

Effects of Voltage Perturbation of the Lingual Receptive Field on Chorda Tympani Responses to Na⁺ and K⁺ Salts in the Rat: Implications for Gustatory Transduction

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ABSTRACT Taste sensory responses from the chorda tympani nerve of the rat were recorded with the lingual receptive field under current or voltage clamp. Consistent with previous results (Ye, Q., G. L. Heck, and J. A. DeSimone. 1993. *Journal of Neurophysiology*. 70:167–178), responses to NaCl were highly sensitive to lingual voltage clamp condition. This can be attributed to changes in the electrochemical driving force for Na⁺ ions through apical membrane transducer channels in taste cells. In contrast, responses to KCl over the concentration range 50–500 mM were insensitive to the voltage clamp condition of the receptive field. These results indicate the absence of K⁺ conductances comparable to those for Na⁺ in the apical membranes of taste cells. This was supported by the strong anion dependence of K salt responses. At zero current clamp, the potassium gluconate (KGlu) threshold was >250 mM, and onset kinetics were slow (12 s to reach half-maximal response). Faster onset kinetics and larger responses to KGlu occurred at negative voltage clamp (–50 mV). This indicates that when K⁺ ion is transported as a current, and thereby uncoupled from gluconate mobility, its rate of delivery to the K⁺ taste transducer increases. Analysis of conductances shows that the paracellular pathway in the lingual epithelium is 28 times more permeable to KCl than to KGlu. Responses to KGlu under negative voltage clamp were not affected by agents that are K⁺ channel blockers in other systems. The results indicate that K salt taste transduction is under paracellular diffusion control, which limits chemoreception efficiency. We conclude that rat K salt taste occurs by means of a subtight junctional transducer for K⁺ ions with access limited by anion mobility. The data suggest that this transducer is not cation selective which also accounts for the voltage and amiloride insensitive part of the response to NaCl.

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INTRODUCTION

Rats perceive sodium and potassium salts as qualitatively distinct taste stimuli. Differences between responses to Na salts and K salts are discernable in the primary taste afferents (chorda tympani) suggesting that different peripheral mechanisms are involved (Erickson, 1963; Frank, Contreras, and Hettinger, 1983; Boudreau, Hoang, Oravec, and Do, 1983), and functional distinctions are maintained in the neuraxis at least to the level of the nucleus tractus solitarius (Schiffman, Lockhead, and Maes, 1983; Scott and Giza, 1990). The Na-K distinction is also observed in hamsters (Hettinger and Frank, 1990) and may be more generally in herbivores and omnivores (Boudreau, Sivakumar, Do, White, Oravec, and Hoang, 1985). Several lines of evidence indicate that the response to NaCl is composed of two peripheral inputs. Part of the Na-evoked chorda tympani (CT) response in the rat develops postnatally; this is the component that is blocked by amiloride (Hill and Bour, 1985). The pharmacology of amiloride and its analogues suggest that this Na taste transducing element is an apical membrane ion channel (Garty and Benos, 1988). Patch clamp recordings from taste cells confirm this (Avenet and Lindemann, 1988; 1991). A second part of the CT response to Na salts is amiloride insensitive as is the entire response to K salts. The amiloride-sensitive component conveys a substantial part of the neural input for Na taste perception because eliminating it by applying amiloride to rat tongues results in the animal failing to distinguish Na from K (Hill, Formaker, and White, 1990; Hettinger and Frank, 1990). The concept of two NaCl detectors is also clear from single unit studies which show that amiloride sensitivity, along with Na^+ ion specificity, resides in a certain taste nerve fiber type (N-fiber type, Hettinger and Frank, 1990), whereas the Cl^- ion dependency is associated primarily with a generalist cation-sensitive fiber type (H-fiber type; Rehnberg, Mackinnon, Hettinger, and Frank, 1993; Smith and Frank, 1993). CT responses under lingual voltage clamp also support the idea of two NaCl detectors (Heck, Persaud, and DeSimone, 1989; Ye, Heck, and DeSimone, 1991, 1993a). NaCl CT responses are composed of a voltage-dependent and a smaller voltage-independent component (Ye et al., 1993a). The voltage-dependent component is the amiloride-sensitive component (Ye et al., 1993a; Ye, Stewart, Heck, Hill, and DeSimone, 1993b); the voltage-insensitive component is the amiloride-insensitive component. The latter depends on the presence of Cl^- in Na salt taste (Formaker and Hill, 1988; Elliott and Simon, 1990).

In this study, we probe the CT response to K salts using the *in situ* lingual voltage clamp method and compare them to Na salt responses. Unlike Na salt responses, K salt responses appear to be mediated by a single, voltage-insensitive transduction mechanism that is markedly diffusion controlled. In addition, anion substitution for Cl^- is more inhibitory than in the case of NaCl. Substituting gluconate for Cl^- increases response thresholds to over 250 mM, lowers response magnitudes, substantially slows onset kinetics, and adds a large rinse-off response. The off response coincides with the collapse of a self-inhibitory, hyperpolarizing field potential. The absence of a voltage-sensitive component to KCl responses in rats suggests that their apical membrane K conductances do not normally have a major role in K salt taste transduction and that K-responding transduction sites lie beyond a paracellular diffusion and resistance barrier.

MATERIALS AND METHODS

Surgical Preparation

The surgical procedure has been described in detail (Heck et al., 1989; Ye et al., 1993a). Sprague-Dawley rats weighing 180–270 gm were preanesthetized with ether and then given an intraperitoneal (IP) injection of sodium pentobarbital (65 mg/kg). Additional injections (12 mg/kg) were administered as needed during the experiment. The rat was placed on an isothermal pad (39°C), the trachea was cannulated, and the head was immobilized with a nontraumatic head holder (Erickson, 1966). The left chorda tympani (CT) nerve was surgically exposed, cut caudally, and placed on a platinum electrode. Petroleum jelly was placed around the CT and a platinum reference electrode was positioned nearby.

Stimulation Chamber and Recording

The stimulation chamber allowed delivery of stimulus solutions to a 7-mm diam section of anterior tongue maintained under current or voltage clamp (Ye et al., 1993a). The tongue sections usually contained ~25 fungiform papillae (Miller, 1976). For stimulation and rinses, a 3-ml aliquot was injected into the chamber at 1 ml/s. The chamber volume was 0.03 ml and rinse and stimulus solutions remained in the chamber for 1.00 min or 1.25 min depending the protocol.

The whole CT neural activity was detected with a battery operated differential amplifier (Ye et al., 1993a). The amplified signal was recorded on a modified Toshiba DX-900 VCR, filtered using a band pass filter (cutoff frequencies 40 hz–3 khz), and fed to an oscilloscope. To obtain integrated CT responses the signal was full-wave rectified and integrated with a time constant of 1.0 s and displayed on one channel of a Linseis TYP 7045 strip chart recorder. An integrator time constant of 200 ms was used for measurement of the time to half-maximal rising phase of the response to 0.5 M KCl.

Current and Voltage Clamp

Transepithelial voltage or current clamp was maintained with a model VCC600 voltage clamp amplifier (Physiologic Instruments, Houston, TX). Ag/AgCl electrodes were used for current passing and voltages were measured with salt bridge electrodes. One of each electrode type was placed beneath the tongue, and one of each was positioned in the chamber or its input tubing (Ye et al., 1993a). The current passing electrode inside the inflow tubing acted as virtual ground. The salt bridge electrolyte gel was obtained from Parker Laboratories Inc. (Orange, NJ). In voltage clamp mode, the clamp drove sufficient current so that the differential voltage (V_{VC}) matched a programmed reference voltage. All voltages were referenced to the mucosal side, and the direction of positive current was taken as the direction of cation flow from mucosa to submucosa. The potential at zero current clamp (V_{CC}) yielded the equivalent of an open-circuit potential.

All voltages were corrected for liquid-junction potentials using the method of Laprade and Cardinal (1983). The voltage clamp values in our experiments were measured relative to V_{CC} ($\Delta V = V_{VC} - V_{CC}$) to correct for individual differences among animals (cf., Ye et al., 1993a). A periodic (15 s) biphasic pulse of either 1 μ A (current clamp) or 20 mV (voltage clamp) was generated for continuously monitoring the transepithelial resistance or conductance. Current and voltage responses were recorded with CT activity on the VCR.

Data Analysis

Integrated CT responses were digitized off-line as previously described (Ye et al., 1993a). The area under the integrated CT response curve for 30 s from the onset of chemically-evoked

neural activity was used as the numerical value of an integrated CT response. Areas were calculated using the computer software, AutoCad (Autodesk Inc., Sausalito, CA).

0.2 M NH_4Cl and 0.1 M NaCl stimuli were applied at the beginning and at the end of each experiment to monitor for possible changes in the responsiveness of the CT nerve. Only preparations that maintained a stable baseline throughout the experiment were used. Fig. 1 shows the initial and final stimulation sequences of NH_4Cl , DKH, NaCl for a typical preparation. The final NH_4Cl stimulation occurred 77 min after the initial. As described previously (Ye et al., 1993a), the percent change in the 0.2 M NH_4Cl response between beginning and end is typically of the order $4.7 \pm 6.3\%$ (mean \pm SEM, $n = 16$). In any given experiment the initial-final NH_4Cl response difference was used to correct all other responses by assuming the rate of change in neural activity during the course of the experiment to be linear. All responses for a given animal were normalized to that of 0.2 M NH_4Cl .

Solutions and Chemicals

Stimulus salts: NH_4Cl , NaCl , KCl and potassium gluconate (KGlu) and buffer salts were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals were reagent grade and were prepared in distilled water. The rinse solution that preceded and followed each test stimulus was 0.01 M KHCO_3 (pH 8.3) (Matsuo and Yamamoto, 1992). After the first of two rinses, NaCl

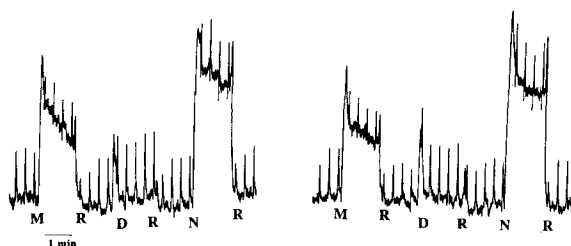


FIGURE 1. Integrated CT records of the initial (*left*) and final (*right*) stimulation sequences at zero current clamp for: 0.2 M NH_4Cl (*M*), DKH (*D*), and 0.1 M NaCl (*N*). *R* denotes the rinse (0.01 M KHCO_3). The two *M* responses were separated by 77 min. The data illustrate typical stability of both the baseline and the responses of stimuli.

depleted Krebs-Henseleit (DKH) buffer was delivered as an artificial saliva. DKH consisted of 6 mM KCl , 2 mM CaCl_2 , 1.2 mM MgSO_4 , 1.3 mM NaH_2PO_4 , 25 mM NaHCO_3 and 5.6 mM glucose, pH 7.5. Test stimuli had pH values between 6.0 and 7.0. A typical stimulation sequence was as follows: test 0; test 1 . . . test i . . . test n ; test 0. Here test 0 represents 0.2 M NH_4Cl (pH 5.6), test 1 is 0.1 M NaCl , test i ($i = 2, 3, \dots, n$) are particular stimuli under study. The test i sequence was run under current and voltage clamp. Between each test solution the following sequence of solutions was applied: rinse; DKH; rinse. The agents tested for their ability to block the CT response to K salts: amiloride, tetraethylammonium chloride (TEA), 4-aminopyridine (4AP), BaCl_2 , CsCl , and acetic acid, were applied in rinse and with the test salts.

RESULTS

NaCl Response under Voltage Perturbation

Fig. 2 (*top row*) shows the CT response to NaCl at zero current clamp and at ± 50 mV. In accordance with earlier results, positive voltage perturbation of the lingual

epithelium caused marked suppression and negative voltage perturbation enhanced the CT response (Ye et al., 1993a). Modulation of the CT response through voltage perturbation follows from the conduction properties of the Na^+ ion detectors, i.e., the epithelial cell Na^+ channels (amiloride-blockable) in taste cell apical membranes (Avenet and Lindemann, 1991; Ye et al., 1993a). For these channels, the driving force for the depolarizing stimulus Na^+ current through the transducer channels is the electrochemical concentration, c_e ,

$$c_e = ce^{-\delta\phi}. \quad (1)$$

Here, c is the Na salt concentration, δ is the fraction of an applied transepithelial perturbation voltage drop across the apical membranes of responding cells, and ϕ is the normalized perturbation potential, $F\Delta V/RT$, where F and RT have their usual thermodynamic meaning, and ΔV ($V_{VC} - V_{CC}$) is the applied perturbation voltage. Because c_e is the complete intensity variable, the CT response as a function of the

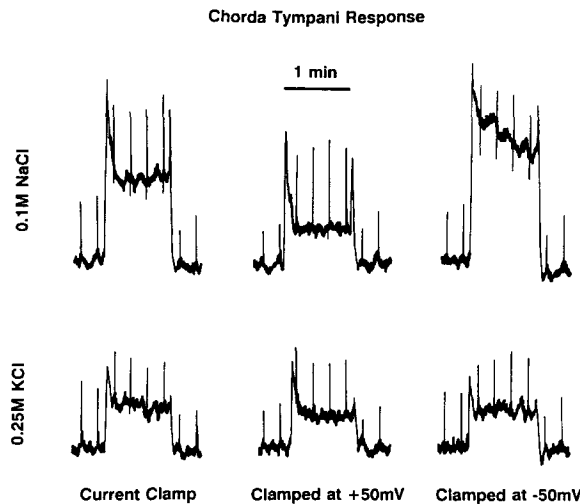


FIGURE 2. Integrated CT records of responses to 0.1 M NaCl (top row) and 0.25 M KCl (bottom row) at current clamp and ± 50 mV. Note the high sensitivity of NaCl and contrasting insensitivity of KCl responses to voltage. These data were obtained from the same animal.

applied NaCl concentration is parameterized by perturbation voltage as seen in Fig. 3. Each curve is described by a modified Beidler equation (Ye et al., 1993b) of the form:

$$R(c, \Delta V) = R_m c_e / (K_m + c_e) \quad (2)$$

where R_m is the maximum response, and K_m is the Na salt concentration giving half-maximal response under unperturbed conditions. With NaCl concentration as the independent variable, K_m appears operationally as a voltage-dependent parameter, viz.

$$K_m(\Delta V) = K_m e^{\delta\phi}. \quad (3)$$

For the data in Fig. 3, $K_m(-50) = 44$ mM, and $K_m(50) = 442$ mM.

KCl Response under Voltage Perturbation

As seen in Fig. 2 (*bottom row*) CT responses to KCl are relatively insensitive to voltage perturbations, in sharp contrast to NaCl responses. Fig. 4 shows that voltage insensitivity was observed over the full 50–500 mM concentration range. Within this range, the KCl CT response functions continue to increase whereas the CT response functions for NaCl have already saturated. This is especially obvious at current clamp and at $\Delta V = -50$ mV (cf. Fig. 3).

Anion Modulation of the K Response

In current clamp mode, up to 0.25 M KGlu produced little, if any, neural response. However, 0.25 M KGlu stimulation was always accompanied by a \sim threefold larger transepithelial potential compared to KCl stimulation at the same concentration (Fig. 5). Neural responses to KGlu were usually measurable at 0.5 M in current clamp

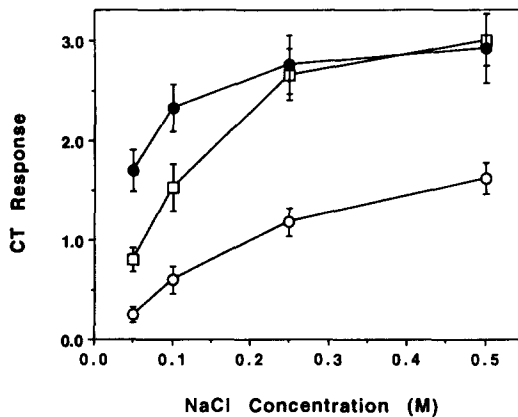


FIGURE 3. CT response as a function of NaCl concentration at zero current clamp (*squares*), -50 mV (*closed circles*), and $+50$ mV (*open circles*). When NaCl concentration is the independent variable, the voltage perturbation appears as a parameter, i.e., an exponential weighting factor on the K_m value (cf. Eq. 3). The unperturbed $K_m = 140$ mM. The increased electrical driving force across the apical channels at -50 mV, results in an enhanced CT response, seen as a reduced K_m (44 mM). The decreased driving force at $+50$ mV causes sup-

pression, seen as an increased K_m (442 mM). (Adapted from Ye et al., 1993*b*). Reprinted with permission of the American Physiological Society.

mode. However, the dynamic characteristics of the response differed from those observed with 0.5 M KCl (Fig. 6). After presentation of 0.5 M KGlu, the integrated CT response slowly increased with a sigmoidal time course usually requiring 10–12 s to reach half-maximum. The CT response showed little, if any, adaptation. At its onset the 0.5 M KGlu stimulus evoked a transepithelial field potential difference of ~ 40 mV (submucosa positive) that collapsed rapidly with stimulus rinse. This was always accompanied by a large, transient, chorda tympani rinse response (cf. Fig. 6). In contrast, stimulation with 0.5 M KCl typically evoked field potential differences of only ~ 15 mV. The CT response had a rapid rising phase (400 ms to half maximum), followed by the usual adaptation phase. Rinse of the stimulus did not evoke off responses in the neural record.

Fig. 7 shows the CT response as a function of KGlu concentration at current clamp and with $\Delta V = \pm 50$ mV. KCl responses were insensitive to voltage perturbation, and comparable results were expected for KGlu. However, while responses to KGlu

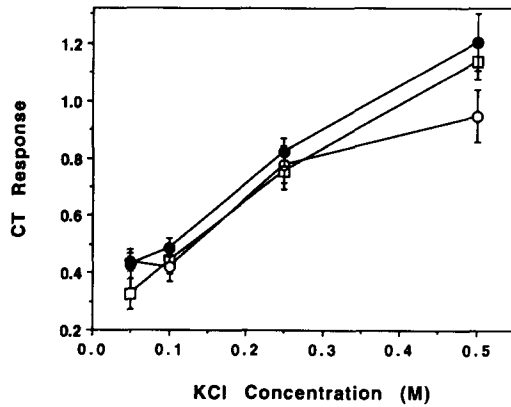


FIGURE 4. CT response as a function of KCl concentration at zero current clamp (squares), -50 mV (closed circles), and $+50$ mV (open circles). KCl responses are generally smaller than NaCl responses, do not saturate with concentration, and are not voltage sensitive.

remained lower than those to KCl at all concentrations and voltage conditions, the KGlu response showed a significant voltage sensitivity. At $\Delta V = -50$ mV, responses to KGlu were significantly greater than those at similar concentrations under current clamp or at $\Delta V = +50$ mV. On the other hand, responses at $\Delta V = +50$ mV exceeded those at current clamp. This is inconsistent with modulation of apical membrane channels with voltage-independent conductance. The time course of the response to KGlu was substantially different under voltage than under current clamp. In Fig. 7 (*inset*) the response to 0.5 M KGlu at -50 mV showed a rapid rising phase followed by adaptation. Therefore, responses to KGlu under voltage clamp, especially at electronegative values, do not display the sluggish response onset observed under current clamp (cf. Fig. 6). The negative voltage clamp response for KGlu follows the dynamic pattern characteristic of KCl responses.

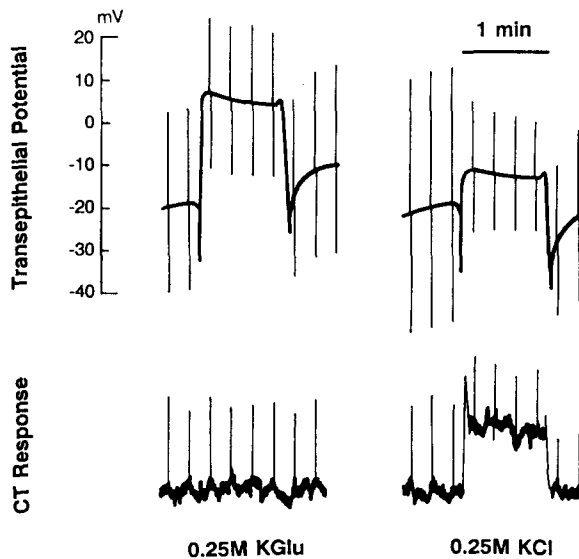


FIGURE 5. A comparison of the evoked transepithelial potential (*top row*) and CT response (*bottom row*) for 0.25 M KGlu and KCl at zero current clamp. The TP of KGlu is \sim threefold higher than that of KCl. This reflects the lower shunt conductance of Glu^- relative to Cl^- (cf. Fig. 10). The higher positive TP (hyperpolarizing on the receptor cells) and lower salt permeability of KGlu are both factors in the failure of KGlu to stimulate at concentrations where the Cl salt is effective. These data were obtained from the same animal.

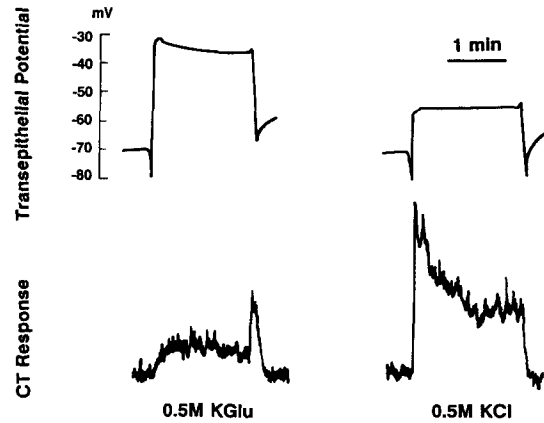


FIGURE 6. Time courses of responses at zero current clamp to 0.5 M KGlu and 0.5 M KCl illustrate strong effect of the anion on the CT response dynamics. The KGlu CT response onset is slow, reflecting strong diffusion control. KCl responses are much less affected because of the higher KCl paracellular shunt permeability (28 times that of KGlu). Note the large response after rinse of KGlu. This coincides with the collapse of the large TP and may represent release of some cells from inhibition by the TP. These data were obtained from the same animal.

Voltage Sensitivity

The relative insensitivity of the K salt CT response to voltage perturbation is readily apparent when expressed as the voltage-sensitivity index (VSI), defined as:

$$\text{VSI} = R(c, -50) - R(c, +50). \quad (4)$$

The VSI for NaCl was 5–10 times that of either K salt (Fig. 8). For KCl the VSI was not significantly different from zero at 50, 100, and 250 mM. The mean VSI for KGlu

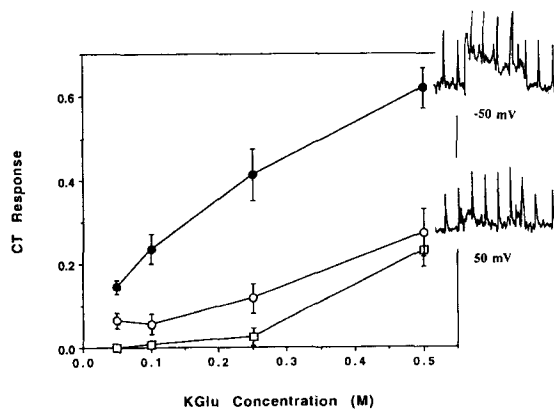


FIGURE 7. CT response as a function of KGlu concentration at zero current clamp (squares), -50 mV (closed circles), and $+50$ mV (open circles). At any concentration and voltage, responses are smaller than those to KCl. Note, however, that at -50 mV, responses are greater than at current clamp or at $+50$ mV. Also note the inversion between current clamp and $+50$ mV with the latter exceeding the former. The inset shows that at -50 mV, the slow onset seen in current clamp (cf. Fig. 6) is

eliminated. This is because K^+ ion transport by the current liberates the K^+ influx from the limits of diffusion control imposed by the low Glu^- shunt permeability. This electrophoretic effect can also account for the inversion. In this case ions are transported from the vascular space to the transducer sites.

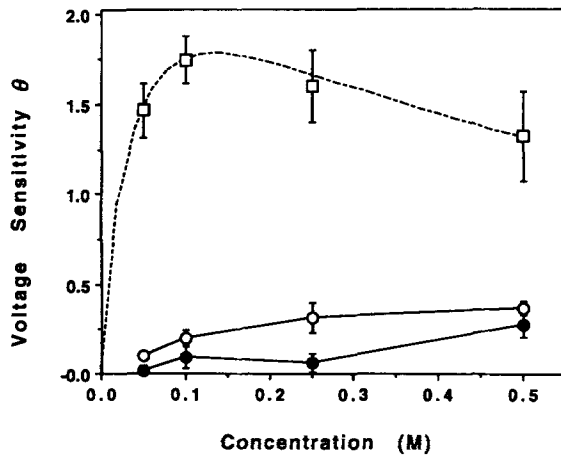


FIGURE 8. Voltage sensitivity for NaCl (squares), KCl (closed circles), and KGlu (open circles). The voltage-sensitivity index (VSI), $[R(c, -50) - R(c, +50)]$, is plotted for each salt. NaCl shows the greatest voltage sensitivity. In this case, the VSI is described accurately by the theoretical function Θ , derived on the assumption that the voltage sensitivity follows from the voltage dependence in the driving force for Na entry across apical membrane channels (see text).

In contrast, KCl and KGlu show virtually no voltage sensitivity. The VSI for KGlu slightly exceeds that for KCl due to the larger electrophoretic effect for KGlu. (The VSI for NaCl is adapted from Ye et al. 1993b). Reprinted with permission of the American Physiological Society.

significantly exceeded that for KCl at 250 mM. The experimental VSI for NaCl was described accurately by the theoretical voltage-sensitivity function, Θ (Ye et al., 1993b). This was obtained by substituting Eq. 2 into Eq. 4. The theoretical function, Θ , is based on the assumption that voltage sensitivity arises from Na^+ influx across apical membrane ion channels. The predicted function, Θ , is plotted through the experimental points for the NaCl VSI in Fig. 8. The function, Θ , has a maximum at $[\text{NaCl}] = K_m = 140 \text{ mM}$, and this is also expressed in the data. The VSI's for KCl and KGlu did not display a maximum over the same concentration range. This fact, as well as the relatively low magnitude of K salt VSIs, is further indication that K^+ ion taste transduction in the rat does not rely an apical ion channel system comparable to that for Na^+ .

Effect of Various Channel Blockers

Tetraethylammonium chloride (5 mM TEA) and 4-aminopyridine (5 mM 4-AP) were tested as possible inhibitors of the response to KGlu. The tongue was first rinsed for 1 min in a solution containing either TEA or 4AP. Then a stimulus solution of either

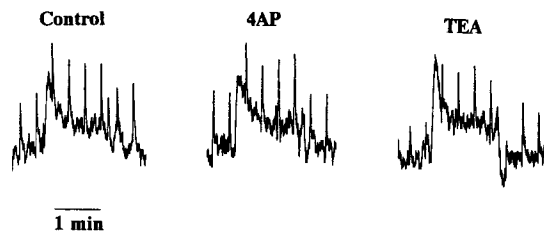


FIGURE 9. CT responses to 0.25 M KGlu at -50 mV voltage clamp. For control, the rinse solution was 0.01 M KHCO_3 . In the treated cases, 5 mM 4AP or TEA was applied in rinse for 1 min before applying KGlu also containing either 4AP or TEA. Neither agent inhibited the CT response.

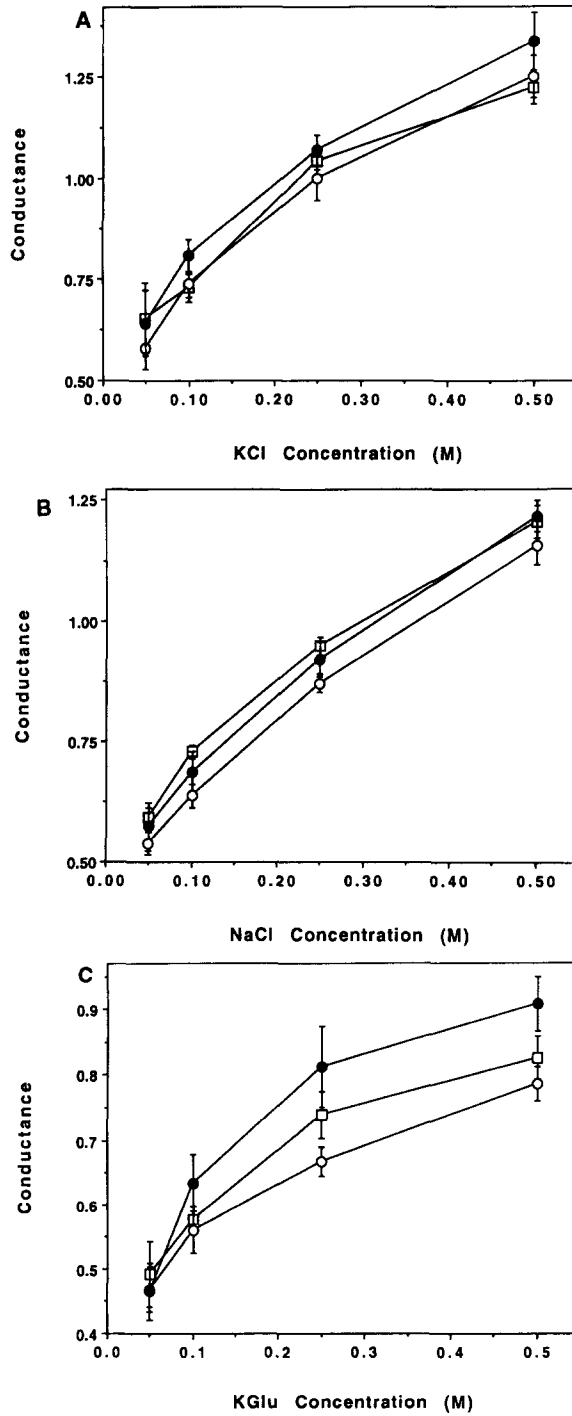


FIGURE 10. (A) Normalized Conductance (relative to that of 0.2 M NH_4Cl) of KCl as a function of KCl concentration for current clamp (squares), -50 mV voltage clamp (closed circles), and +50 mV voltage clamp (open circles). Note lack of voltage dependence and general similarity to the CT response vs KCl concentration function (Fig. 4). (B) Same for NaCl. Note similarity of conductances to KCl showing no paracellular shunt selectivity for Na over K. In contrast to KCl, NaCl CT responses do not correlate with conductance. (C) The conductances of KGlu are the smallest, but conductances at -50 mV are significantly greater than those at +50 mV. Similar characteristics are seen in the CT response as a function of KGlu concentration (cf. Fig. 7).

0.25 M or 0.5 M KGlu containing the same test agent was applied. KGlu was chosen because its relatively high shunt resistance should focus K ion interactions with the apical taste cell domain. Trials were done in current clamp or at -50 mV voltage clamp. Under negative voltage clamp the driving force for K entry through apical K^+ -channels should be increased, assuming K^+ -channels are present. Fig. 9 shows typical voltage clamp responses of 0.25 M KGlu for control and 4AP and TEA-treated cases. No significant differences in CT response were observed between control presentations and TEA or 4AP-treated cases. $BaCl_2$, (5 mM), CsCl (10 mM), and amiloride (0.1 mM) were tried in the same protocol with no significant effect on KGlu response. Acetic acid (0.1 M) in the rinse produced a CT response that was additive with that of KGlu. These results are in accord with the voltage clamp data indicating that apical membrane K^+ -channels are not major transducers for K salt taste in rats.

Transepithelial Conductance

The change in transepithelial conductance with increasing KCl concentration (relative to the conductance of 0.2 M NH_4Cl) is shown in Fig. 10A. There were no significant differences in conductances at current or voltage clamp for any KCl concentration. At each clamp voltage the conductance was a monotonically increasing function of KCl concentration, but with decreasing slope. A comparison with Fig. 4 shows that the KCl conductance and CT response have similar concentration dependence and are independent of clamp voltage. Fig. 10B shows that the NaCl transepithelial conductance behaved much like that of KCl with respect to concentration and voltage dependence. However, unlike the KCl case, NaCl conductance correlated poorly with the CT response in both voltage and concentration dependence (Fig. 3). A distinct voltage dependence was seen in the case of the KGlu conductance (Fig. 10C). The mean conductances at $\Delta V = -50$ mV significantly exceeded those at $\Delta V = +50$ mV at 0.25 M and 0.5 M. This rectification probably arises from the higher ionic conductance of K^+ relative to that of gluconate ions. As with KCl, there is a correlation between transepithelial conductance and CT response (cf. Fig. 7). In this case, however, there is a significantly higher conductance at $\Delta V = -50$ mV, correlated with the significantly higher CT response.

ANALYSIS

Diffusion Control

K salt permeability from CT response onset. The low voltage sensitivity of the KCl response (Fig. 8), poor CT responses and large field potential differences for KGlu relative to KCl (Fig. 5), the sluggish onset kinetics of the CT response to 0.5 M KGlu (Fig. 6), and good correlation of K-salt CT responses with transepithelial conductance suggest that K^+ ion sensing is diffusion-controlled and takes place largely below the tight junctions. The sluggish onset in the KGlu response (Fig. 6) can be attributed to the diffusional delay in the K salt concentration increase at the subjunctional transduction sites. Assuming a single thin barrier between the applied stimulus and the subjunctional sites, the intercellular K^+ ion concentration, $c_i(t)$ varies according to

$$dc_i/dt = (P_{KA}/\ell)(c - c_i) \quad (5)$$

where P_{KA} is the permeability coefficient of the salt KA and the length ℓ is the intracellular volume divided by the diffusion area. Implicit here is the assumption of constant extracellular volume. We have also assumed that all tight junctions are identical, and that the measured transepithelial conductance is a good approximation to that of the shunt. With these assumptions the early time course is then:

$$c_i(t) = c + (c_i(0) - c) \exp(-P_{KA}t/\ell) \quad (6)$$

where c is the applied concentration of KA, and $c_i(0)$ is the initial intercellular K concentration. The equilibration half time for KA is:

$$\tau_{KA} = 0.693/(P_{KA}/\ell). \quad (7)$$

Thus, the ratio of permeability coefficients for KCl and KGlu can be estimated from the measured half times in the rise of the CT responses for KCl and KGlu, viz:

$$P_{KCl}/P_{KGlu} = \tau_{KGlu}/\tau_{KCl} \quad (8)$$

From the data on 0.5 M salts, $\tau_{KCl} = 0.42$ s and $\tau_{KGlu} = 12$ s, which gives:

$$P_{KCl}/P_{KGlu} = 28 \quad (9)$$

K Salt Permeability from Conductance

Current across the paracellular shunts is well described by a modified Goldman equation (Fuchs, Larsen, and Lindemann, 1977),

$$I_i = z_i F P_i \phi (c_{io} - c_{ii} \exp[z_i \phi]) / (\exp[z_i \phi] - 1) \quad (10)$$

where I_i is the current contributed by the i th ion, z_i is the valence (+1 or -1), F is Faraday's constant, P_i is the permeability coefficient, $\phi = F\Delta V/RT$, where ΔV is the transepithelial voltage, c_{io} is the outside ion concentration, and c_{ii} is the ion concentration in the subjunctional region. In the modified form, P_i is concentration dependent (Fuchs et al., 1977)

$$P_i = P_{mi} K_{mi} / (K_{mi} + c_{io} + c_{ii}) \quad (11)$$

where P_{mi} is the maximum permeability and K_{mi} is a constant with units of concentration. The conductance contributed by the i th ion, G_i is:

$$G_i = dI_i/d\phi. \quad (12)$$

The sum of G_i over all ions produces the total conductance. To correct for variation in individual preparations, conductances for NaCl, KCl, and KGlu at each concentration were normalized to that at 0.2 M NH_4Cl before pooling. A least squares fit of the data (as plotted in Fig. 8) yielded the values of P_{mi} (dimensionless due to normalization) and K_{mi} in Table I.

Using these ionic permeabilities, estimates of the paracellular K salt permeabilities can be obtained from:

$$P_{KA} = 2P_K P_A / (P_K + P_A) \quad (13)$$

where A is either chloride or gluconate. Using Table I and Eq. 13, $P_{KCl} = 0.00495$ and $P_{KGlu} = 0.000174$. The ratio of permeabilities, $P_{KCl}/P_{KGlu} = 0.00495/0.000174 = 28$, confirms that found from CT onset kinetics (vide supra).

CT Response K_m Values: Correction for Diffusion

In diffusion control, the actual stimulus K concentration in the microenvironment of the transducer site is lower than that applied to the tongue. This results in an overestimate of the true K_m value in the CT response vs K salt concentration curve. This may be corrected by noting that the K salt stimulus is supplied at a rate given by Eq. 5 and that the CT response, R , will be proportional to that rate,

$$R_m c_i / (K_m + c_i) = \alpha P_{KA} (c - c_i). \quad (14)$$

Here, R_m is the maximum CT response to K^+ ion, K_m is the dissociation constant between K^+ ions and the subjunctional transducer sites (presumably ion channels), c_i is the local, intercellular K^+ ion concentration, c is the applied concentration of KA, P_{KA} is its permeability coefficient, and α is a proportionality constant.

TABLE I
Relative Values of Ion Permeability Parameters Derived from Lingual Conductance Measurements

Ion	$P_{mi} \times 10^2$	K_{mi} <i>mol/liter</i>
Na ⁺	1.19 ± 0.16	0.133
K ⁺	1.19 ± 0.20	0.110
Cl ⁻	0.312 ± 0.075	0.767
Gluconate	0.00876 ± 0.050	16.6

The values were obtained by fitting the conductance vs salt concentration data shown in Fig. 8 to Eqs. 10–12. The assumed values of the ion concentrations in the subjunctional region (c_i) are as follows: [Na⁺] = 50 mM; [K⁺] = 10 mM; [Cl⁻] = 50 mM; [gluconate] = 0.

The extent to which the system is diffusion controlled is given by the dimensionless diffusion-control modulus, Λ ,

$$\Lambda = R_m / \alpha P_{KA} K_m. \quad (15)$$

Λ is analogous to a Thiele modulus for diffusion-controlled chemical reactions (Aris, 1975). Systems with Λ values greater than unity are diffusion controlled. From Eq. 15, it is clear that relatively small values of P_{KA} will produce diffusion-controlled conditions. When:

$$\Lambda + 1 \gg c / K_m, \quad (16)$$

the local concentration, c_i , is approximately

$$c_i \approx c / (\Lambda + 1). \quad (17)$$

The CT response, corrected for diffusion, is then:

$$R = R_m c / [K_m (\Lambda + 1) + c]. \quad (18)$$

Diffusion control is therefore seen as an apparent increase in K_m , i.e.,

$$K_a = K_m (\Lambda + 1) \quad (19)$$

where K_a is the apparent value of K_m . Eq. 18 has been used to fit the current clamp data for KCl and results are displayed in Fig. 11 A. By least squares fit: $R_m = 1.76 \pm 0.25$, $K_a(\text{KCl}) = 294 \pm 84$ mM. Current clamp data for KGlu showing greater diffusion control can be fit to the straight line (Fig. 11 A):

$$R = [R_m/K_m(\Lambda + 1)]c. \quad (20)$$

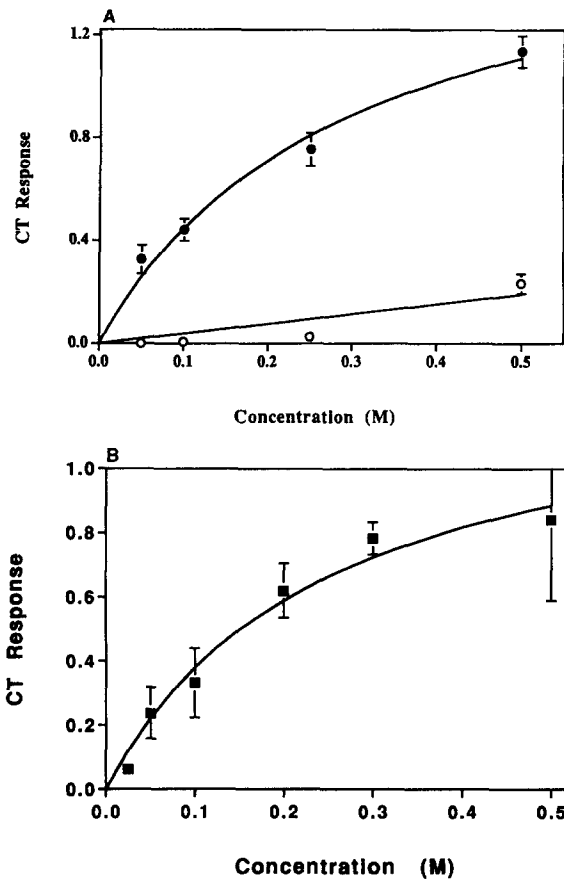


FIGURE 11. (A) CT response at current clamp as a function of concentrations of KCl (closed circles) and KGlu (open circles). Data points for KCl are from Fig. 4. The KCl curve was drawn from the hyperbolic function given in Eq. 18. Least squares fit gave: $R_m = 1.76$, and $K_a(\text{KCl}) = 294$ mM. The K_m corrected for diffusion control is 134 mM. Diffusion control produces a slight right-hand shift along the concentration axis. The data for KGlu are from Fig. 7. The KGlu data were fit to a straight line assuming pseudo-first-order conditions (Eq. 20). $K_a(\text{KGlu}) = 4.68$ M. Diffusion-control with Glu^- produces a very large right-hand shift along the concentration axis. (B) The amiloride-insensitive (AI) and voltage-insensitive (VI) component of the CT response to NaCl (squares). The hyperbolic curve was drawn according to Eq. 18. Least squares fit gave: $R_m = 1.34$ and $K_a(\text{NaCl}) = 250$ mM. The K_m value corrected for diffusion control is 129 mM; this does not differ from that for KCl.

Least squares fit gives a slope of $(3.76 \pm 0.50) \times 10^{-4}$. From the fits for KCl and KGlu we then generate two equations that can be solved simultaneously for the actual K_m and for α .

$$K_m + 1.76/\alpha P_{\text{KCl}} = 294 \quad (21)$$

$$K_m + 1.76/\alpha P_{\text{KGlu}} = 4690 \quad (22)$$

Using the values of P_{KCl} and P_{KGlu} obtained above, the results are: $K_m = 134$ mM, and $\alpha = 2.22$.

The NaCl CT response is similar to the KCl response in that it has an amiloride-insensitive (AI) and a voltage-insensitive (VI) component. Fig. 11 B shows that component. The curve is drawn according to Eq. 18 with least square parameters: $R_m = 1.34$ and $K_a = 250$ mM, which are seen to be close to those for KCl. The actual K_m for the voltage-insensitive part of the NaCl response is 129 mM, essentially identical to that for KCl.

Table II compares the parameters of the AI and VI component of the NaCl response with the responses for KCl and KGlu. We note that Λ_{KCl} and Λ_{NaCl} are about unity. Thus, diffusion for the chloride salts is not negligible, but is also not a major impediment to stimulation of subjunctional transducer sites. On the other hand $\Lambda_{\text{KGlu}} = 34$ shows that KGlu responses are severely diffusion limited. These effects are more clearly seen in a comparison of chemoreception efficiency, $\eta(c)$, the CT

TABLE II
The Effect of Diffusion Control on Chemoreception Efficiency for KCl and KGlu Responses, and the Voltage-independent Part of the NaCl Response

	K_a	K_m	Λ	$\eta(0.1)$	$\eta(0.5)$
	<i>M</i>	<i>M</i>			
NaCl	0.250	0.129	0.94	0.65	0.84
KCl	0.234	0.134	1.2	0.70	0.86
KGlu	4.68	0.134	34.0	0.05	0.12

Diffusion limits access of subjunctional transduction sites to both K- and Na-salts. Λ is the diffusion-control modulus (cf. Eq. 15), and $\eta(0.1)$ and $\eta(0.5)$ are respectively, the chemoreception efficiencies at salt concentrations of 0.1 and 0.5 M. Diffusion increases the apparent K_m (i.e., K_a) for each stimulus. For NaCl and KCl this results in approximate doubling of K_m . Values of Λ near unity indicate that diffusion control is moderate for the Cl-salts, but chemoreception efficiency is, nevertheless, reduced 30–35% at 0.1 M. For KGlu, diffusion limitations imposed by gluconate are sufficiently severe to render KGlu a marginal stimulus even at 0.5 M.

response in diffusion control relative to the ideal response'in the absence of diffusion barriers. This is:

$$\eta(c) = (K_m + c)/(K_a + c). \quad (23)$$

At 0.1 M, Table II shows that AI and VI responses for NaCl and KCl responses are 65 and 70% efficient, respectively, whereas KGlu responses are only 5% efficient. At 0.5 M, NaCl and KCl efficiencies have increased to 84 and 86%, respectively; the KGlu response is 12%.

Electrophoretic Effect of Voltage on KGlu Response

The sluggish diffusion-controlled onset kinetics of the CT response to KGlu under current clamp (Fig. 6) were not observed under negative voltage clamp (Fig. 7). Under a current, K^+ ion influx is no longer limited solely by the low mobility of the gluconate ion. The K^+ ion influx, J_K , is also augmented by the current, I (Katchalsky

and Curran, 1965), viz.,

$$J_K = P_{KA}(c - c_i) + t_K I / F. \quad (24)$$

Here, t_K is the transport number of K^+ across the paracellular diffusion barrier. With current, the effective stimulus K^+ concentration is:

$$c_i = (c + t_K I / FP_{KA}) / (\Lambda + 1). \quad (25)$$

At -50 mV, the current rapidly moves additional K^+ ions from the stimulus into the subjunctional regions, accounting for the faster kinetics of the response and increased magnitude. However, at $+50$ mV, the responses are still slightly larger than current clamp responses despite the reversed current. This may be due to current moving cations (Na^+ as well as K^+) from the vascular fluid space into the taste bud. This could also result in a cation concentration increase and, therefore, a slight taste response.

DISCUSSION

Contrasting Voltage Effects on NaCl and KCl Responses

Ion-transporting epithelia, including the dorsal lingual epithelium (Mierson, Heck, DeSimone, Biber, and DeSimone, 1985), move ions through parallel transcellular and paracellular pathways. The topology of these pathways is depicted schematically in Fig. 12 (Fidelman and Mierson, 1989; Reuss, 1992). A transcellular Na^+ ion pathway that includes an amiloride-blockable component accessible from the apical side has been demonstrated (Mierson et al., 1985). Based on patch clamp recordings from taste cells (Avenet and Lindemann, 1988) and fungiform papillae (Avenet and Lindemann, 1991), and the selective amiloride blockage of Na^+ salt stimulation of the chorda tympani in most herbivores and omnivores, there is general agreement that apical membrane Na^+ channels in taste cells are transducers for Na salt taste. Given the topology shown in Fig. 12, it is possible to perturb the potential across apical membrane transducer channels. If they are present, controlled changes in the stimulus ion electrochemical concentration (cf. Eq. 1) will be reflected as increases or decreases in CT nerve activity (Ye et al., 1993a,b). These expectations are fulfilled for $NaCl$ (Figs. 1 and 2). It should be noted that the CT response is a continuous function of the electrochemical concentration (Ye et al., 1993a,b; and Fig. 3). That is, changes in CT nerve spike frequency are graded, reflecting the changing intensity in the Na^+ electrochemical concentration. The apical membrane potential difference exerts significant control on the intensity of the Na^+ stimulus is significant as evidenced by the 10-fold shift with perturbation voltage in the apparent K_m in the CT response vs $NaCl$ concentration curve. Differences in apical membrane potential among cells could account for much of the variation in their response properties, and might be a variable by which receptor sensitivity is either up or down regulated. Overall, our results indicate that the taste nerves fire initially at a rate proportional to the depolarizing Na^+ current crossing taste cell apical membranes.

Taste cells also have voltage-gated Na^+ , K^+ , and Ca^{2+} channels (Roper, 1983; Kashiwayanagi, Miyake, and Kurihara, 1983; Kinnamon and Roper, 1988; Béhé, DeSimone, Avenet, and Lindemann, 1990). Their role in transduction is unknown,

but it seems reasonable that they contribute to taste quality coding, or at least to activating Ca^{2+} channels involved in transmitter release (Kinnamon and Cummings, 1992). For Na^+ taste, however, voltage-gated channels in taste receptor cells must also be subject to control that maintains the proportionality between incoming stimulus current and the frequency of evoked neural spikes, insuring receptor sensitivity to continuous changes in stimulus intensity (electrochemical concentration).

In the case of KCl, the voltage-dependent modulation of the CT response is absent, as evidenced by a nearly zero VSI (Fig. 8). This contrasts with the high-voltage sensitivity observed with NaCl (cf. Fig. 8). Two possible reasons for the absence of voltage-sensitivity are (a) there is a nonconducting K^+ -transducing element in the apical membrane of taste cells (a K^+ - H^+ antiporter, for example), or (b) the K^+ ion transducer is a K^+ ion channel, that is not in the apical membrane. The strong anion dependence in the K salt response, both in magnitude and time course, argues against an apical membrane K^+ transduction site. The fact that the K^+ -channel blockers used in this study failed to alter K^+ responses when applied to the tongue

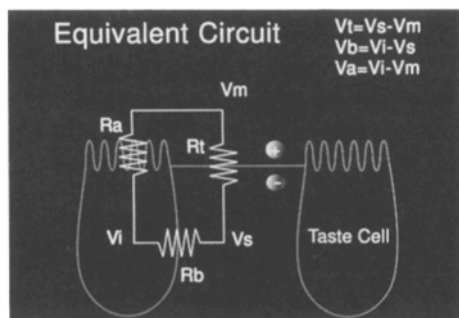


FIGURE 12. Schematic drawing of series-parallel network showing the relation of the transepithelial pathway to the paracellular pathway. Note that on topological grounds the receptor potential (V_b) can be influenced by the apical membrane and the transepithelial potential differences (V_a and V_t , respectively). The quantities in the figure are: V_m , mucosal potential, V_s , submucosal potential, V_i , intracellular potential, R_a , apical membrane resistance, R_b , basolateral membrane resistance, R_t , paracellular shunt resistance.

also argues against an apical site. While the slight voltage sensitivity observed in the K Glu responses may initially suggest an apical channel, the anion dependence of the voltage sensitivity makes this unlikely. The fact that K Glu CT responses at positive voltage clamp exceed those at current clamp is also inconsistent with an apical channel mechanism for K salt responses. As shown in the analysis, the small voltage sensitivity for K Glu and the inversion of the current clamp and positive voltage clamp responses can be explained by paracellular electrophoretic transport of K^+ and Na^+ ions. Kim and Mistretta (1993) report partial inhibition of the rat CT response to K salts with 4AP, and enhancement of the NaCl response. They reported small but significant effects at 0.25 M K salts, and larger effects at lower K salt concentrations. We did not look for 4AP effects below 0.25 M, however, at 0.25 M we saw no effect of 4AP (cf. Fig. 9) even when using K Glu under negative voltage clamp. These conditions should have been optimal for demonstrating the action of K^+ -channel blockers on putative apical K^+ channels. This negative result along with the other evidence presented here suggests that apical K^+ channels in rat taste cells are not a

major factor in K salt transduction. In this respect, the distribution of K⁺ channels in rat taste cells appears to differ markedly from that in *Necturus* taste cells (Kinnamon and Roper, 1988), where they are located at the cell apical pole. On the other hand, mammalian taste cells have many of the properties of ion transporting epithelia, where apical membrane K⁺ channels are not always represented. Recent work has demonstrated the absence of apical membrane K⁺ channels from esophageal and buccal epithelia (Khalbuss, Alkiek, Marousis, and Orlando, 1993).

Anion Effects

Substituting a larger ion for chloride resulted in a far greater inhibition of the CT responses to K salts than to Na salts (Elliott and Simon, 1990; Ye et al., 1991, 1993a; Rehnberg et al., 1993). KGlu CT responses in current clamp were not regularly observed at concentrations below 0.25 M. At 0.5 M, response onset was slow and large rinse responses were prominent. Rinse responses have been reported after stimulation of the rat CT with potassium benzoate (Miller, 1971; Sato and Beidler, 1979). Benzoate and gluconate probably have similar paracellular mobilities. If K⁺ ion transduction sites must be reached by diffusion across a paracellular barrier, benzoate responses, like those of gluconate, should also be highly diffusion controlled. Responses to KGlu are further self inhibited by the high transepithelial field potentials produced by KGlu diffusion through the cation-selective shunt pathways (cf. Fig. 6). Rinsing rapidly collapses the hyperpolarizing transepithelial field potential. This could result in a transient depolarization of the receptor cells, and a transient neural response. Fig. 12 illustrates how the topology of the peripheral taste sensors establishes a relation between the receptor potential and the transepithelial field potential, viz:

$$V_b = V_a - V_t. \quad (26)$$

Here, V_b is the intracellular potential referenced to the submucosal side, V_a is the apical and V_t is the transepithelial potential, both referenced to the mucosal side. This relation is supported by intracellular recordings (Sato and Beidler, 1979) showing that rat taste cells hyperpolarize at low concentrations of potassium benzoate and depolarize when it is rinsed. Large hyperpolarizing field potentials generated by potassium benzoate are probably responsible for the suppression of NaCl responses reported by Miller (1971) and Sato and Beidler (1979). In Miller's experiments (Miller, 1971), the suppressive effect of potassium benzoate on the NaCl response was exerted from a region surrounding the taste papilla that was stimulated with NaCl. Because V_t is established in the paracellular regions, ion flow from the surrounding intercellular space into that of the NaCl-stimulated papilla would rapidly hyperpolarize that papilla. Clearly, shunt mediated potentials can have important modulatory effects on taste responses and may be responsible for some mixture suppressions.

The large electropositive values of V_t for KGlu follow from the restricted diffusion of gluconate. The retarding effect of gluconate on the response can be removed by recording at negative voltage clamp (Fig. 7), further evidence that the slow onset kinetics of the KGlu response in current clamp are the result of severe diffusion control. Under these conditions K⁺ ions are transported by the inward current

independently of gluconate. The response at negative voltage clamp now shows rapid onset kinetics similar to those seen with the higher mobility Cl^- ion.

Diffusion Control and Chemoreception Efficiency

The K_m of the CT response vs concentration curve for NaCl (Fig. 3) appears as a voltage-dependent parameter. This reflects the ionic character of the stimulus, the apical membrane locus of the transducing channel, and the fact that concentration comprises only a part of the intensity. Diffusion limitations also influence the apparent K_m , in this case always causing an increase over the molecular K_m . The extent to which this occurs depends on the diffusion-control modulus (Λ in Eq. 15). For KCl, a Λ of 1.2 (see Table II) shows that CT responses are moderately diffusion-controlled. The result is an overestimate of the true K_m for K^+ activation of its transducer by $\sim 75\%$. Similarly, K_m for the voltage insensitive (VI) part of the NaCl response was overestimated by 94%. Diffusion limitation reduces the efficiency of the responses (η , cf. Eq. 23) to KCl and the VI part of the NaCl response. However, at 0.1 M, responses to the VI part of NaCl and to KCl are $\sim 70\%$ of what could be achieved if the diffusion barrier were absent. At 0.5 M, efficiency increases to $\sim 85\%$, i.e., the steeper the diffusion gradient, the greater the utilization of the transduction sites. For KGlu, however, Λ is 34 and the efficiency of the response at 0.1 M is only $\sim 5\%$. These severe diffusion limitations imposed by gluconate only result in an efficiency increase to 12% when the KGlu concentration is 0.5 M. The location of virtually all of the K^+ transduction sites beyond a paracellular diffusion barrier is clearly the reason that K salt anion effects are more pronounced than in Na^+ transduction. On the other hand, the presence of apical membrane Na^+ channels restricts significant diffusion effects only to the voltage-insensitive part of the Na salt response.

CONCLUSIONS

Na Salts

The rat peripheral taste system is comprised of two separate transduction pathways for Na^+ ion detection. One is a taste cell apical membrane Na^+ channel that can be characterized and probed by its sensitivity to lingual voltage perturbations. The second is accessible through the paracellular shunt pathway (cf. Fig. 12) and lies below a diffusion and resistance barrier (probably the tight junctional complexes). Due to the electrical connectivity imposed by epithelial tissue structure, the two pathways are not completely independent. One type of interaction, sensed in the cellular pathway, can arise from transepithelial potentials set up in the paracellular pathway by electrodiffusion across the shunt barrier (DeSimone, Heck, Mierson, and DeSimone, 1984; Harper, 1987; Elliott and Simon, 1990). This is the source of the first anion effect in Na salt taste (Ye et al., 1991, 1993a). Recordings from N-fibers by Rehnberg et al. (1993) nicely illustrate this anion effect in cross-adaptation experiments. N-fibers were adapted to NaCl for several seconds. The NaCl was then replaced with the same concentration of sodium acetate. Most fibers showed a sudden drop in spike frequency. In the reverse paradigm, most fibers adapted to sodium acetate showed increased spike activity when NaCl replaced sodium acetate. Sodium

acetate produces larger electropositive field potentials than NaCl (Ye et al., 1991). According to Eq. 26, replacing NaCl with sodium acetate will hyperpolarize the taste cells supplied by N-fibers, while replacing sodium acetate by NaCl will further depolarize taste cells, temporarily increasing their excitability. The fact that the apical membrane Na^+ channel is available to all Na salts insures that CT responses to them are kinetically similar with respect to the early onset of the response and adaptation.

The second peripheral sensor for Na salts is insensitive to voltage perturbations and amiloride. This system contributes significantly when Cl^- is the anion, but not when it is acetate or gluconate. This is the source of the second anion effect in Na salt taste (Ye et al., 1991, 1993a). The higher shunt conductance of the chloride salts relative to gluconate (Fig. 10) results in a paracellular Cl^- ion permeability that is ~ 36 times that of gluconate (cf. Table I). This gives Na^+ ions access to the submucosal transduction sites. These are probably ion channels, but that is not established unambiguously. The VI component of the NaCl CT response is, however, a saturating function of NaCl concentration (Fig. 11 B) with apparent K_m (K_a in Table II) of 0.25 M. Correction for diffusion control shows that it is actually ~ 0.13 M, about the same as K_m for the apical Na^+ channel (0.14 M). This Cl^- -dependent system may be on a separate cell type subserved by H fibers described by Rehnberg et al. (1993). Alternatively, H fiber responses could be the nonselective responses of free nerve endings. In any event NaCl, among the Na salts, appears to be uniquely suited to take advantage of both peripheral inputs to higher brain centers. This may be important in the encoding of NaCl as the most perceptually salty of the Na salts (Smith and Frank, 1993).

K Salts

In the rat, an apical membrane K^+ ion conductive pathway analogous to that for Na^+ does not appear to be a major contributor to K salt taste transduction. However, apical membrane K^+ ion conducting channels in epithelia, can sometimes be activated under the influence of hormones (Van Driessche, Aelvoet, and Erlj, 1987). The possibility that apical K^+ ion conducting channels in rat taste cells may be activated under some circumstances should not, therefore, be ruled out (Kim and Misretta, 1993). However, the paracellular shunt is a principal transduction pathway for K salts and is, therefore, diffusion limited. For this reason, CT responses to KCl are dynamically very different from those to KGlu and potassium benzoate (Sato and Beidler, 1979). The diffusion limited K_m (K_a in Table II) for KCl is 0.234 M, very similar to that for the VI part of the NaCl response. The K_m corrected for diffusion is 0.134 M, also essentially the same as that for NaCl. The kinetic equivalence of the diffusion-controlled responses to NaCl and KCl follows from the fact that the shunt pathway, while cation selective, does not distinguish between Na^+ and K^+ . This is seen in the similar conductance vs concentration curves (Fig. 8, A and B) for NaCl and KCl and in Table I where the derived paracellular permeability coefficients are equivalent for both ions. There is, therefore, no selectivity in the transport process. Moreover, the equivalence of the K_m values corrected for diffusion further suggests that the paracellular transduction process does not distinguish between Na^+ and K^+ . This is consistent with the fact that H fiber responses in rats and hamsters are about

equal for NaCl and KCl (Hettinger and Frank, 1990), and that the paracellular response is thought to be expressed in H fibers (Rehnberg et al., 1993). A salt such as KCl, while not a good stimulus, illustrates the existence of a diffusion-controlled process in transduction. It also illustrates how substances that generate high field potentials can inhibit or suppress neural activity in taste cells. Field potential effects may, therefore, be critical in some types of mixture interactions.

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