A MODEL FOR THE STIMULATION OF TASTE RECEPTOR CELLS BY SALT

JOHN A. DESIMONE *and* STEVEN PRICE

From the Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

ABSTRACT A taste cell mucosal surface is regarded as a planar region containing bound anionic sites and openings to ionic channels. It is assumed that the bulk aqueous properties of the exterior phase are not continuous with the surface but terminate at a plane near the surface. The region between the (Stern) plane and the membrane is regarded as having a lower dielectric constant than bulk water. This fact admits the possibility of ion pair formation between fixed sites and mobile cations. Mobile ion pairs entering the region may also bind to a fixed anionic site. Thus, it is assumed that mobile cations and ion pairs are potential determining species at the surface. Binding cations neutralizes surface charges, whereas binding mobile ion pairs does not. This competition accounts for the observed anion effect on stimulation of taste receptors by sodium salts. The potential profile is constructed by superimposing the phase boundary potentials with an ionic diffusion potential across the membrane. The model accounts for the anion effect on receptor potential, pH effects, the reversal of polarity when cells are treated with $FeCl_3$, and the so-called "water reponse," depolarization of the taste cell upon dilution of the stimulant solution below a critical lower limit. The proposed model does not require both bound cationic and anionic receptors, and further suggests that limited access to a Stern-like region continuous with membrane channels may generally serve to control transport of ions.

INTRODUCTION

Current thinking on the mechanism by which taste receptor cells are stimulated by salts originated with a study by Beidler (2) in 1954. He observed that the integrated neural response in the chorda tympani of the rat could be related to the concentration of salt flowing onto the tongue by a simple adsorption isotherm. He proposed that salts could adsorb to the membranes of taste receptor cells and that the response of the cell is proportional to the amount adsorbed. In order to account for the fact that not all salts are equally potent taste stimuli he elaborated a theory in which the receptor cell is supposed to be stimulated by the cation and inhibited by the anion (2–5). Thus, different sodium salts would not be equally stimulatory because of their differing inhibitory anions. Since changes in intracellular potentials elicited by salts were shown to be linearly related to changes in frequency of discharge of the chorda tympani, integrated activity of the nerve gives an indirect measure of receptor potentials (31).

Kamo et al. (15, 16) have recently demonstrated that the zeta potentials near the surface of phospholipid vary with the salt in the media in a manner paralleling the varia-

tion in receptor potentials of taste receptor cells in the same media. Like taste receptor cell potentials, the zeta potentials of phospholipids are reversed by application of $FeCl_3$. Furthermore, instead of simple salts depolarizing from negative potentials toward zero, cells or phospholipids treated with $FeCl_3$ are depolarized by simple salts from positive potentials toward zero.

The theory that taste receptor cells have sites through which cations can excite and anions can inhibit has difficulty in accounting for some of these observations. A phospholipid film is essentially a sheet of negative charge. It should, therefore, attract cations and repel anions. That is, the anion concentration in the immediate vicinity of the lipid should be considerably reduced relative to that in the bulk solution. This makes the anion unattractive as a candidate for interaction with the membrane. Treating the phospholipid with $FeCl_3$ should convert it into a sheet of positive charge. Depolarization by simple salts could then involve only anions, the cation of the salt being excluded by electrostatic repulsion. We present here an alternative hypothesis. It is proposed that the interaction of simple salts with the cells involves an initial change in the surface potential caused by the binding of ions of charge opposite to that of the membrane sheet. In the immediate region of the membrane, neutral ion pairs compete with ions for binding sites on the membrane. As will be discussed below, this model not only obviates the difficulty of trying to bring an inhibitory ion into contact with a region from which it is electrostatically excluded, it is also able to quantitatively account for the receptor potentials, the effects of $FeCl_3$ and of pH on salt taste responses, and the increased hyperpolarization with increased salt concentration (water response) which often accompany the receptor potentials. It is also in accord with the evidence against the existence of specialized receptor molecules for the recognition of salts by taste receptor cells.

THE MODEL

In constructing a model of salt taste reception, we recognize that the local ionic environment in the vicinity of the cell surface differs markedly from the bulk phases in contact with it. There are two important reasons for this. First, the presence of fixed charge mainly in the form of carboxyl and phosphate moieties; and second, the restricted orientational mobility of water which, along with the non-polar membrane lipid, results in a lower dielectric constant near the surface (24, 28). The role of surface charge and the double layer of mobile ions has long been recognized as a major factor in colloid stability (24, 28). More recently the role of surface charge has been considered in treatments of ion permeability changes in nerve conduction (6, 12, 21), the kinetics of surface enzymic reactions (8, 9, 13) and cell adhesion (22). Fig. 1 represents a taste cell outer surface showing fixed negative charge sites located within a region of low dielectric constant of thickness δ. The region beyond δ extending to infinity is assumed to be aqueous. We note the analogy with the so-called compact (Stern) and diffuse (Gouy) regions often considered in electrochemistry and colloid science (24, 28).

It is well known that a variety of interactions between ionic species and surfaces can

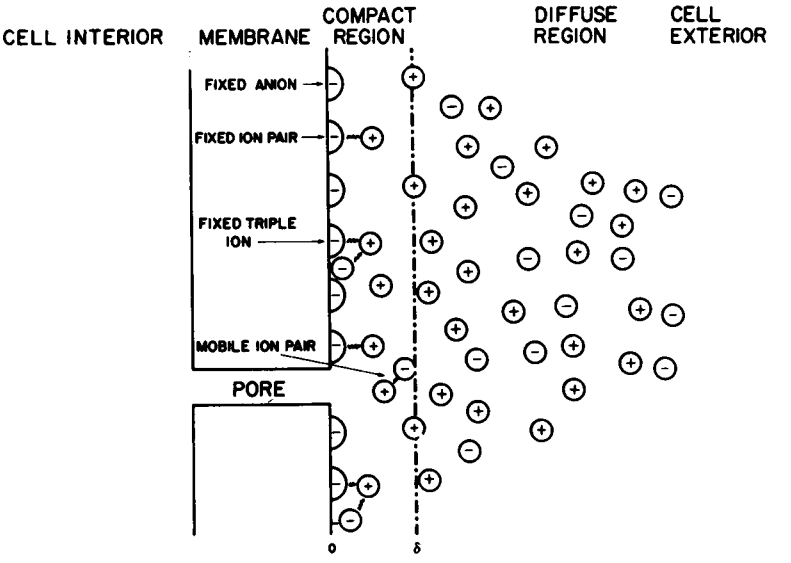

FIGURE 1 A schematic representation of a taste cell membrane. It is assumed that the outer surface contains fixed anion sites. A compact region of thickness δ is characterized by low dielectric content. The diffuse region is aqueous.

occur. The most important is, of course, electrostatic. However, the quasi-nonaqueous nature of the surface region opens the possibility to further interactions. These include covalent attachment to the surface, specific adsorption due to London-van der Waals attraction and ion pair formation between mobile and fixed charges in the local region of low dielectric constant. Ion pair formation between simple salts is well investigated in media of low dielectric constant, generally by potentiometric or conductance techniques (11, 25). The ion pair equilibria

$$Na^+ + X^- \rightleftharpoons Na^+X^-,$$

where X^- is chloride, formate, acetate, or propionate, have been studied and the reported association constants in a medium of dielectric constant 19 are 187, 236, 297, and 248, respectively (25). Triple ion associations of the form $Y^-M^+X^-$ are evidently also possible and are consistent with the conductance data (27). It is clear that while co-ions are nearly electrostatically excluded from the surface region, neutral ion pairs (dipolar species) can readily approach the surface and perhaps further interact with it by forming a triple-ion complex with surface fixed charges. The chances of this occurring are improved if specific adsorption between similar hydrophobic moieties of the mobile ion pair and the surface can occur. Rice and Nagasawa (26) have reviewed the evidence for specific interactions (site binding) between simple ions and polyelectrolytes as opposed to nonspecific electrostatic association (volume binding). They concluded that both types of association are possible and cite evidence for local ion pair association. More specifically, Strauss and Ross (29, 30) using both electro-

phoretic and membrane equilibration methods provide convincing evidence for the binding of alkali cations to synthetic polyphosphates. The law of mass action holds, provided electrostatic weighting of the cation concentration is considered. Mikulecky and Tobias (19), however, note the formal similarities between treatments which include ion pair association and those which include only electrostatic interaction. Our treatment of taste cells will include the hypothesis of ion pair formation in what should be a favorable local region near the surface. While the precise local conditions have not been specified, the concept of ion binding is certainly consistent with current thinking on the nature of the taste receptor-ion interaction (2–5). There is evidence which suggests that charged phospholipids such as phosphatidyl serine may be particularly significant as receptor groups. The summated response in the rat to various concentrations of HCl and NaOH, gives as a function of pH, an apparent pK of about 1.8 suggesting a phosphate H^+ receptor (4). The role of charged phospholipids as mediators of ion permeability is well-established in the work of Mikulecky and Tobias on Millipore phospholipid membranes (19, 32).

Designating the fixed ionic sites as R^-, we have in addition to equilibrium I, cation binding to the fixed sites,

$$R^- + Na^+ \rightleftharpoons R^-Na^+, \tag{IIa}$$

$$R^- + H^+ \rightleftharpoons RH. \tag{IIb}$$

Triple ion associations formed by mobile ion pairs and fixed sites are

$$R^- + Na^+X^- \rightleftharpoons R^-Na^+X^-. \tag{III}$$

It is important to note that processes II*a* and II*b* result in neutralization of surface charge whereas process III does not. The binding of a cation can therefore lead to depolarization of the surface, whereas the binding of a neutral ion pair does not. The fraction of surface bearing charge is given by

$$\beta = ([R^-] + [R^-Na^+X^-])/([R^-] + [R^-Na^+X^-] + [R^-Na^+] + [RH]). \tag{1}$$

From the equilibria I–III we have

$$k_1 = [Na^+X^-]/\gamma^2[Na^+]_\delta[X^-]_\delta \tag{2}$$

$$k_{2a} = [R^-Na^+]/[R^-][Na^+]_\delta \tag{3}$$

$$k_{2b} = [RH]/[R^-][H^+]_\delta \tag{4}$$

$$k_3 = [R^-Na^+X^-]/[R^-][Na^+X^-]. \tag{5}$$

Here the constants k_i are in liters per mole and γ is the mean activity coefficient of NaX. Assuming the ions in the aqueous region to be distributed according to the Boltzmann Law we have

$$[M^+]_\delta = [M^+]_\infty e^{-\phi\delta} \quad \text{for cations} \tag{6A}$$

$$[A^-]_\delta = [A^-]_\infty e^{\phi\delta} \quad \text{for anions} \tag{6B}$$

where $\phi = e\psi/kT$ and e is the electronic charge, ψ is the potential, k is Boltzmann's constant, and T the absolute temperature. Using Eqs. 2–6, the fraction of bound charge is

$$\beta = \frac{1 + k_1 k_3 \gamma^2 [Na^+]_\infty^2}{1 + k_1 k_3 \gamma^2 [Na^+]_\infty^2 + (k_{2a}[Na^+]_\infty + k_{2b}[H^+]_\infty)e^{-\phi_\delta}}, \tag{7}$$

where electroneutrality in the bulk phase requires $[Na^+]_\infty = [X^-]_\infty$. The electrostatic potential in the aqueous region is governed by Poisson's equation,

$$d^2\psi_2/dx^2 = -4\pi\rho/\epsilon_2, \tag{8}$$

where ρ is the mobile charge density and ϵ_2 is the dielectric constant of the aqueous phase. The boundary conditions at infinity are

$$\psi_2(\infty) = \left.\frac{d\psi_2}{dx}\right]_\infty = 0. \tag{9}$$

The boundary conditions at $x = \delta$ are

$$\epsilon_1 \left.\frac{d\psi_1}{dx}\right]_\delta = \epsilon_2 \left.\frac{d\psi_2}{dx}\right]_\delta, \tag{10}$$

$$\psi_1(\delta) = \psi_2(\delta) = \psi(\delta), \tag{11}$$

where ψ_1 refers to the potential in the compact region. We shall assume that the volume charge density in the compact region is negligible. Therefore, the potential is obtained from a solution of Laplace's equation (24, 28). Thus, the region is essentially modeled as a parallel plate capacitor with charge density σ and plate separation δ,

$$\psi_1(x) = \psi(\delta) - (4\pi\sigma/\epsilon_1)(x - \delta). \tag{12}$$

The surface charge density is given by

$$\sigma = -e\beta R_t, \tag{13}$$

where R_t is the total surface site density.

Combining Eqs. 10, 12, and 13 we can write

$$\left.\frac{d\phi_2}{dx}\right]_\delta = \frac{4\pi e^2 \beta R_t}{\epsilon_2 kT}. \tag{14}$$

The potential in the aqueous region beyond the compact zone satisfies the Poisson-Boltzmann equation. In specifying the charge density in this region we, of course, consider ions of the test salt, Na^+ and X^-. Dissolved carbon dioxide will also contribute H^+ and HCO_3^- ions. Accordingly the volume charge density is

$$\rho = e([Na^+] + [H^+] - [X^-] - [HCO_3^-]).$$

Combining this relation with the Boltzmann relations (Eq. 6) and the boundary condi-

tions (Eq. 9), the solution to Eq. 8 is

$$d\phi_2/dx = -2\kappa \sinh(\phi_2/2), \tag{15}$$

where

$$\kappa^2 = 8\pi e^2([Na^+]_\infty + [H^+]_\infty)/\epsilon_2 kT,$$

and $1/\kappa$ is the Debye length. Combining Eq. 14 and 15 we write

$$\sinh \frac{|\phi_\delta|}{2} = R_t \left[\frac{\pi e^2}{2\epsilon_2 kT([Na^+]_\infty + [H^+]_\infty)}\right]^{1/2} \beta([Na^+]_\infty, [H^+]_\infty, |\phi_\delta|). \tag{16}$$

Eq. 16 enables us to determine $|\phi_\delta|$ as a function of the bulk solution concentrations. Boundary conditions in the form of Eq. 16, which determine the surface potential as a function of the concentration of the potential determining ions, have been extensively discussed (21, 22, 28).

By numerically solving Eq. 16 for ϕ_δ in terms of the bulk salt concentration, it is possible to correlate the depolarization of the surface with increases in salt concentration. Indeed, Kamo et al. (15. 16) find that the ζ-potential of liposomes made from tongue lipid extracts changes with salt concentration similarly to the observed receptor potentials across taste cell membranes. The ζ-potential is similar to our ϕ_δ. Of course changes in the surface potential can be correlated with measured membrane potentials only because each may depolarize with increasing salt concentration. They are, in fact, very different quantities although recent studies have shown that ion permeability across membranes is greatly influenced by surface phenomena (18, 33). The receptor potential is not a direct measure of the surface potential, but the latter may contribute to it by determining in part the inner and outer phase boundary potential drops. This point of view is developed in the next section.

REPRESENTATION OF THE RECEPTOR POTENTIAL

We now derive an expression for the membrane potential which allows us to quantitatively account for several key observations concerning the nature of salt taste reception; viz., the anion effect on the response of rat taste receptors to four different Na^+ salts, the pH independence between values of 3-11 (23), reversal of polarization when the cells are treated with $FeCl_3$ and the water response wherein a salt concentration regime exists for which dilution produces depolarization (4).

We assume that in addition to the fixed surface sites, the membrane contains a lower density of pores. Moreover, the observable membrane potential is taken to be a superposition of the inner and outer surface potential drops and a transmembrane diffusion potential. This view is, of course, analogous to that encountered with ion exchange membranes (14). For simplicity we assume the membrane to be perfectly cation selective and that ion fluxes are due to inward Na^+ and outward K^+ movements. Within the membrane the transport equations are

$$J_i = -D_i[(dc_i/dx) + c_i(d\phi/dx)], \qquad i = Na^+, K^+ \tag{17}$$

where we have ignored cross-coupling effects including those relating to osmotic water flow. In the zero current condition, $J_{Na^+} = -J_{K^+}$. Integration of Eq. 17 across the membrane gives the diffusion potential (7, 14). For simplicity we shall ignore contributions from interior cations other than K^+, in which case we can write.

$$\phi_i - \phi_0 = \ln[D_{Na^+}[Na^+]_o/D_{K^+}[K^+]_i)]. \tag{18}$$

Here ϕ_i and ϕ_o are reduced potentials, respectively, at the inner and outer surfaces. D_{Na^+} and D_{K^+} are membrane diffusion coefficients. $[Na^+]_o$ is the concentration at the outer channel entrances (identical to $[Na^+]_\delta$ discussed previously), and $[K^+]_i$ is the concentration at the inner channel entrances. The total membrane potential includes the phase boundary contributions which must be added to Eq. 18. The outer compact zone contributes

$$\phi_o - \phi_\delta = 4\pi e\sigma\delta/\epsilon_1 kT. \tag{19}$$

This may be regarded as the potential drop across the exterior molecular capacitor. Eq. 19 may be recast with the aid of Eqs. 10 and 15, viz.

$$\phi_o - \phi_\delta = (-2\epsilon_2/\epsilon_1)\kappa\delta\sinh(|\phi_\delta|/2). \tag{20}$$

In the absence of significant triple ion complexing at the surface, the potential difference across the compact zone may tend to zero in two ways, each corresponding to neutralization of surface charge. In the first case, increasing the salt concentration causes ϕ_δ to asymptotically approach zero, i.e. maximum electrostatic shielding. In the second case, decreasing the bulk salt concentration causes $|\phi_\delta|$ to increase; this causes the surface cation concentration to remain high. The high surface cation concentration including surface H^+ ion is then available to neutralize surface charge. In effect the surface charge density can have a maximum at some critical salt concentration. At this point the potential drop across the compact zone reaches a maximum negative value. The effects of high surface potential on counterion concentration have been discussed by Morawetz (20). In his analysis of the polyelectrolyte rod model of Fuoss, Katchalsky, and Lifson, he observes that over an eightfold dilution of polyelectrolyte, the fraction of counterion found less than 15 Å from the rod surface remains essentially constant. The reason for this is the compensating effect of increasing surface potential in the face of decreasing bulk counterion levels (in other words, an increasing Boltzmann factor, $e^{-\phi_\delta}$, with dilution).

The last remaining contribution is the Gouy potential, i.e. the potential of the diffuse double layer. This is

$$\phi_\delta = \ln([Na^+]_\infty/[Na^+]_o). \tag{21}$$

This part of the membrane potential is closely analogous to the Donnan potential discussed in the theory of ion exchange membranes (18). The two potentials arise as a result of a constraint on the mobility of a charged species. Whether the charge is distributed in a separate phase (the Donnan case) or constrained to a surface (the Gouy case) does not affect, at least qualitatively, the distribution of mobile ions and the sign

of the potential. A good example of this is the treatment of surface charge effects on surfactant film pressures. Both the Donnan and Gouy approaches give essentially similar predictions (1). Another example is the modeling of potentials across membranes separating KCl solutions as discussed by Eriksson (10). Again both Donnan and Gouy potentials fit the data well.

To complete the expression for total membrane potential we shall assume the same surface considerations also apply to the interior. We therefore develop expressions similar to Eqs. 19 and 20, viz.

$$\phi_{-\delta} - \phi_i = -4\pi e\sigma_i\delta/\epsilon_1 kT, \tag{22}$$

and

$$\phi_{-\delta} - \theta = \ln([K^+]_\infty/[K^+]_i). \tag{23}$$

Here σ_i is the interior membrane charge density. The potential difference in Eq. 22 is assumed across a region of thickness δ. The interior Gouy potential is given by Eq. 23, where θ is the reduced membrane potential, eV/kT, and V is the measurable membrane potential. Combining Eqs. 18, 19, 21, 22, and 23 gives

$$\theta = -4\pi e^2\beta R_i\delta/\epsilon_1 kT + \ln[Na^+]_\infty + \ln(D_{Na^+}/D_{K^+}\lambda_{K^+}[K^+]_\infty), \tag{24}$$

where we have used Eq. 13 and defined λ_{K^+} by

$$\ln\lambda_{K^+} = 4\pi e\sigma_i\delta/\epsilon_1 kT. \tag{25}$$

As a result of cell homeostasis the third term on the right-hand side of Eq. 24 can be taken as constant. The term, $\ln[Na^+]_\infty$, increases monotonically with external Na^+ ion concentration. The first term can become more positive by any process which causes β to decrease. This can occur upon increasing the bulk salt concentration, for this increases the likelihood of ion pair charge neutralization. On the other hand β may also decrease upon dilution at low salt concentration. As already mentioned the reason for this is that the nearly unscreened surface potential, ϕ_δ, can increase the local Na^+ concentration by the Boltzmann factor even as the bulk salt concentration decreases. The tendency toward ion pairing increases and β falls. This last effect, depolarization upon dilution, accounts for the "water response" (4) and will be described more fully below. One further effect is in evidence if competitive triple-ion formation occurs. In this case the first term may become more negative as the salt concentration increases. This is due to an increase in β because charged triple ion complexes prevent sodium ions from forming depolarizing ion pairs. Thus, it is conceivable that a taste cell may produce its maximum salt response at some intermediate salt concentration and thereafter decrease as the salt load increases. In any case the observed effect will depend on the chosen values of k_i, R_i, δ, ϵ_1, and ϵ_2 and the resulting sum of the first two terms. Clearly a decreasing first term may be modified by an increasing second term leading, for example, to an apparent saturation effect.

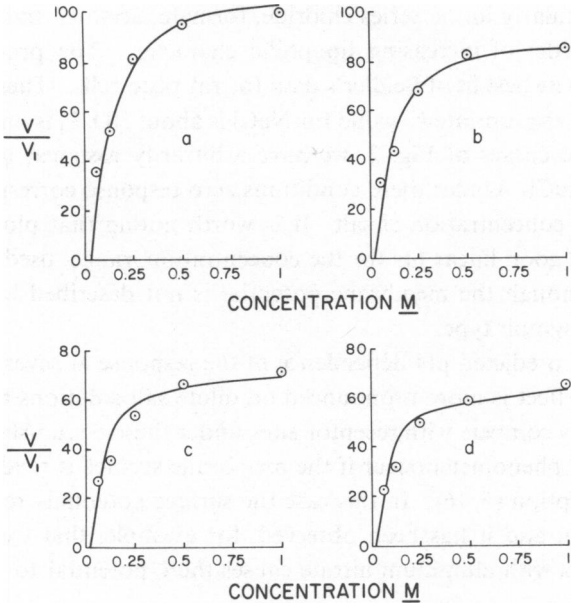

FIGURE 2 Eq. 24 has been fit to the data of Beidler (5) for the observed response of rat taste cells to sodium chloride (*a*), sodium formate (*b*), sodium acetate (*c*), and sodium propionate (*d*). The equation and the data were normalized to a 100% response for 1 M NaCl. The constant term in Eq. 24 expressed in millivolts is 141 mV, and 100% response is 71.8 mV. The values for the parameters are as follows: $R_t = 3.3 \times 10^{13}$ sites/cm^2, $\epsilon_1 = 20$, $\epsilon_2 = 80$, $\delta = 3.8$ Å, $k_{2a} = 3.45$ M^{-1}, $k_{2b} = 10^3$ M^{-1}, pH = 6.5. The values for $k_1 k_3$ are (*a*) 20 M^{-2}, (*b*) 40 M^{-2}, (*c*) 70 M^{-2}, (*d*) 90 M^{-2}.

CORRESPONDENCE BETWEEN THEORY AND DATA

In Fig. 2 we give the membrane potential V as a function of the bulk salt concentration for four sodium salts in contact with rat taste cells showing the "anion effect" discovered by Beidler (2-5). A value of 3.45 was assumed for k_{2a} which is consistent with typical binding constants obtained by Strauss and Ross (29) for Na^+ binding to synthetic polyelectrolytes; k_{2b} was taken as 10^3, a value which sensibly fits the fact of pH independence of salt taste reception between values of 3 and 11 (2-5, 23). We need not specify separately the constants k_1 and k_3 because only their product appears in Eq. 7. The value of k_1 is of course strongly dependent on the local dielectric constant. Kay (17) reports a value of 0.2 for NaCl in water, however at a dielectric constant of 19, an ion pair association constant of 187 can be calculated for NaCl (25). It is unlikely that sodium formate, acetate, or propionate show appreciable ion pairing in water. In media of lower dielectric constant ion pair equilibria involving these salts do occur (25). The constant k_3 measures the strength of association of the mobile ion pairs with the fixed anionic sites. It is well known in the case of ion exchangers that the membrane prefers the counterion with the nonionic moiety most like the nonionic parts of the matrix (14). If taste cells behave analogously, it is reasonable to assume that k_3

should increase regularly in the series chloride, formate, acetate, and propionate, because this is the order of increasing lipophilic character. The products k_1k_3 were chosen to produce the best fit of Beidler's data for rat taste cells. These are 20, 40, 70, and 90 M^{-2}. If the appropriate k_1 value for NaCl is about 200, k_3 is on the order of 0.1. In constructing the curves of Fig. 2, we have arbitrarily assigned a value of 100% response to 1 M NaCl. Under these conditions zero response corresponds to a small non-zero threshold concentration of salt. It is worth noting that plots of $[Na^+]_\infty/V$ vs. $[Na^+]_\infty$ result in good linear fits for the concentration range used (as shown by Beidler [5]), even though the membrane potential is not described by an adsorption equation of the Langmuir type.

Fig. 3 shows the predicted pH dependence of the response at several NaCl concentrations. The pH effect is more pronounced on dilute salt solutions because H^+ ions may more favorably compete with receptor sites under these circumstances. It is clear that the same basic phenomena occur if the membrane surface is rendered positive by specific Fe^{3+} adsorption (5, 16). In this case the surface potentials reverse sign. The effect is well known and it has been observed, for example, that treating negatively charged glass beads with aluminum nitrate causes the ζ-potential to become positive (24).

Beidler (4) has shown that the salt taste receptors of certain species may sometimes decrease in spontaneous activity as the concentration of certain salts in contact with the cells increases. He interprets this effect as the superposition of inhibitory anion and excitatory cation binding to separate receptor sites. Potassium benzoate on rabbit taste receptors causes an initial hyperpolarization with increasing salt at low concentration (between 0 and about 10 mM) followed by depolarization at higher concentrations. An alternative interpretation in terms of the present model arises from the fact that H^+ and Na^+ determine the surface charge density by specific association in addition to the electrostatic screening effect of the double layer. As already described, in the absence of significant triple-ion competition, high salt screens the surface and thereby lowers the surface potential, but the higher density of cations still results in significant surface cation concentrations. Therefore β, the fraction of surface charge, is lowered by cation binding with depolarization resulting. We have also seen that low salt concentration leaves the surface unscreened, resulting in higher surface potentials $|\phi_\delta|$. The surface cation concentration is always higher than the bulk by the Boltzmann factor, so in the manner previously described dilution of the bulk results in increased surface cation concentrations. The increased surface cation concentration again results in a lower density of fixed sites, β decreases, and depolarization again results, this time with dilution. Fig. 4 shows the effect for two cases involving only H^+ and Na^+ competition (no triple-ion competition). Curve 1 has $\delta = 2.2$ Å and curve 2, $\delta = 22$ Å; in each case $\epsilon_1 = 2$, and $k_{2a} = 0.1$.

The work of Kamo et al. (16) on Millipore filters containing bovine tongue lipids and liposomes prepared from bovine tongue lipids suggests an important role for phase boundary potentials in the measured receptor potential of taste cells stimulated by

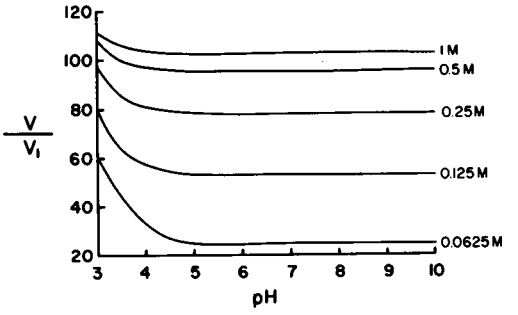

FIGURE 3

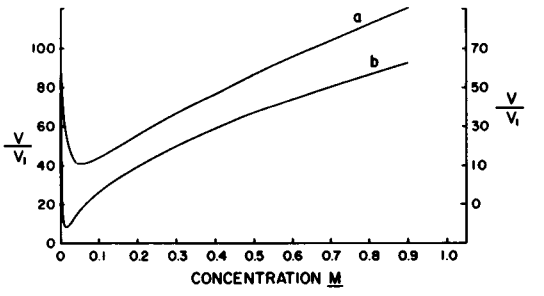

FIGURE 4

FIGURE 3 Response as a function of pH for NaCl for each of the concentrations shown. Parameters are the same as given in Fig. 2.

FIGURE 4 (*a*) Right-hand ordinate: Membrane potential as a function of salt concentration showing depolarization above and below 50 mM salt. Parameters are $R_t = 6.6 \times 10^{13}$ sites/cm^2, $\epsilon_1 = 2, \epsilon_2 = 80, \delta = 22$ Å, $k_{2a} = 0.1$ M^{-1}, $k_{2b} = 10^3$ M^{-1}, $k_3 = 0$. (*b*) Left-hand ordinate: Membrane potential with a minimum at about 10 mM salt, $\delta = 2.2$ Å. Potentials are normalized with the value at 1 M salt.

salts. They have also modeled the reversal of sign that occurs in the potential of membranes when treated with $FeCl_3$. On the other hand, the Millipore-lipid membrane does not show conductance changes similar to those produced by salts on taste cell membranes. Whereas diffusion potentials may be small in the Millipore model system, ion permeation is probably quite important in vivo. Therefore a diffusion potential should also be included to account for the current passed across the membrane. Kamo et al. (16) also discuss this possibility. We have focused mainly on Beidler's classic data which was obtained from a series of sodium salts. Since taste cells respond to other cations it is possible that these too are permeable and if present contribute to the diffusion potential. The present analysis can easily be generalized to include several cations (e.g. see ref. 7 or 14) and does not assume the existence of exclusive sodium or potassium channels.

The model presented in this paper supposes that ions may bind to fixed sites present on the membrane. In this respect there are similarities to Beidler's taste equation based on the binding of cations as in Langmuir adsorption (1–4). If the receptor sites are negatively charged, the surface concentrations of both Na^+ and H^+ ions are higher than in the bulk phase. If the bulk salt concentration is lowered at constant pH, the H^+ ion concentration near the surface increases. This results in neutralization of surface charge. At higher salt concentrations Na^+ binding likely predominates. A plot of surface charge density vs. NaCl concentration should show a maximum negative value at an intermediate salt concentration. Such a plot is presented by Kamo et al. (16) constructed from ζ-potential measurements on liposomes. Thus in considering salt binding to taste cell membranes, the local salt concentration is the important quantity and local pH effects may be critical particularly at low salt concentrations.

SUMMARY

The effects of differing anions, pH, charge reversal, and the water response in salt taste reception can all be interpreted in terms of a simple physicochemical model. The essential features are: (*a*) the region near the cell outer surface allows neutral ion pairs to compete with cations for a *single* anionic receptor; (*b*) the receptor-ion and receptor-ion pair complexes are potential determining; (*c*) the receptor potential is a superposition of phase boundary and diffusion potentials; (*d*) nonpolar adsorption can account for increased triple ion binding constants in order to explain anion effects; (*e*) pH and charge reversal effects are readily understood; and (*f*) the water response can be interpreted as a local effect due to the interplay of electrostatic screening and specific binding of cations.

The authors thank Doctors D. C. Mikulecky and L. M. Beidler for timely criticism and S. K. DeSimone for help with the calculations.

This work was supported by Research Grant DE-03018, by Research Career Development Award DE-12433 (to S. Price) from the National Institute of Dental Research, National Institutes of Health, and an A. D. Williams Research Grant (to J. A. DeSimone).

Received for publication 16 December 1975.

REFERENCES

1. ADAMSON, A. W. 1967. Physical Chemistry of Surfaces. 2nd edition. Interscience Publishers, New York. 184.
2. BEIDLER, L. M. 1954. Theory of taste stimulation. *J. Gen. Physiol.* **38**:133.
3. BEIDLER, L. M. 1961. Taste receptor stimulation. *Progr. Biophys. Biophys. Chem.* **12**:107.
4. BEIDLER, L. M. 1967. Anion influences on taste receptor response. *In* Olfaction and Taste. II. T. Hayashi, editor. Pergamon Press, New York. 509.
5. BEIDLER, L. M. 1971. Taste receptor stimulation with salts and acids. *In* Handbook of Sensory Physiology, Volume IV, Part 2. Taste. L. M. Beidler, editor. Springer Verlag, New York. 200.
6. CHANDLER, W. K., A. C. HODGKIN, and H. MEVES. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol.* **180**:821.
7. CIANI, S., G. EISENMANN, and G. SZABO. 1969. A theory for the effects of neutral carriers such as the macrotetralide actin antibiotics on the electric properties of bilayer membranes. *J. Membrane Biol.* **1**:1.
8. DESIMONE, J. A. 1975. The effects of surface charge and the double layer on the rate of surface catalyzed enzymatic reactions. *Biophys. J.* **15**(2, pt. 2):12*a*. (Abstr.).
9. ENGASSER, J., and C. HORVATH. 1975. Electrostatic effects on the kinetics of bound enzymes. *Biochem. J.* **145**:431.
10. ERIKSSON, E. 1951. The significance of pH, ion activities, and membrane potentials in colloidal systems. *Science (Wash. D.C.).* **113**:41.
11. FUOSS, R. M., and C. A. KRAUS. 1933. Properties of electrolyte solutions. III. The dissociation constant. *J. Am. Chem. Soc.* **55**:1019.
12. GILBERT, D. C., and G. EHRENSTEIN. 1970. Effect of divalent cations on potassium conductance of squid axons: determination of surface charge. *J. Gen. Physiol.* **55**:822.
13. GOLDSTEIN, L., Y. LEVIN, and E. KATCHALSKI. 1964. A water-insoluble polymeric derivative of trypsin. II. Effect of polyelectrolyte carrier on the kinetic behavior of the bound trypsin. *Biochemistry.* **3**:1913.
14. HELFFERICH, F. 1962. Ion Exchange. McGraw-Hill Book Co., New York.
15. KAMO, N., M. MIYAKE, K. KURIHARA, and Y. KOBATAKE. 1974. Physicochemical studies of taste reception. I. Model membrane stimulating taste receptor potential in response to salts, acids and distilled water. *Biochim. Biophys. Acta.* **367**:1.

16. KAMO, N., M. MIYAKE, K. KURIHARA, and Y. KOBATAKE. 1974. Physicochemical studies of taste reception. II. Possible mechanism of generation of taste receptor potential induced by salt stimuli. *Biochim. Biophys. Acta.* **367**:11.
17. KAY, R. L. 1960. An application of the Fuoss-Onsager conductance theory to the alkali halides in several solvents. *J. Am. Chem. Soc.* **82**:2099.
18. MCLAUGHLIN, S. G. A., G. SZABO, and G. EISENMAN. 1971. Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* **58**:667.
19. MIKULECKY, D. C., and J. M. TOBIAS. 1964. Phospholipid-cholesterol membrane model. *J. Cell. Comp. Physiol.* **64**:151.
20. MORAWETZ, H. 1961. *In* Polyelectrolyte Solutions. S. A. Rice and M. Nagasawa, editors. Academic Press, New York. 207.
21. NELSON, A. P., P. COLONOMOS, and D. A. MCQUARRIE. 1975. Electrostatic coupling across a membrane with titratable surface groups. *J. Theor. Biol.* **50**:317.
22. NINHAM, B. W., and V. A. PARSEGIAN. 1971. Electrostatic potential between surfaces bearing ionizable groups in ionic equilibrium with physiologic saline solution. *J. Theor. Biol.* **31**:405.
23. OGAWA, H. 1969. Effects of pH on taste responses of the chorda tympani nerve of rats. *Jpn. J. Physiol.* **19**:670.
24. OVERBEEK, J. T. G. 1969. *In* Colloid Science. I. H. R. Kruyt, editor. American Elsevier Publishing Co., New York. 115.
25. PURLEE, E. L., and E. GREENWALD. 1957. The measurement of acid dissociation constants and ion-pair dissociation constants in solvents of intermediate dielectric constants. Carboxylic acids and their sodium and potassium salts in 70% dioxane—30% water. *J. Am. Chem. Soc.* **79**:1372.
26. RICE, S. A., and M. NAGASAWA. 1961. Polyelectrolyte Solutions. Academic Press, New York. 427.
27. ROBINSON, R. A., and R. H. STOKES. 1959. Electrolyte Solutions. 2nd edition. Butterworths, London. 402.
28. SMITH, A. L. 1973. *In* Dispersion of Powders in Liquids. G. D. Parfitt, editor. John Wiley & Sons, Inc., New York. 86.
29. STRAUSS, V. P., and P. D. ROSS. 1959. Counterion binding by polyelectrolytes. III. Stability constants for the binding of univalent cations by PO_3^--groups of polyphosphates from electrophoresis measurements. *J. Am. Chem. Soc.* **81**:5295.
30. STRAUSS, V. P., and P. D. ROSS. 1959. Counterion binding by polyelectrolytes. IV. Membrane equilibrium studies of the binding of univalent cations by long-chain polyphosphates. *J. Am. Chem. Soc.* **81**:8299.
31. TATEDA, H., and L. M. BEIDLER. 1964. The receptor potential of the taste cell of the rat. *J. Gen. Physiol.* **47**:479.
32. TOBIAS, J. M., D. P. AGIN, and P. PAWLOWSKI. 1962. Phospholipid-cholesterol membrane model. Control of resistance by ions or current flow. *J. Gen. Physiol.* **45**:989.
33. VAN LAMSWEERDE-GALLEZ, D., and A. MEESSEN. 1975. The role of proteins in a dipole model for steady-state ionic transport through biological membranes. *J. Membr. Biol.* **23**:103.