

NaCl, 150 mM NaCl, 500 mM NaCl, 150 mM KCl, and 150 mM *N*-methyl-D-glucammonium chloride. Previous studies (Mierson et al., 1985) have shown that I_{sc} is relatively insensitive to pH over the range of 4–7.4. Hence, these electrolyte solutions were unbuffered and were bubbled with 100% O_2. The submucosal side, however, was always maintained in buffered (pH 7.4) K-H.

All chemicals were reagent grade. Ouabain octahydrate, phloridzin (phloretin-2′-β-D-glucoside), and collagenase were obtained from Sigma Chemical Co., St. Louis, MO. Amiloride was a gift from Dr. E. G. Cragoe of Merck, Sharp & Dohme, West Point, PA. ^{22}Na and ^{36}Cl were from New England Nuclear, Boston, MA; test solutions were 0.2–0.3 μCi/ml. 3-*O*-methyl-D-glucose (methyl-^{3}H) was from ICN Radiochemicals, Irvine, CA; test solutions were 1 μCi/ml.

Statistics. Results are expressed as means ± SE. Differences were considered significant if the *P* value, calculated from the paired Student's *t* test, was <0.05.

Neurophysiological Experiments

For the neurophysiological experiments, adult beagles weighing 7–10 kg were anesthetized with sodium pentobarbital infused intravenously. Supplemental doses were administered as necessary to maintain anesthesia. A tracheal cannula was inserted and the dog was placed on a respirator. Blood pressure was recorded throughout the experiment. Access to the chorda tympani nerve was obtained after its exit from the tympanic bulla by dissection through the left lateral aspect of the head. Initially, a flap of skin overlying the temporomandibular joint and the masseter was cut and retracted from the region of the auditory canal toward the muzzle. The attachments of the masseter were freed below the temporomandibular joint, continuing along the margin of the mandible to below its genu. The bulk of the muscle was removed and the mandible was cut with rongeurs diagonally upward from just above the genu, thus freeing the condyloid process from the rest of the jaw. This piece was retracted upward and outward, disarticulating the joint without traumatizing the underlying tissue. The chorda tympani could be located, along with branches of the trigeminal nerve, in this underlying tissue immediately below a thin muscular layer. The chorda tympani was freed from connective tissue, cut at its cephalic end, and suspended from a platinum-iridium hook electrode. A mixture of petrolatum and mineral oil was used to insulate the nerve and prevent its dehydration.

Electrical signals were differentially amplified with respect to an indifferent electrode placed against local connective tissue and bandwidth-limited. The resulting signal was rectified and integrated with a time constant of 10 s. The integrated signal was displayed on a strip-chart recorder and served as a measure of the neural taste response.

The tongue was exposed and stimulated by flowing ~10 ml of solution from a syringe for 5 s over the anterior dorsal surface. Excess stimulus was allowed to drain from the tongue, but the tongue was not rinsed for at least 30 s. The volume of the rinse solution was 10–15 ml.

RESULTS

Sugar Concentration-Response Relations

The canine dorsal lingual epithelium in vitro responds to solutions of various sugars with a dose-dependent increase in either the open-circuit potential or the short-circuit current (I_{sc}). Fig. 1 *A* shows I_{sc} as a function of mucosal sugar concentration for glucose, fructose, or sucrose in K-H. Each concentration-response relation was fitted to a three-parameter empirical relation (Hill equation) employing a least-squares criterion. For glucose, K_m was 0.15 M and the Hill coefficient (n) was 3.6

(Fig. 1 *A*). Each of the other sugars could be similarly described and the respective equation parameters are given in the legend to Fig. 1. At a concentration of 0.5 M, glucose and fructose produced approximately the same I_{sc}, whereas sucrose produced significantly less (Fig. 1 *A* and Table I). In another series of experiments,

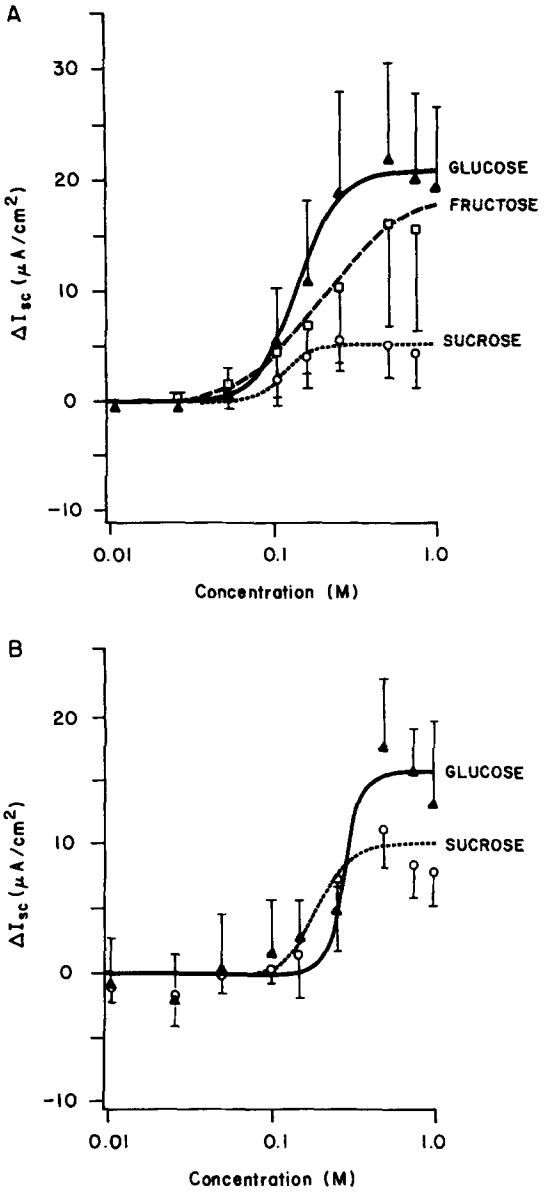

FIGURE 1. (*A*) I_{sc} as a function of the mucosal concentration of glucose, sucrose, or fructose in K-H for the canine dorsal lingual epithelium in vitro. ΔI_{sc} was calculated relative to I_{sc} with no sugar present. The concentration-response function for each tissue was generated by successively increasing the sugar concentration. Each point is a mean ±SEM (number of tissues =11 [glucose], 4 [fructose], 5 [sucrose]). The smooth curve represents the equation $\Delta I_{sc} = K_1 x^n/(K_m^n + x^n)$, where x is the sugar concentration in moles per liter and n is the Hill coefficient. For glucose (triangles), K_1 = 20.8 μA/cm², K_m = 0.15 M, and n = 3.6. For fructose (squares), K_1 = 18.3 μA/cm², K_m = 0.20 M, and n = 1.9. For sucrose (circles), K_1 = 5.2 μA/cm², K_m = 0.11 M, and n = 5.2. (*B*) ΔI_{sc} for glucose or sucrose dissolved in 30 mM NaCl (four tissues for each sugar). For glucose (triangles), K_1 = 16.1 μA/cm², K_m = 0.28 M, and n = 8.3. For sucrose (circles), K_1 = 10.0 μA/cm², K_m = 0.20 M, and n = 5.7.

sugars were dissolved in 30 mM NaCl as the supporting medium. This mucosal solution produces near-zero transepithelial potential and approximates the Na concentration in saliva at basal rates of stimulation (cf. DeSimone et al., 1984). Fig. 1 *B* shows the concentration-response relation for glucose and sucrose in 30 mM NaCl.

At 0.5 M sugar, glucose evoked the larger response (Fig. 1 *B* and Table I). With 30 mM NaCl as the supporting electrolyte, the data were not as well described by the Hill equation. Mannose and 3-*O*-methylglucose in K-H also evoked an increase in I_{sc}, which was dose dependent (data not shown). With K-H on both sides of the tissue, glucose added to the submucosal solution caused a slow decrease in I_{sc} and an increase in resistance with increasing concentration.

Effect of NaCl Concentration on the Sucrose-mediated Increase in I_{sc}

The magnitude of the response to 0.5 M sucrose depended on the NaCl concentration in the mucosal supporting medium. Sucrose solutions at 0.5 M in NaCl at concentrations of ≤10 mM produced negligible inward I_{sc} (data not shown). However, in 30 mM NaCl, 0.5 M sucrose stimulated its maximal observed I_{sc} (Table I). In K-H, 0.5 M sucrose stimulated significantly less current (Table I). When the NaCl concentration was 0.5 M, the addition of 0.5 M sucrose resulted in no further increase in I_{sc}

TABLE I

Effects of Sugars on In Vitro Canine Lingual Epithelium

Sugar Electrolyte	Glucose 30 mM NaCl	Glucose K-H	Sucrose 30 mM NaCl	Sucrose K-H	Sucrose 500 mM NaCl	Fructose K-H
I_{sc}, baseline ($\mu A/cm^2$)	−0.5 ± 2.1	27.0 ± 1.8	−0.3 ± 1.1	26.0 ± 2.3	148 ± 43	23.3 ± 3.8
I_{sc}, with sugar ($\mu A/cm^2$)	17.2 ± 1.4	48.9 ± 5.2	11.3 ± 1.1	31.3 ± 3.1	130 ± 40	39.3 ± 8.4
Change in I_{sc}	17.8 ± 2.6	21.9 ± 4.0	11.6 ± 1.5	5.3 ± 1.6	−18 ± 4	16.1 ± 4.8
R, baseline ($\Omega \cdot cm^2$)	708 ± 59	654 ± 62	996 ± 169	535 ± 64	184 ± 49	421 ± 42
R, with sugar ($\Omega \cdot cm^2$)	941 ± 114	582 ± 60	1362 ± 246	620 ± 86	195 ± 45	425 ± 32
Percent change in R	+32 ± 8	−11 ± 4	+37 ± 17	+15 ± 4	+7 ± 4	+2 ± 6
n	4	11	4	5	2	4

The sugar concentration was always 0.5 M. n = number of experiments.

above the value in 0.5 M NaCl alone (Table I). Hence, the current stimulated by sucrose peaks between 10 and 143.7 mM Na (the concentration in K-H) and then declines. On the other hand, glucose responses in 30 mM NaCl were not significantly different from those in K-H (Table I).

Resistance Changes Owing to the Presence of Sugars

For all the sugars and electrolyte concentrations investigated, changes in resistance produced by the sugars at a fixed electrolyte concentration relative to baseline conditions were small compared with the resistance changes observed when the electrolyte concentrations were varied. With the 30 mM NaCl supporting medium, both 0.5 M glucose and 0.5 M sucrose resulted in a small but statistically significant increase in resistance (cf. Table I). For other sugars or media investigated, resistance changes were <20%, up to 1 M sugar.

Comparison of Salt- and Sugar-mediated Increase in I_{sc}

The response of this epithelium to NaCl stimulation has been described in detail (DeSimone et al., 1984). NaCl- and sugar-stimulated increases in I_{sc} show a number of differences. Fig. 2 shows the time course of I_{sc} when a tissue was treated first with 0.5 M sucrose (trace *A*) and subsequently with 0.25 M NaCl (trace *B*). In the first case, the baseline was established in K-H. The tissue resistance calculated from the 3-mV pulse was 759 $\Omega \cdot cm^2$. At *S*, the mucosal solution was replaced with 0.5 M sucrose in K-H. The current increased at nearly constant resistance. In a given preparation, the initial rise in current produced by sugars was always slower than the rise in current produced by increasing the NaCl concentration above baseline concentration. In trace *B*, the baseline was established in a modified buffer consisting of K-H with the NaCl removed. This established a Na concentration of 26.5 mM. At *N*, the mucosal solution was replaced by 0.25 M NaCl. The resulting time course was

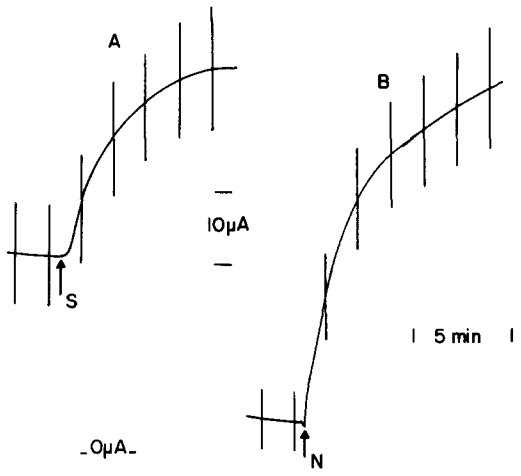

FIGURE 2. Comparison of the time course of the increase in I_{sc} due to 0.5 M sucrose in K-H (*A*) and to 0.25 M NaCl (*B*) in a collagenase-treated preparation of canine lingual epithelium. Current excursions were in response to a bipolar 3-mV voltage pulse; the length of excursion is proportional to tissue conductance.

the two-component hyperosmotic response typical of a NaCl stimulus (DeSimone et al., 1984). Beginning at *N*, the salt response consisted of a rapid first component, followed by an inflection point and a slower second component. This was accompanied by a continuous decrease in resistance from 1,328 to 664 $\Omega \cdot cm^2$. The rapid first component was never observed with sugar stimulation at a fixed concentration of supporting electrolyte.

As seen in Fig. 3 *A*, as the NaCl concentration was varied between 0.01 and 1 M (open circles), I_{sc} varied from close to 0 to >100 $\mu A/cm^2$. The resistance changes corresponding to this are shown in Fig. 3 *B*. At 0.01 M NaCl, the tissue resistance was >1,200 $\Omega \cdot cm^2$. With increasing NaCl concentration, it fell continuously to ~100 $\Omega \cdot cm^2$ at 1 M NaCl. Over the same range of concentrations, glucose dissolved in K-H resulted in smaller changes in current, as seen in Fig. 3 *A* (filled circles). In contrast to salt stimulation, the resistance fell only slightly with glucose stimulation over the entire concentration range (Fig. 3 *B*).

Amiloride Effect on the Sugar-mediated Increase in I_{sc}

The sugar-mediated increase in I_{sc} is amiloride sensitive in isosmotic supporting electrolytes (DeSimone et al., 1984) and in 30 mM NaCl. Fig. 4 shows the time course of I_{sc} following the addition of 0.1 mM amiloride to 0.5 M glucose in 30 mM NaCl. In

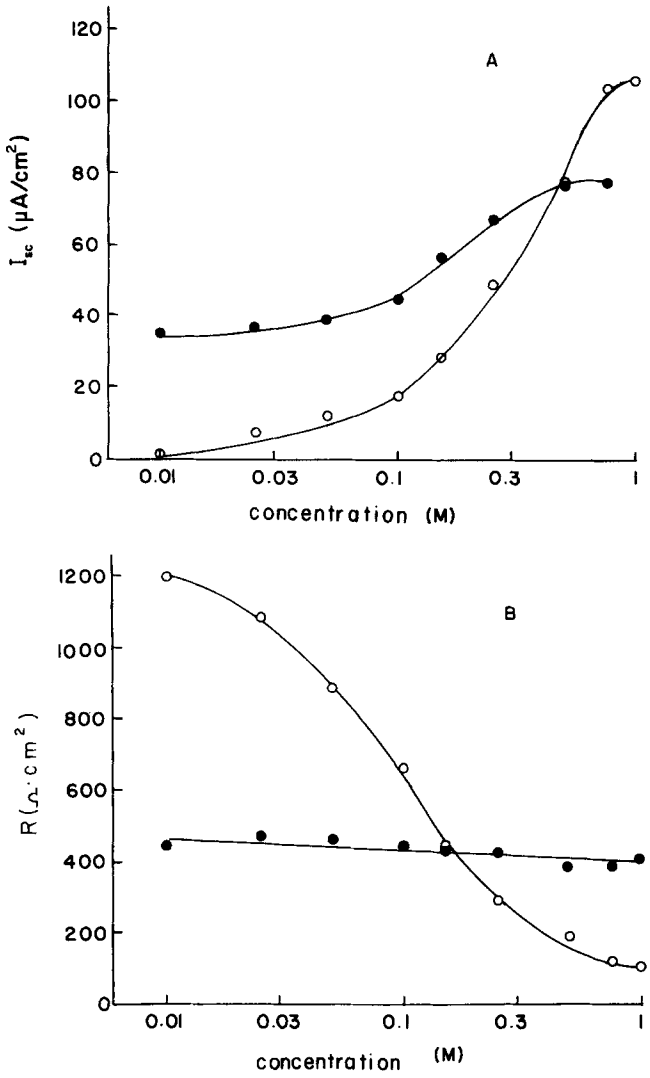

FIGURE 3. Comparison of the effect of D-glucose in K-H (filled circles) and of NaCl (open circles) on I_{sc} (*A*) and on the tissue resistance (*B*) in one canine lingual preparation.

three experiments, amiloride resulted in a mean reduction in I_{sc} of 73 ± 4% in the presence of glucose in 30 mM NaCl. Similar results were obtained with sucrose in 30 mM NaCl. Amiloride also inhibited the I_{sc} produced by glucose, sucrose, or fructose in K-H. These results suggest that sugars increase I_{sc} by specifically increasing

the Na flux through the transporting cells of the lingual mucosa. This was confirmed by measuring the Na unidirectional fluxes directly (*vide infra*).

Effects of 3-O-Methylglucose and Phloridzin

In order to rule out a metabolic effect of glucose, we performed experiments with 3-*O*-methylglucose, a nonmetabolizable glucose analogue used extensively as a substrate for the intestinal glucose carrier (Schultz and Zalusky, 1964). 3-*O*-methylglucose in 30 mM NaCl produced a dose-dependent increase in I_{sc}. In the small intestine and kidney, glucose or its 3-*O*-methyl derivative binds to a site on the apical membrane glucose carrier. Phloridzin competes with glucose for this site, effectively blocking sugar entry and stoichiometrically linked Na ion transport (Schultz and Zalusky, 1964). In our experiments, phloridzin added to the mucosal solution (0.5

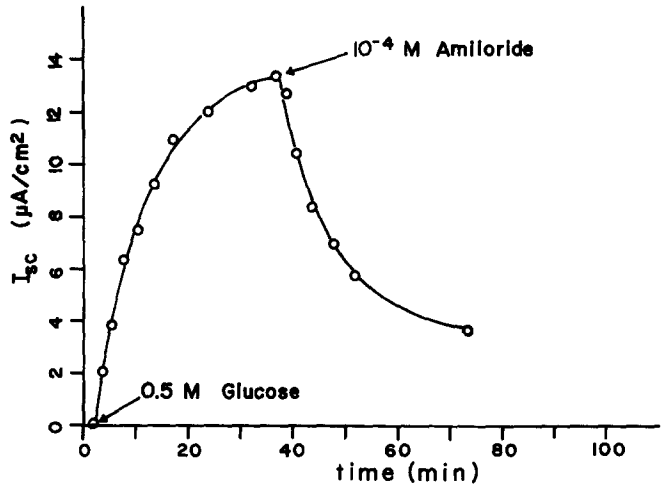

FIGURE 4. Time course of increase in I_{sc} due to 0.5 M glucose in 30 mM NaCl. In 30 mM NaCl, I_{sc} was zero. Adding 0.5 M glucose in 30 mM NaCl caused I_{sc} to increase toward a steady state. At the indicated point, the mucosal solution was replaced by 0.5 M glucose in 30 mM NaCl with 0.1 mM amiloride. This resulted in the indicated decline in I_{sc}.

mM, 5 mM, or saturated solution) had no effect on the ability of 0.5 M 3-*O*-methylglucose to increase I_{sc}.

Fluxes of Na, Cl, and 3-O-Methylglucose

While it is clear that the presence of various sugars causes increased ion transport across the lingual mucosa, it is not known if the sugar itself is translocated by a process stoichiometrically linked to ion transport, nor is it known precisely which ion (or ions) is responsible for the increased current. To answer these questions, we measured simultaneously the unidirectional fluxes of ^{22}Na, ^{36}Cl, and [^{3}H]3-*O*-methylglucose under short-circuit conditions. K-H was selected as the supporting medium for the following reasons. (*a*) A symmetrical electrolyte medium under short-circuit constraints eliminates transmural passive electrolyte driving forces. (*b*)

Among the media investigated, isosmotic K-H supported a consistently robust sugar-mediated increase in I_{sc}. (*c*) In K-H, transepithelial resistance was relatively constant over a range of sugar concentration. [^{3}H]3-*O*-methylglucose was the sugar of choice because it elicits an increase in I_{sc} but is not metabolized within the cells.

The unidirectional fluxes were obtained first in the absence of and then in the presence of unlabeled 0.5 M 3-*O*-methylglucose added to the mucosal solution. The results are summarized in Table II. The addition of the unlabeled sugar resulted in a doubling of I_{sc}. Concurrently, there was a doubling of the unidirectional mucosal-to-submucosal Na flux. Before the addition of unlabeled sugar, the net Na flux accounted for 53% of I_{sc} and Cl secretion accounted for 27% of I_{sc}. The residual flux

TABLE II

Effects of 3-O-Methylglucose on the In Vitro Canine Lingual Epithelium

	No sugar	With sugar	*P*
I_{sc} ($\mu eq/cm^2 \cdot h$)	0.72 ± 0.06	1.44 ± 0.12	<0.0005
R ($\Omega \cdot cm^2$)	724 ± 51	682 ± 62	<0.025
PD (mV)	13.2 ± 0.4	24.2 ± 1.1	<0.0005
J_{ms}^{Na} ($\mu eq/cm^2 \cdot h$)	0.83 ± 0.08	1.63 ± 0.19	<0.0005
J_{sm}^{Na} ($\mu eq/cm^2 \cdot h$)	0.43 ± 0.06	0.32 ± 0.04	<0.025
J_{net}^{Na} ($\mu eq/cm^2 \cdot h$)	0.40 ± 0.08	1.31 ± 0.16	<0.005
J_{ms}^{Cl} ($\mu eq/cm^2 \cdot h$)	0.49 ± 0.05	0.70 ± 0.14	<0.05
J_{sm}^{Cl} ($\mu eq/cm^2 \cdot h$)	0.65 ± 0.09	0.68 ± 0.09	>0.3
J_{net}^{Cl} ($\mu eq/cm^2 \cdot h$)	−0.17 ± 0.08	0.02 ± 0.07	<0.05
J_{ms}^{glu} ($\mu M/cm^2 \cdot h$)	0.032 ± 0.004	2.39 ± 0.27	<0.0005
J_{sm}^{glu} ($\mu M/cm^2 \cdot h$)	0.036 ± 0.003	0.033 ± 0.003	<0.01
J_{net}^{glu} ($\mu M/cm^2 \cdot h$)	−0.004 ± 0.002	2.36 ± 0.27	<0.0005
J_{net}^{Na}/I_{sc}	0.53 ± 0.08	0.89 ± 0.05	<0.0005
J^R/I_{sc}	0.20 ± 0.06	0.14 ± 0.03	>0.15

n = 12 (pairs of tissues). Values are means ± SE. *P* values were determined by the Student's *t* test for paired variants. 3-*O*-methylglucose (0.5 M) was added on the mucosal side in K-H. The submucosal solution was always K-H. Values in absence of sugar were obtained after a 30-min equilibration period. Values in presence of sugar were obtained 1 h after adding the glucose derivative. J_{ms}, unidirectional mucosal-to-submucosal flux; J_{sm}, unidirectional submucosal-to-mucosal flux; J_{net}, net flux. A positive value for J_{net} indicates absorption. J^R, residual, or unmeasured, ion flux $[I_{sc}/F - (J_{net}^{Na} - J_{net}^{Cl})]$. Electrical parameters for each tissue pair were averaged and counted as single value.

was 20% of the current. In the presence of 0.5 M 3-*O*-methylglucose, net Na flux accounted for 89% of the current, Cl secretion was abolished, and the residual flux was reduced to 14%.

The difference in the Na influx in the presence and absence of sugar was 0.80 μeq/cm^2·h. This represents the sugar-activated Na ion influx. The change in the sugar influx upon the addition of 0.5 M 3-*O*-methylglucose was 2.36 μeq/cm^2·h. The sugar/Na influx ratio was therefore not 1:1, but rather ~3:1. This observation alone, however, does not constitute evidence in favor of a common pathway for both Na and sugar absorption. Fig. 5 shows that over the first isotope-collecting

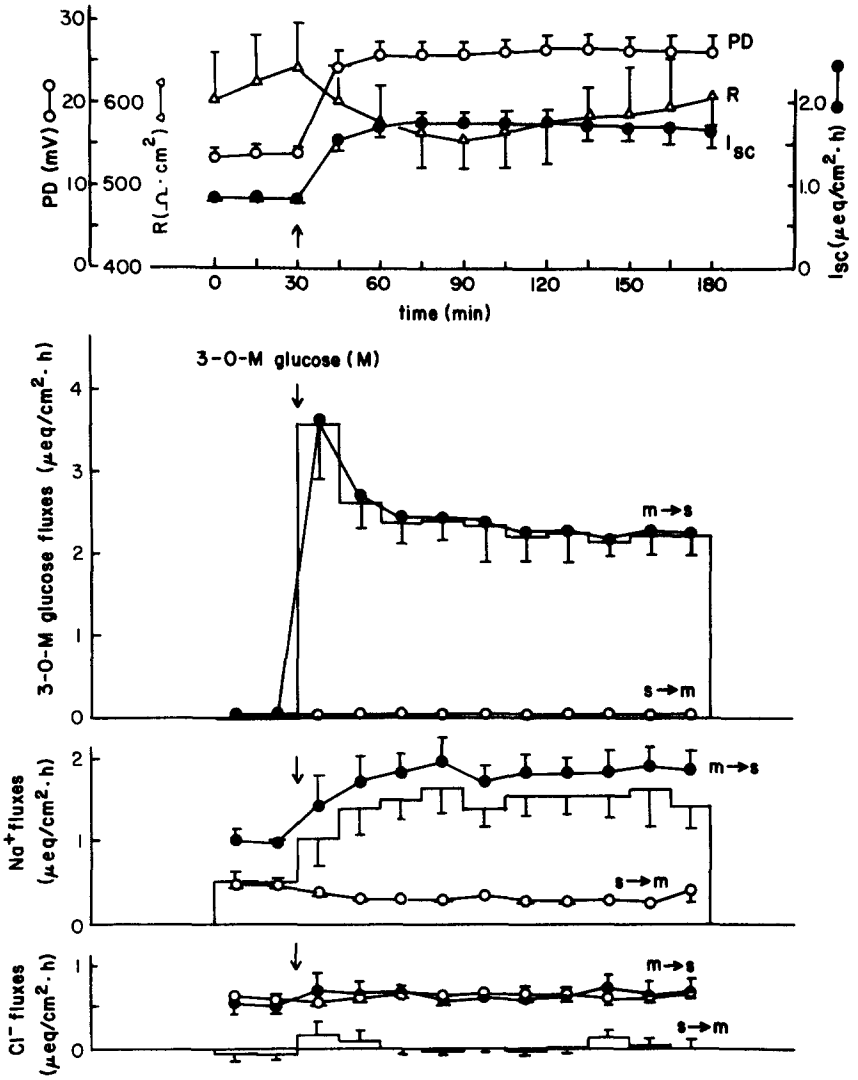

FIGURE 5. Effects of unlabeled 3-*O*-methylglucose (0.5 M added to mucosal solution) on electrical parameters (PD, I_{sc}, and R), Na, Cl, and 3-*O*-methylglucose fluxes in canine lingual epithelium (n = 4). Sugar was added in K-H. Unidirectional flux from mucosal to submucosal side: m→s; unidirectional flux from submucosal to mucosal side: s→m. The histogram represents net flux.

period, the ratio of sugar to Na influx was considerably greater than 3:1. This figure also shows that the Na and sugar fluxes reached steady state 15–30 min after addition of sugar. The electrical parameters and the ion fluxes maintained their steady state values for at least 2.5 h after sugar addition. Hence, each tissue served as its own control when inhibitors were added.

Effects of Ouabain and Amiloride on the Na and Sugar Fluxes

If the sugar and Na fluxes are tightly coupled at some point in their passage across the epithelium, then any agent that blocks Na transport should simultaneously reduce the sugar flux. If they traverse the tissue by parallel routes, blocking Na transport should have no effect on the transport of the sugar. Experiments using ouabain or amiloride showed the first hypothesis to be incorrect. Fig. 6 shows the effects of ouabain on I_{sc} and Na fluxes after steady state conditions were achieved in the presence of 3-*O*-methylglucose. I_{sc} and the net Na absorption decreased by 93 ± 2 and 91 ± 9%, respectively, whereas influx of 3-*O*-methylglucose (not shown) was unaffected. Similarly, amiloride caused reductions in both I_{sc} (51 ± 4%) and net Na absorption (40 ± 8%) (cf. Fig. 7) with no decrease in sugar influx. Thus, much like

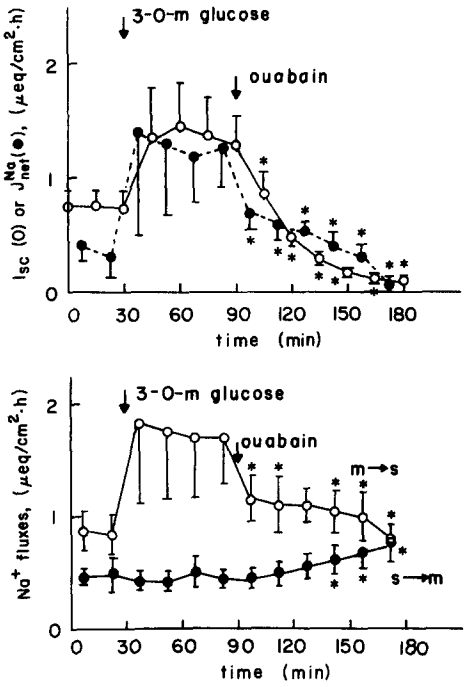

FIGURE 6. Effects of 3-*O*-methylglucose and ouabain on I_{sc} and Na fluxes in canine lingual epithelium ($n = 4$). Sugar (0.5 M) was added on the mucosal side, ouabain (9×10^{-4} M) was added on the submucosal side, and K-H was on both sides of the tissue. The symbols represent the same quantities as in Fig. 5. Asterisks indicate a significant difference from the collection period just before adding the inhibitor ($P < 0.05$, paired Student's t test).

the hyperosmotic salt response (Mierson et al., 1985), there was both an amiloride-sensitive and an amiloride-insensitive Na flux as a result of sugar stimulation.

Permeability Coefficient of 3-O-Methylglucose

The insensitivity of the sugar influx to both inhibitors of Na influx supports the second hypothesis; viz., the pathways for sugar and Na absorption are independent. Given the concentration gradient of sugar across the epithelium and using the values for the unidirectional fluxes in Table II, the flux ratio does not differ within experimental error from that predicted by the Ussing flux equation. Hence, we conclude that sugar transport is by passive diffusion. With this assumption, we can calculate an apparent permeability coefficient for 3-*O*-methylglucose of (1.31 ± 0.15) × 10^{-6} cm/s.

Effect of Cation Substitution on Sugar-mediated Increase in I_{sc}

The ion flux determinations prove that the sugar-mediated increase in current is carried by Na in K-H. To determine the specificity of the response for Na, experiments were done using supporting electrolytes free of Na. In these experiments, the increase in current evoked by 0.5 M glucose dissolved in either 0.15 M KCl or 0.15 M *N*-methyl-D-glucammonium Cl (GACl) was compared with the current evoked by 0.5 M glucose in 0.15 M NaCl in the same tissue. In each case, the percent increase in I_{sc} was calculated relative to a baseline containing only the supporting electrolyte. To compare the results for a given supporting electrolyte relative to the results for a

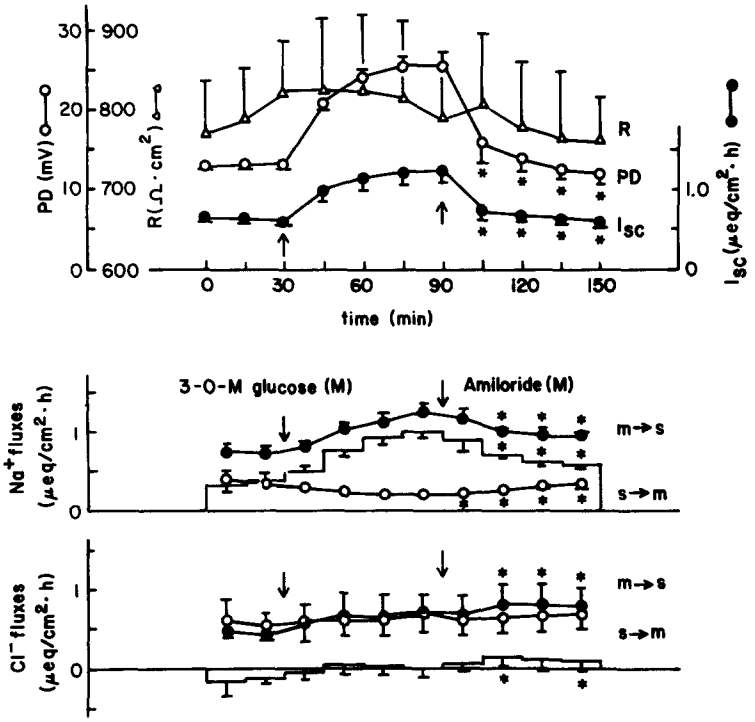

FIGURE 7. Effects of 3-*O*-methylglucose and amiloride on electrical parameters and Na and Cl fluxes in canine lingual epithelium ($n = 4$). Sugar (0.5 M) and amiloride (10^{-4} M) were added on the mucosal side. Symbols are the same as in Fig. 6.

supporting medium of NaCl, the parameter r was calculated as follows: r = (percent increase in I_{sc} for test ion)/(percent increase in I_{sc} for NaCl) × 100.

The results are summarized in Table III. In each group of experiments, both KCl and GACl were less effective in a given tissue relative to NaCl as a supporting medium. On the average, a KCl medium resulted in 28% of the current above baseline compared to the NaCl control. GACl was least effective, giving a value of r not significantly different from zero. The cellular mechanism responsible for the sugar-mediated increase in current therefore has a preference for Na. This result is independent of whether the value of I_{sc} for the supporting electrolyte with the test ion

was greater than (KCl case) or less than (GACl case) the I_{sc} for NaCl before the addition of glucose.

Response of the Chorda Tympani to NaCl, KCl, Sucrose, and Fructose

Amiloride inhibition of the NaCl response and recovery kinetics. The chorda tympani responded well to 0.5 M NaCl applied to the tongue. Fig. 8 *A* shows a typical control response to 0.5 M NaCl above a baseline established by rinsing the tongue in 30 mM NaCl. The first time marker under the record of integrated spike activity indicates the duration of stimulus application; the second time marker indicates the application of rinse solution. The first record to the right of the control was obtained after a rinse of the tongue for 1 min in 30 mM NaCl containing 800 μM amiloride; at the second time marker, the normal rinse of 30 mM NaCl was applied. The response was inhibited by 66%. In no case was the inhibition 100%. After a second rinse, recovery of the NaCl response was followed in time with a succession of stimulus applications and rinses. In Fig. 8 *B*, the peak response to NaCl relative to the peak control response is shown during the recovery phase. The time course of

TABLE III

Dependence of Glucose Response In Vitro on Identity of Cation

Group	Electrolyte	I_{sc}, no glucose	I_{sc}, with glucose	Percent increase in I_{sc} due to glucose	r
		$\mu A/cm^2$	$\mu A/cm^2$		
1	NaCl	28.6 ± 3.9	52.6 ± 18.2	74.0 ± 35.7	100
	KCl	35.7 ± 4.8	46.7 ± 13.2	26.1 ± 18.1	27.6 ± 8.3
2	NaCl	31.2 ± 4.1	61.9 ± 17.0	90.2 ± 32.8	100
	GACl	18.7 ± 3.0	21.1 ± 5.6	9.7 ± 12.1	6.1 ± 8.7

n = 3. Values are means ± SE. The salt concentration was always 0.15 M; the glucose concentration was always 0.5 M. For the definition of r, see text.

recovery was always exponential, which indicates that recovery from inhibition by amiloride is a first-order process. Recovery data were fitted to the equation

$$r(t) = A - Be^{-kt},$$

where $r(t)$ is the normalized response to 0.5 M NaCl at time t during the recovery phase, and A, B, and k are constants.

The ratio B/A is the maximum extent of inhibition and k is a first-order rate constant. These empirical constants have been interpreted in terms of a two-site transport model in which amiloride competes with Na for one of the sites but not the other (DeSimone and Ferrell, 1985). The presence of an amiloride-insensitive Na-transduction element would explain the residual responsiveness to NaCl in the presence of amiloride. Similarly, in terms of this model, recovery from inhibition could be interpreted as the decay of a complex between the amiloride-sensitive site and the drug, a process that would be expected to follow first-order kinetics. Table IV shows the average values of B/A and k for three canine preparations, along with the values of the same parameters previously found for the rat. The differences in the

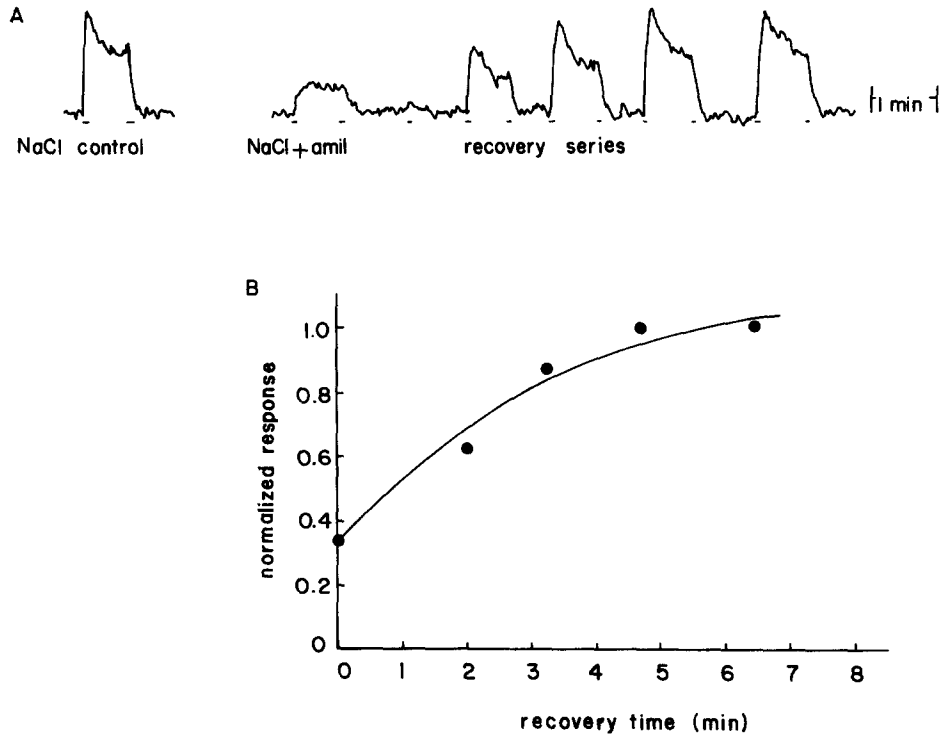

FIGURE 8. (*A*) Integrated response of the canine chorda tympani to 0.5 M NaCl above a baseline established by rinsing the tongue in 30 mM NaCl. In the first record to the right of the control, the tongue was rinsed for 1 min in 30 mM NaCl containing 800 μM amiloride; subsequently, recovery of the NaCl response was followed with a succession of NaCl stimuli. The line pairs under each trace indicate the application of stimulus and rinse solution (30 mM NaCl). (*B*) Magnitude of the peak response to NaCl relative to the peak control response during the recovery phase. The time course of the recovery was exponential; see text.

maximum extent of inhibition and the recovery rate constant between the dog and the rat were not significant.

Relative responses of NaCl and KCl. Fig. 9 shows the responses of the chorda tympani to NaCl and KCl in the same preparation and the effect of amiloride on the responses. At 0.5 M, KCl was always a more effective stimulus than NaCl. This

TABLE IV

Comparison of Amiloride Inhibition/Recovery Time Course Parameters of the Canine and Rat NaCl Chorda Tympani Response

Species	*B/A*	*k*
Dog	0.71 ± 0.04 (n = 3)	0.24 ± 0.03 (n = 3)
Rat*	0.78 ± 0.02 (n = 4)	0.19 ± 0.01 (n = 4)

Values are means ± SE.

*From DeSimone and Ferrell (1985).

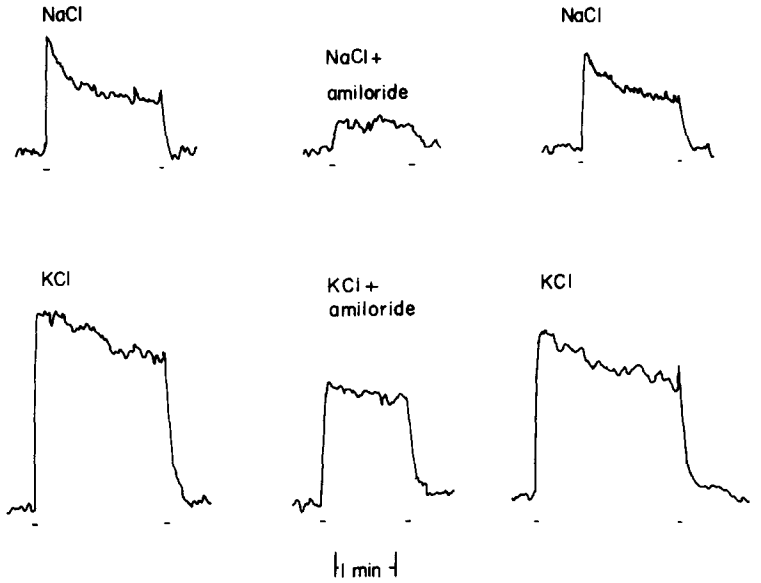

FIGURE 9. Integrated response of the chorda tympani to 0.5 M NaCl (upper trace) or 0.5 M KCl (lower trace) in the same preparation, the effect of amiloride (800 μM) on the response, and the response after recovery from amiloride.

agrees with the results of Beidler et al. (1955). To test the effect of amiloride, the tongue was rinsed for 1 min in 30 mM NaCl containing 800 μM amiloride. The test stimulus containing amiloride was then applied. As can be seen in Fig. 9, amiloride blocked part of the KCl response as well as that due to NaCl. This is in contrast to its relatively small effect on the KCl response in rats (Schiffman et al., 1983; Heck et al., 1984; Brand et al., 1985) and hamsters (Herness, 1987). The KCl response in the dog, however, was affected by amiloride to a significantly smaller extent than the NaCl response (cf. Table V). Moreover, the percent of inhibition by amiloride of the chorda tympani responses due to NaCl and KCl was comparable to the percent of inhibition by amiloride of I_{sc} evoked by NaCl and KCl in in vitro preparations of canine lingual epithelium, as shown in Table V.

TABLE V

Comparison of the Percent Inhibition by Amiloride of the NaCl and KCl I_{sc} Values In Vitro with the Canine Chorda Tympani Responses

Stimulus	Percent inhibition of I_{sc}	Percent inhibition of chorda tympani response
0.5 M NaCl	68.8 ± 0.8* (n = 4)	79.7 ± 4.1 (n = 7)
0.5 M KCl	39.8 ± 4.2* (n = 4)	39.0 ± 5.4 (n = 3)

Values are means ± SE. The integrated chorda tympani response is measured 30 s after stimulus onset.

*From DeSimone et al. (1984).

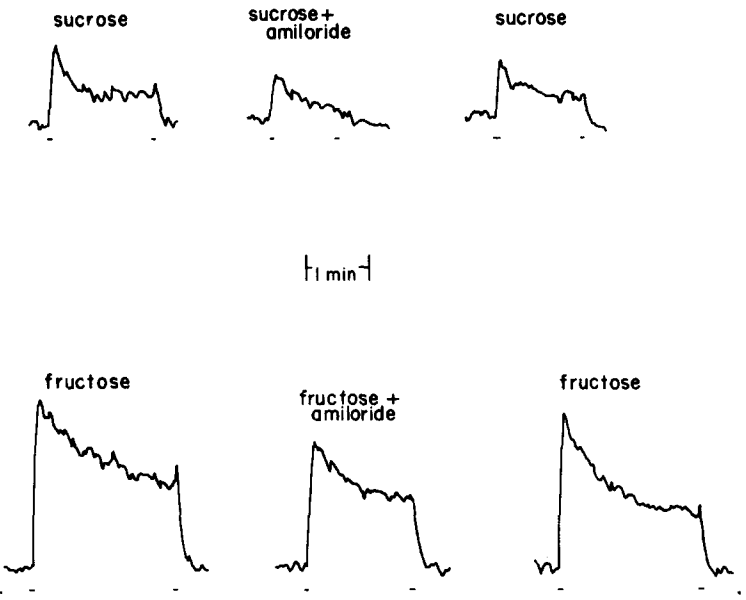

FIGURE 10. Integrated response of the chorda tympani to 1.0 M sucrose (upper trace) or fructose (lower trace). From left to right: control response of 1 M sugar dissolved in 30 mM NaCl; effect of amiloride on the test response; recovery. The tongue was rinsed with amiloride (800 μM in 30 mM NaCl) for 1 min before applying the sugar stimulus with amiloride. Adapting and rinse solutions were 30 mM NaCl. Test solutions contained 30 mM NaCl, 800 μM amiloride, and 1 M sugar.

Amiloride inhibition of the responses to sucrose and fructose. The canine chorda tympani responded well to sucrose and fructose, with fructose the better of the two stimuli at a given concentration. This agrees with published results (Andersen et al., 1962; Ferrell, 1984*a*). The tongue was adapted to 30 mM NaCl. A control stimulus of 1 M fructose or 1 M sucrose in 30 mM NaCl was then applied. The effect of amiloride was determined by rinsing the tongue in 30 mM NaCl containing 800 μM amiloride for 1 min and then applying the 1-M sugar stimulus dissolved in a solution of 30 mM NaCl and 800 μM amiloride. Fig. 10 shows that both sucrose and fructose responses were reduced significantly by amiloride. Recovery from inhibition was

TABLE VI

Inhibition by Amiloride of the Canine Chorda Tympani Response to Sucrose and Fructose

Stimulus	Percent inhibition of peak response	Percent inhibition of response at 30 s
1 M sucrose	31.8 ± 3.4 (n = 5)	39.6 ± 14.4 (n = 5)
1 M fructose	23.5 ± 1.3 (n = 3)	27.4 ± 2.0 (n = 3)

Values are means ± SE.

observed in all cases, but unlike recovery of the NaCl response, recovery of the sugar response was sometimes incomplete. Table VI summarizes the inhibition of the sucrose and fructose responses.

DISCUSSION

Comparison with Sugar Effects on Other Epithelia

The only characteristic common to both the canine lingual sugar-mediated current source and the intestinal carrier for galactose and glucose is a preference for Na ion. The most striking difference is that the lingual system is not at all dedicated to sugar transport. The labeled sugar 3-*O*-methylglucose was transported across the tissue, but not by a process coupled to Na influx. Blockage of Na transport, either at the apical membrane by amiloride or at the basolateral membrane by ouabain, had no effect on the transport of 3-*O*-methylglucose. The value of the calculated permeability coefficient of 3-*O*-methylglucose for the canine tongue is similar to those found for the passive permeability of leaky epithelia to sugars. Loeschke et al. (1971), for example, reported a value of $1–2 \times 10^{-6}$ cm/s for the permeability coefficient of 3-*O*-methylglucose across the bullfrog jejunum. Mistretta (1971) showed the rat lingual epithelium in vitro to be permeable to [^{14}C]glucose, [^{3}H]fructose, and [^{14}C]mannitol. Our value of the permeability coefficient for 3-*O*-methylglucose is about seven times larger than that obtained by Mistretta (1971) for glucose. This is reasonable agreement considering the differences in species, tissue preparation, and glucose derivatives. There is indirect evidence that glucose also permeates the cat lingual epithelium (Hallbäck et al., 1979). Based on our results, sugar transport across the canine lingual epithelium is a passive process. However, the sugar-activated Na influx is both ouabain and amiloride sensitive, and occurs under symmetrical short-circuit conditions. These results indicate a transcellular route mediated by the cell Na pumps.

There are additional distinguishing features of the canine sugar-mediated increase in current. Activation of the canine lingual system is not restricted to monosaccharides, and the current is not inhibited by phloridzin, as the intestinal glucose carrier is. While the canine lingual system and the intestinal system both increase I_{sc} as a function of the sugar concentration, their respective kinetics are quite distinct. Both show saturation, but the K_m for glucose is 150 mM in the lingual system (this article) and 4 mM in the intestinal system (Schultz and Zalusky, 1964). In addition, the Hill coefficients for the lingual and intestinal systems are 3.6 and 1, respectively. As Table I shows, 0.5 M glucose elicits comparable values of current in both 30 mM NaCl and K-H (143.7 mM Na). However, the efficiency of intestinal glucose absorption is significantly reduced at NaCl concentrations below 120 mM (Bosačkova and Crane, 1965). Finally, Na-linked glucose transport across the intestine is found in most species, whereas saccharide-stimulated Na transport across the dorsal lingual epithelium is not generally present.

The effects of hyperosmotic sucrose on the transport properties of a variety of tissues have been reported. In each case, the effect is unlike that observed in the canine tongue. When hyperosmotic sucrose in Ringer's was placed on the mucosal side of the *Necturus* gallbladder, the submucosal potential became electronegative

with respect to the mucosa and the transepithelial resistance increased significantly (Reuss and Finn, 1977). These effects were due to osmotic flow through the paracellular shunts. The increased resistance probably reflected collapse of the intercellular spaces, and the transepithelial potential was a streaming potential through a partially cation-selective shunt. Intracellular potential measurements showed that the apical cell membranes increased in conductance to Na, K, and Cl nonspecifically. In contrast, in the canine tongue, sugars produced the opposite change in transepithelial potential with little or no change in resistance. In addition, flux measurements and mucosal ion substitution showed that the current was primarily due to Na (cf. Tables I and II). However, as shown in Table III, in a high-K medium, K ions could also support a current, though a much smaller one. In a tighter transporting epithelium, the frog skin, 1 M sucrose in Ringer's resulted in a sharp drop in I_{sc} and a marked increase in resistance (Zeiske and Van Driessche, 1984). Sugars evidently decrease the Na permeability of the frog skin, whereas they have the opposite effect on the canine tongue. Large increases in the resistance of the small intestine were observed when hyperosmotic mannitol was placed in the mucosal solution (Madara, 1983). This was interpreted as an osmotic effect. The canine lingual frenulum gave small submucosa-negative potentials when hyperosmotic sucrose was placed in the mucosal bath (Siegal et al., 1976). These were regarded as streaming potentials. Again, this is opposite in direction to what was observed in the case of the dorsal lingual epithelium.

The fact that the sugar-mediated increase in current appears to be an unique property of the canine dorsal lingual epithelium is further indication that its function is neither absorption nor strictly osmotic regulation. Rather, the results of this article support a chemosensory function. A sugar-activated lingual Na transport system as found in the dog is not universally present among mammals. This would be consistent with the surprising heterogeneity of the sweet taste submodality among mammals.

Contrast with Lingual NaCl Response

In contrast to salt stimulation, resistance changed little over the entire range of sugar concentrations. Because the major changes in tissue resistance appear to arise in paracellular regions of the tissue (DeSimone et al., 1984), this suggests that sugars increase the ion flow through the transporting cells without affecting the paracellular shunt conductance. Thus, one important difference between currents stimulated by salts and those stimulated by sugars is the difference in ionic pathways across the tissue involved with each type of stimulus. Typically, a salt stimulus gives rise to a ouabain-sensitive and a ouabain-insensitive I_{sc} (DeSimone et al., 1984), which suggests both a transcellular and a paracellular ion flow. Sugar-stimulated ion flow in K-H is eliminated under ouabain, which suggests a transcellular route.

The presence of an amiloride-sensitive component in the sugar-mediated current of the canine tongue suggests that, at least for this species, the binding of sugars to cells on the tongue is followed by an increase in apical membrane Na transport. If these cells include those innervated by the taste nerves, the binding of sugars followed by increased ion influx may serve as a sugar taste transduction mechanism. The cells may be directly depolarized if Na enters through a channel, or they may be

depolarized by a secondary event if Na entry occurs via an electroneutral mechanism. The Na pathways associated with sugar appear to be distinct from those that mediate the response to NaCl. In the latter case, amiloride is more effective in blocking currents at hyperosmotic NaCl concentrations than at NaCl concentrations near the salt taste threshold (DeSimone et al., 1984; DeSimone and Ferrell, 1985; Simon and Garvin, 1985). However, amiloride blocks a major part of the sugar-mediated short-circuit current even at a NaCl concentration of 30 mM (cf. Fig. 4). This indicates that sugars are capable of expressing ion pathways that are usually not observed in their absence. On the other hand, it is a well-documented psychophysical result that NaCl at concentrations at or below the salt taste threshold is sweet to humans (Bartoshuk et al., 1978). It is possible that a small proportion of the normally sugar-activated pathways might be only partially coupled or entirely decoupled from the sugar-binding site. In the absence of sugar, at low concentrations of Na, an Na influx might occur in a small but significant population of cells normally responsive to sweet stimuli. The sweetness of low Na concentrations for humans follows if one type of sweet receptor is Na-linked.

The ability of high NaCl concentrations to inhibit the dog chorda tympani response to sucrose suggests that sugar binding itself may be directly affected by Na. If the canine tongue is adapted to 0.5 M NaCl, the addition of a mixture of 0.5 M NaCl plus 0.5 M sucrose produces no additional chorda tympani activity (Andersen et al., 1963). We carried out the analogous experiment in vitro. After a steady state current was reached in response to 0.5 M NaCl, a mixture of 0.5 M NaCl plus 0.5 M sucrose was added to the mucosal side. The sugar was ineffective in increasing the current under these conditions (cf. Table I). Thus, the failure of the chorda tympani to respond to sucrose after adaptation to a high salt concentration correlates well with the failure of sucrose to elicit an additional Na current at high salt concentrations. One possible mechanism is that very high NaCl concentrations change the conformation of the sugar-binding sites, decreasing the fraction of sugar molecules bound. The net effect would be a failure to activate fully the ion-transport mechanism associated with sweet reception. This experiment is consistent with the conclusion of Hyman and Frank (1980) that some mixture taste interactions may arise at the peripheral level, perhaps the receptor level.

Chorda Tympani Response

The response of the canine chorda tympani to 0.5 M NaCl was amiloride sensitive to about the same extent as that of the rat. As Table IV shows, there is no significant difference between the dog and the rat in either the maximum extent of inhibition of the NaCl response (B/A) or the time course of recovery. This is a good indication that both the dog and the rat have dual receptor systems for NaCl, as indicated by the presence of both amiloride-sensitive and amiloride-insensitive responses. Agreement in the extent of amiloride inhibition and the kinetics of recovery between rat and canine preparations suggests a common transduction mechanism for NaCl between these species. Agreement between the percent inhibition by amiloride of the I_{sc} and the canine chorda tympani response to 0.5 M NaCl (Table V) supports the idea that the taste receptor is a Na-transport pathway. This is consistent with

recent work on the α_2-adrenergic receptor where there is good evidence that cell Na entry is an integral part of the receptor function (Nunnari et al., 1987).

The canine chorda tympani response to KCl was also amiloride sensitive, as seen in Fig. 9 and Table V. However, the inhibition was significantly smaller than that for NaCl. Again, there was good agreement in the percent of inhibition by amiloride of the I_{sc} owing to KCl in vitro and the inhibition of the chorda tympani response. The results suggest that K ions may also be able to use, in part, the amiloride-sensitive Na pathway. In this respect, the canine KCl response differs significantly from that in rodents. However, it may account for the fact that, in humans, KCl has both a salty and bitter taste. The saltiness may arise from K being able to use the Na system to some extent.

Both fructose and sucrose gave robust chorda tympani responses, with fructose being the better stimulus. Again, unlike rodent responses to sugars, the canine responses were significantly inhibited by amiloride, as seen in Table VI. These results suggest that amiloride-sensitive, sugar-stimulated Na influx, demonstrated in vitro, is connected with saccharide taste reception and transduction. The precise nature of the Na pathway is unclear. The Na-transport system that is activated by α_2-adrenergic receptors is an electroneutral Na/H exchanger and is amiloride sensitive (Nunnari et al., 1987). Here, too, smaller effects with K were also noted. It is possible that the canine saccharide receptor may also be stimulating Na influx by this route.

The temporal characteristics of the chorda tympani response to both salts and sugars are notably different from those of the I_{sc} in vitro. The change in current develops over a longer time course in response to both salt and sugar stimuli. This appears to be a characteristic of the in vitro preparation per se. This conclusion is based on results from a rat preparation (Heck et al., 1985; DeSimone et al., 1987), where both the chorda tympani response and the I_{sc} can be measured simultaneously. Unfortunately, this preparation cannot be used to study sugar responses, because the rat sugar response is not accompanied by a change in current. However, the time course of the I_{sc} response to salts coincides with the chorda tympani response and is notably faster than the I_{sc} response to salt stimuli in vitro. The slower response in vitro probably reflects the loss of optimal blood-tissue exchange with consequent reduced metabolic efficiency.

Heterogeneity of Sweet Response

In the absence of a sugar stimulus, 80% of I_{sc} resulted from Na absorption (53%) and Cl secretion (27%). In the presence of sugar, the net Na flux accounted for ~90% (Table II) of I_{sc} and net Cl transport was negligible. The overall effect of the sugar was a 100% increase in Na influx and a 43% increase in Cl influx. The smallest effects were on the residual flux, which was reduced 6% after sugar stimulation. Only 40–50% of the Na flux was blocked by amiloride, which suggests that two separate Na pathways can be activated by sugars. The increase in Cl influx may indicate the presence of Cl-linked Na influx. This might explain the portion of Na influx that is amiloride insensitive. The presence of two sugar-sensitive Na pathways, with different dependences on sugar concentration, is probably the source of Hill coeffi-

cients >1 in the dose-response relations. The Hill equation only empirically represents the actual dynamics. The fitted curves in Fig. 1 emphasize, through the high values of the Hill coefficients, that a sugar concentration range exists wherein small changes in concentration result in large changes in current. The large values of n per se suggest that a single highly cooperative mechanism is unreasonable. The sugar-sensitive ion-transport mechanisms may be distributed in more than one cell type, as indicated by the results of Tonosaki and Funakoshi (1984*a*, *b*). They have shown that two sucrose-responsive taste cell types are present in the mouse. Each cell type depolarized in the presence of sucrose but with different conductance characteristics. The H cell depolarized in response to sucrose with an increase in resistance, which suggests a decrease in K conductance. The D cell depolarized with no change in resistance. The reversal potential for this type was always more positive than the cell resting potential, which suggests an inward Na current. The failure to detect a change in resistance in the D cell may indicate the presence of an electrogenic, but nonconducting, ion-exchange element.

The incomplete block of the canine chorda tympani response by amiloride indicates that in the dog, as in other species, there is more than one sweet taste mechanism. The fact that amiloride partially blocks the taste of sugars in humans (Schiffman et al., 1983) is consistent with a human Na-linked sweet receptor. The only other mammal for which a link between the presence of sugar and Na influx across the lingual epithelium has been established is the cat (Hallbäck et al., 1979), although a direct tie to gustatory transduction in that case is yet to be established. The amiloride-sensitive mechanism is evidently absent from some species, as pointed out in the Introduction. It seems reasonable to conclude that the rat, hamster, and gerbil do not use a Na-linked sugar taste mechanism to any significant extent. Hyman and Frank (1980) conducted a comprehensive single-fiber analysis of the chorda tympani response of the hamster to mixtures of sucrose and electrolytes. Their data indicate that transduction for sucrose taste in the hamster is not Na-activated.

Species variability has also been demonstrated for other sweet taste inhibitors. Gymnemic acid blocked the human sweet taste response (Diamant et al., 1965). However, it was not effective in the squirrel monkey (Snell, 1965) or in three species of Old World monkeys (Hellekant et al., 1974; Hellekant, 1977). It was also ineffective in the rat, rabbit, and pig (Hellekant, 1976). An effect approximately equal to its potency in humans was recently found in the chimpanzee (Hellekant et al., 1985). It is interesting to note that, like amiloride, gymnemic acid gives partial suppression of the canine chorda tympani sweet response (Hellekant, 1976). Thus, the dog and humans share sensitivity to both gymnemic acid and amiloride as inhibitors of the sweetness of sucrose. Interestingly, Ferrell (1984*b*) showed that dogs prefer cyclamate to saccharin, which is the same as the human preference for these sweeteners.

It is not clear whether Na ion pathways in the dorsal tongue are activated directly by the sugar or whether a second messenger may be involved. Salivary-level Na concentrations appear sufficient to drive an inward Na flux across the apical membranes of lingual epithelial cells. The normally high levels of salivary K ions may also allow for inward K flow, even though the sugar-stimulated ion pathway is less selec-

tive for K ions. The presence of apical ion pathways does not rule out the possibility of a transduction mechanism involving a second messenger or perhaps some sort of active process at the apical membrane. In addition, studies of the possible effects of Ca ions and pH have not been made. These of course are fertile areas for future investigation.

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REFERENCES

Aceves, J., and D. Erlij. 1971. Sodium transport across the isolated epithelium of the frog skin. *Journal of Physiology.* 212:195–210.

Andersen, H. T., M. Funakoshi, and Y. Zotterman. 1962. Electrophysiological investigation of the gustatory effect of various biological sugars. *Acta Physiologica Scandinavica.* 56:362-375.

Andersen, H. T., M. Funakoshi, and Y. Zotterman. 1963. Electrophysiological responses to sugars and their depression by salt. *In* Olfaction and Taste. Y. Zotterman, editor. Macmillan Publishing Co., New York, NY. 1:177–192.

Bartoshuk, L. M., C. Murphy, and C. T. Cleveland. 1978. Sweet taste of dilute NaCl: psychophysical evidence for a sweet stimulus. *Physiology & Behavior.* 21:609–613.

Beidler, L. M., I. Y. Fishman, and C. W. Hardiman. 1955. Species differences in taste responses. *American Journal of Physiology.* 181:235–239.

Biber, T. U. L., and T. L. Mullen. 1977. Effect of inhibitors on transepithelial efflux of Na and nonelectrolytes in frog skin. *American Journal of Physiology.* 232:C67–C75.

Blochaviak, P., and W. Jakinovich, Jr. 1985. Suppression of the rat's chorda tympani responses by taste inhibitors. *Association for Chemoreception Science Abstracts.* No. 102.

Bosačkova, J., and R. J. Crane. 1965. Studies on the mechanism of intestinal absorption of sugars. VIII. Cation inhibition of active sugar transport and ^{22}Na influx into hamster small intestine, in vitro. *Biochimica et Biophysica Acta.* 102:423–435.

Brand, J. G., J. H. Teeter, and W. L. Silver. 1985. Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Research.* 34:207–214.

DeSimone, J. A., and F. Ferrell. 1985. Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. *American Journal of Physiology.* 249:R52–R61.

DeSimone, J. A., G. L. Heck, and S. K. DeSimone. 1982. Evidence for an amiloride-sensitive ion channel in canine lingual epithelium. *Association for Chemoreception Sciences Abstracts.* No. 6.

DeSimone, J. A., G. L. Heck, S. Mierson, and S. K. DeSimone. 1984. The active ion transport properties of canine lingual epithelia in vitro. Implications for gustatory transduction. *Journal of General Physiology.* 83:633–656.

DeSimone, J. A., G. L. Heck, and K. Persaud. 1987. Transmural inward currents coincide with neural excitation and adaptation to NaCl in the rat. *Chemical Senses.* 12:652. (Abstr.)

Diamant, H., B. Oakley, L. Strom, C. Wells and Y. Zotterman. 1965. A comparison of neural and psychophysical responses to taste stimuli in man. *Acta Physiologica Scandinavica.* 64:67–74.

Ferrell, F. 1984*a*. Gustatory nerve response to sugars in neonatal puppies. *Neuroscience & Biobehavioral Reviews.* 8:185–190.

Ferrell, F. 1984*b*. Preference for sugars and nonnutritive sweeteners in young beagles. *Neuroscience & Biobehavioral Reviews.* 8:199–203.

Hallbäck, D.-A., M. Jodal, and O. Lundgren. 1979. Vascular anatomy and tissue osmolality in the filiform and fungiform papillae of the cat's tongue. *Acta Physiologica Scandinavica.* 105:469–480.

Heck, G. L., S. Mierson, and J. A. DeSimone. 1984. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science.* 223:403–405.

Heck, G. L., M. E. Welter, and J. A. DeSimone. 1985. Simultaneous recordings of the transepithelial lingual potential and integrated neural response of the rat. *Chemical Senses.* 10:427–428. (Abstr.)

Hellekant, G. 1976. On the gustatory effects of gymnemic acid and miraculin in dog, pig and rabbit. *Chemical Senses and Flavor.* 2:85–95.

Hellekant, G. 1977. Effects of miraculin and gymnemic acid in the rhesus monkey (*Macaca mulatta*). *In* Food Intake and Chemical Senses. Y. Katsuki, M. Sato, S. F. Takagi, and H. Oomura, editors. University of Tokyo Press, Tokyo. 201–210.

Hellekant, G., E. C. Hagstrom, Y. Kasahara, and Y. Zotterman. 1974. On the gustatory effects of miraculin and gymnemic acid in the monkey. *Chemical Senses and Flavor.* 1:137–145.

Hellekant, G., C. Hård Af Segerstad, T. Roberts, H. Van Der Wel, J. N. Brouwer, D. Glaser, R. Haynes, and J. W. Eichberg. 1985. Effects of gymnemic acid on the chorda tympani proper nerve responses to sweet, sour, salty and bitter taste stimuli in the chimpanzee. *Acta Physiologica Scandinavica.* 124:399–408.

Herness, M. S. 1987. Effect of amiloride on bulk flow and iontophoretic taste stimuli in the hamster. *Journal of Comparative Physiology.* 160:281–288.

Hyman, A. M., and M. E. Frank. 1980. Sensitivities of single nerve fibers in the hamster chorda tympani to mixtures of taste stimuli. *Journal of General Physiology.* 76:143–173.

Jakinovich, W., Jr. 1985. Stimulation of the gerbil's gustatory receptors by methyl glycopyranosides. *Chemical Senses.* 10:591–604.

Loeschke, K., D. Hare, and T. Z. Czáky. 1971. Passive sugar flux across frog jejunum in vitro. *Pflügers Archiv.* 328:1–20.

Madara, J. L. 1983. Increases in guinea pig small intestinal transepithelial resistance induced by osmotic loads are accompanied by rapid alterations in absorptive-cell tight-junction structure. *Journal of Cell Biology.* 97:125–136.

Mierson, S., S. K. DeSimone, and J. A. DeSimone. 1987*a*. Sugar-induced ion transport in canine lingual epithelium *in vitro*. *Federation Proceedings.* 46:1273. (Abstr.)

Mierson, S., S. K. DeSimone, G. L. Heck, and J. A. DeSimone. 1987*b*. Sugar-induced sodium transport in canine lingual epithelium and sweet taste transduction. *Abstracts of the 9th International Biophysics Congress.* 130.

Mierson, S., J. A. DeSimone, G. L. Heck, and S. K. DeSimone. 1982. Canine lingual epithelium has amiloride-sensitive ion channels. *The Physiologist.* 25:335. (Abstr.)

Mierson, S., G. L. Heck, S. K. DeSimone, T. U. L. Biber, and J. A. DeSimone. 1985. The identity of the current carriers in canine lingual epithelium in vitro. *Biochimica et Biophysica Acta.* 816:283–293.

Mistretta, C. M. 1971. Permeability of tongue epithelium and its relation to taste. *American Journal of Physiology.* 220:1162-1167.

Ninomiya, T., T. Mizukoshi, T. Higashi, and M. Funakoshi. 1984. Differential responsiveness of two groups of rat chorda tympani fibers to ionic chemical and electrical tongue stimulations. *Proceedings of the 18th Japanese Symposium on Taste and Smell.* 145–148.

Nunnari, J. M., M. G. Repaske, S. Brandon, E. J. Cragoe, Jr., and L. E. Limbird. 1987. Regulation of porcine brain α_2-adrenergic receptors by Na^+, H^+ and inhibitors of Na^+/H^+ exchange. *Journal of Biological Chemistry.* 262:12387–12392.

Reuss, L. and A. L. Finn. 1977. Effects of luminal hyperosmolality on electrical pathways of *Necturus* gallbladder. *American Journal of Physiology.* 232:C99–C108.

Sato, M. 1985. Sweet taste receptor mechanisms. *Japanese Journal of Physiology.* 35:875–885.

Schiffman, S. S., H. Cahn, and M. G. Lindley. 1981. Multiple receptor sites mediate sweetness: evidence from cross adaptation. *Pharmacology Biochemistry and Behavior.* 15:377–388.

Schiffman, S. S., E. Lockhead, and F. W. Maes. 1983. Amiloride reduces the taste intensity of Na^+ and Li^+ salts and sweeteners. *Proceedings of the National Academy of Sciences.* 80:6136–6140.

Schultz, S. G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. *Journal of General Physiology.* 47:1043–1059.

Siegal, I. A., K. T. Izutsu, and J. Burkhart. 1976. Transfer of alcohols and ureas across the oral mucosa measured using streaming potentials and radioisotopes. *Journal of Pharmaceutical Sciences.* 65:129–131.

Simon S. A., and J. L. Garvin. 1985. Salt and acid studies on canine lingual epithelium. *American Journal of Physiology.* 249:C398–C408.

Simon, S. A., R. Robb, and J. L. Garvin. 1986. Epithelial responses of rabbit tongues and their involvement in taste transduction. *American Journal of Physiology.* 251:R598–R608.

Snell, T. S. 1965. The responses of the squirrel monkey chorda tympani to a variety of taste stimuli. M. S. Thesis, Brown University. Cited in Hellekant et al., 1985.

Soeda, H., and F. Sakudo. 1985. Electrical responses to taste chemicals across the dorsal epithelium of bullfrog tongue. *Experientia.* 41:50–51.

Tonosaki, K., and M. Funakoshi. 1984*a*. Intracellular taste cell responses of mouse. *Comparative Biochemistry and Physiology.* 78A:651–656.

Tonosaki, K., and M. Funakoshi. 1984*b*. The mouse taste cell response to five sugar stimuli. *Comparative Biochemistry and Physiology.* 79A:625–630.

Vlahopoulos, V., and W. Jakinovich, Jr. 1986. Antagonism of the gerbil's sucrose taste response by *p*-nitrophenyl α-D-gluocopyranoside and chloramphenicol. *Journal of Neuroscience.* 6:2611–2615.

Zeiske, W., and Van Driessche. 1984. The sensitivity of apical Na^+ permeability in frog skin to hypertonic stress. *Pflügers Archiv.* 400:130–139.