Model for the dynamic responses of taste receptor cells to salty stimuli

I. Function of lipid bilayer membranes

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ABSTRACT The dynamic response of the lipid bilayer membrane is studied theoretically using a microscopic model of the membrane. The time courses of membrane potential variations due to monovalent salt stimulation are calculated explicitly under various conditions. A set of equations describing the time evolution of membrane surface potential and diffusion potential is derived and solved numerically. It is shown that a rather simple membrane such as lipid bilayer has functions capable of reproducing the following properties of dynamic response observed in gustatory receptor potential. Initial transient depolarization does not occur under Ringer adaptation but does under water. It appears only for comparatively rapid flows of stimuli, the peak height of transient response is expressed by a power function of the flow rate, and the membrane potential gradually decreases after reaching its peak under long and strong stimulation. The dynamic responses in the present model arise from the differences between the time dependences in the surface potential ϕ_s and the diffusion potential ϕ_d across a membrane. Under salt stimulation ϕ_d cannot immediately follow the variation in ϕ_s because of the delay due to the charging up of membrane capacitance. It is suggested that lipid bilayer in the apical membrane is the most probable agency producing the initial phasic response to the stimulation.

INTRODUCTION

Taste sensation is triggered by a variation in the electric potential of gustatory receptor membranes induced by chemical stimuli. The induced potential variation, usually depolarization, generates impulses in gustatory nerve fibers (Kimura and Beidler, 1961; Akaike et al., 1976; Sato and Beidler, 1983). The responses of the receptor potential to chemical stimuli as well as the neural responses generally consist of large initial transient depolarization (phasic response) and subsequent steady depolarization (tonic response) (Sato, 1977, 1980). The response in the steady state (the tonic response) has received intensive study on the assumption that it contains most of the taste sensory information (Marowitz and Halpern, 1977; Price and DeSimone, 1977; Sato, 1980; Kurihara et al., 1986).

It has been pointed out based on various experiments that the phasic response plays an essential role in the recognition of taste quality (Wang and Bernard, 1970; Halpern and Tapper, 1971; Smith and Frank, 1972; Halpern and Marowitz, 1973; Heck and Erickson, 1973; Smith and Bealer, 1975; Marowitz and Halpern, 1977). It seems clear evidence for the importance of phasic response that rats can qualitatively discriminate within the duration of the phasic response to decide quickly whether to ingest certain substances (Halpern and Tapper, 1971; Halpern and Marowitz, 1973). It may also be evidence that the taste intensities perceived by many people increase monotonically with the flow rate of stimulus (Meiselman and Bose, 1977) because the phasic response increases with the flow rate but the tonic response does not (Smith and Bealer, 1975; Kashiwagura et al., 1980).

It is quite important that the mechanism of the dynamic responses of receptors including both the phasic and tonic depolarizations be clarified. Some researchers have proposed models describing how the observed time courses of the response intensities arise. Heck and Erickson (1973) described the time course of the gustatory neural response based on a model in which the response was proportional to the adsorption rate of stimulants on the receptor membrane (Paton, 1961). Kamo et al. (1980) assumed that receptor sites occupied by chemical stimulants changed their conformation from active to inactive and the response intensities were proportional to the number of occupied sites with active conformation. They showed that the dynamic responses of receptors arose from the kinematical processes for the stimulant adsorption on the sites. These models, however, have not shown how the adsorption of stimulants induces various changes in the receptor potential. Recently, Heck et al. (1989) and Fidelman and Mierson (1989) developed models in which channels and pumps cooperate to produce dynamic potential responses. The models simulated intracellular depolarization and subsequent repolarization in response to a salt stimulus which may account for gradual adaptation of response. Fidelman and Mierson (1989) took a network thermodynamic approach and investigated in detail the effects of various kinds of ion channels, pumps, and paracellular pathway in the epithelium on the potential variation. The effect

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of the lipid bilayer membrane was not considered in either model.

In this paper we study the effect of the lipid bilayer on the dynamic potential variation under salt stimulation. We present a microscopic model of lipid bilayer membrane to investigate by what mechanism the phasic response is induced. We suggest based on the present study and the observed results that the lipid bilayer in the apical membrane is the most probable candidate for the salt taste transduction pathway causing the phasic response. The observed duration of the phasic response is at most of the order of seconds in both the receptor potential response and the neural one (Sato, 1976, 1977, 1980; Kurihara et al., 1986). We show that the lipid bilayers produce sharp phasic responses with the same order of time constants as the observed ones. On the other hand, the dynamic responses due to the cooperation of ion channels with ion pumps have time constants of the order of several tens of seconds (Fidelman and Mierson, 1989; Heck et al., 1989) which are much longer than the observed ones, whereas the time constants of the responses due to the tight junctions forming the paracellular pathway are of the order of milliseconds, which are much shorter. Some experimental evidence has also indicated the importance of lipid bilayers in the phasic response. The phasic responses to sodium salts are inhibited only partially by the application of amiloride, although the tonic responses to some organic salts are completely inhibited by amiloride (Formaker and Hill, 1988). This indicates that there exists another pathway of the phasic response except for amiloride-sensitive sodium channel. The phasic response is preferentially suppressed by the action of anesthetics (Kashiwagura et al., 1976). There are many observed results that anesthetics act preferentially on the lipid membranes (Shieh et al., 1976; Vanderkooi et al., 1977; Trudell, 1977; Yokono et al., 1981; Janoff et al., 1981; Yoshida et al., 1983; Ueda and Kayama, 1984).

We have calculated explicitly the time courses of variations in the membrane potential of lipid bilayer induced by the salt stimulation. A set of equations describing the time evolution of the surface potential and the diffusion potential across a membrane is derived and solved numerically. Time dependence of the membrane potential arises not only from the time course of stimulus concentration change but also from the process that displacement current charges the membrane capacitance. It is shown based on the present computer simulation that such rather simple membranes like lipid membranes have the functions of reproducing various observed properties of the dynamic responses of receptors. These properties are: the phasic response appears depending on the adapting solution and on the flow rate of stimuli, the peak height of phasic response is expressed by the power function of the flow rate, and the membrane potential shows a gradual decrease after reaching its peak, i.e., adaptation phenomena, under long and strong stimulation. The properties of dynamic response originate essentially from various combinations of the rapid variation of surface potential with the slow change in diffusion potential.

A system approach such as in the network model (Fidelman and Mierson, 1989) is necessary for a comprehensive understanding of the taste transduction mechanism of both phasic and tonic responses. Then, it is quite important to investigate the characteristics of each of the main elements in the transduction system in detail to put the system approach into practice. Lipid bilayer is one such element. This is the other reason why we focus on the lipid bilayer in the present study. Many experiments suggest that the contribution of the lipid bilayer, as well as the ion channel proteins, is important to the tonic response besides phasic one. Recently, a lot of research has shown that amiloride-blockable Na^+ channels in the apical membranes of taste cells provide a prominent transduction mechanism for salt stimulation (Schiffman et al., 1983; Heck et al., 1984; DeSimone et al., 1981, 1984; Brand et al., 1985; DeSimone and Ferrell, 1985; Hill and Bour, 1985; Mierson et al., 1985; Simon and Garvin, 1985; Hettinger and Frank, 1987; Herness, 1987; Avenet and Lindemann, 1988; Hellekant et al., 1988; Heck et al., 1989). However, much experimental evidence indicates that some other mechanisms are involved in the response of a taste cell to salt stimulus (Schiffman et al., 1983; Heck et al., 1984; DeSimone et al., 1984; Brand et al., 1985; DeSimone and Ferrell, 1985; McPheeters and Roper, 1985; Hill and Bour, 1985; Yoshii et al., 1986; Hettinger and Frank, 1987; Herness, 1987; Hellekant et al., 1988; Formaker and Hill, 1988; Nakamura and Kurihara, 1988; Heck et al., 1989). The surface potential and/or phase-boundary potential induced by the surface charges of the lipid bilayer membranes are the most probable agencies producing the other mechanism (DeSimone and Price, 1976; Kurihara et al., 1978, 1986). Many experiments indicate the important contribution of the surface charges to the taste transduction mechanism. Salt stimuli evoke quite small, if any, resistance change across the receptor membranes of some taste cells (West and Bernard, 1978; Tonosaki and Funakoshi, 1984*a*, 1984*b*). Divalent salts generally evoke larger potential variation but smaller resistance changes than monovalent salts with the equimolar concentration (Akaike et al., 1976; Sato, 1980; Kurihara et al., 1986). It is highly probable (Aiuchi et al., 1976; DeSimone and Price, 1976) that the significant dependence of salt response on the anion species arises through the anion dependence of the changes in surface potential. It has been shown (Yoshii and Kurihara, 1983;

Sugawara et al., 1989) that the surface charges play a key role in the water response in which depolarization is induced by decreasing the ion concentration in the stimulus solution. It has been suggested based on the observed correlation between the responses to NaCl and HCl (Ozeki and Sato, 1972; Price and DeSimone, 1977) that the transduction process is at least partially developed by a common mechanism. The lipid bilayer membrane is one of the most probable candidates for agents of the common mechanism because there have been impressive studies demonstrating marked similarities between the behavior of lipid bilayers and salty and sour taste receptors (Price and DeSimone, 1977; DeSimone and Heck, 1980). DeSimone et al. (1980) showed that the variation in the surface pressure of charged phospholipid membranes due to stimulant flow was one of the most probable candidates for triggers of the responses to salt and acid stimuli.

In the early postnatal rats, there are no amiloride-sensitive pathways for salt taste (Hill and Bour, 1985). When the receptor membrane has no pathway specific to sodium salts, it is highly possible that the present model explains appropriately the main features of tonic responses as well as phasic response in such systems.

THEORETICAL MODEL

Model for bilayer membrane

We consider a model system in which a lipid bilayer membrane is in aqueous solution and divides the solution into two regions. These correspond to the external and internal regions of a taste receptor cell. Lipid molecule consists of a hydrophilic polar head group and two hydrophobic alkyl chains. Some lipid molecules have electric dipoles originating from choline residues and phosphate groups in their polar head regions, whereas other kinds of lipids have net negative electric charges. The lipid bilayers considered are a mixture of these, twitterionic and ionizable, molecules. We consider a model bilayer membrane with surface layers including both charges and dipoles. We can reasonably assume that the distribution of the charges and dipoles is smoothed over the entire membrane surface because the lateral exchange rate of lipid molecules is quite high ($\sim 10^7$/s) (Smith and Oldfield, 1984).

The surface charges produce a diffused electric double layer in the solution and result in a surface potential. The dipoles cause a potential jump at the membrane surface as shown in Fig. 1. In addition, a transmembrane electric potential, that is, the diffusion potential, appears due to the permeation of ions through the hydrophobic region. The membrane potential is defined as the potential difference between the two bulk solutions across the membrane. Thus, the membrane potential ϕ_m is given by

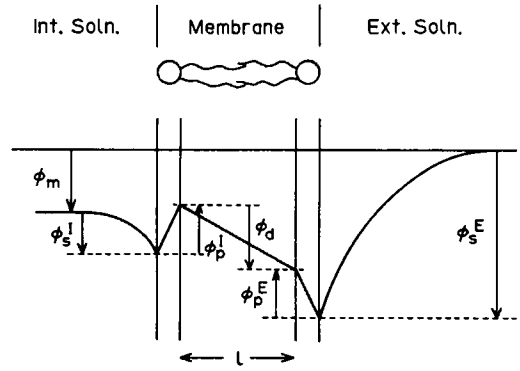

FIGURE 1 Diagrams of the model membrane system and membrane potential. l: thickness of alkyl chain region; ϕ_m: membrane potential; ϕ_s^I and ϕ_s^E: surface potentials; ϕ_d: diffusion potential; ϕ_p^I and ϕ_p^E: potential differences due to dipoles in polar heads.

$$\phi_m = \phi_s^E + \phi_p^E - \phi_d - \phi_p^I - \phi_s^I \quad (1)$$

as shown in Fig. 1, where ϕ_s^I and ϕ_s^E are the surface potentials at the internal and external surfaces, respectively, ϕ_p^I and ϕ_p^E the potentials due to surface dipole moments, and ϕ_d the diffusion potential. We hereafter consider the dynamic response of ϕ_m to chemical stimuli through the response of each component potential.

The ion species considered are a kind of alkaline ion M^+ and a kind of halogen ion A^-, besides proton H^+ and hydroxide OH^-. The cations are adsorbed on the negatively charged heads of ionizable lipids and modify the surface charge densities of the membrane. We assume that the ions are not adsorbed on the dipolar heads. We do not consider any conformation changes in the membrane induced by the chemical stimulation in the present paper.

Using the above model we studied how the membrane potential ϕ_m varies every second under the salt stimulation given as follows. We varied the concentrations of M^+ and A^- in the external bulk solution with time, whereas the ionic concentrations are fixed in the internal bulk solution.

Basic equations for membrane potential

We derive the basic equations by which the time dependence of membrane potential is determined. The

membrane potential ϕ_m is determined by the external and internal surface potentials ϕ_s^E and ϕ_s^I, the external and internal dipole potentials ϕ_p^E and ϕ_p^I, and the diffusion potential ϕ_d, as shown in Fig. 1 and Eq. 1. The dipole potentials are independent of time because we assumed that no ions are adsorbed on the dipoles. For simplicity, we consider only cases where both the internal and external membrane surfaces include equivalent dipoles. Then

$$\phi_p \equiv \phi_p^I = \phi_p^E, \tag{2}$$

where ϕ_p is determined by the dipole density in the polar head region.

We obtained an equation determining ϕ_d in the following way. The total electric current J flowing through the membrane is the sum of ionic and displacement currents and is expressed as

$$J = F \sum_\nu z_\nu \Phi_\nu + \epsilon_m \frac{\partial E_m}{\partial t}, \tag{3}$$

where the symbol ν stands for ion species ($\nu = H$ for proton, OH for hydroxide, M for alkaline ion, and A for halogen ion), Φ_ν is the flux (mol/m^2 · s) of the ion ν, z_ν is its valency, ϵ_m is the dielectric constant of the alkyl chain region, F is the Faraday constant, and E_m is the electric field in the alkyl chain region. We can reasonably assume that the electric field is constant spatially in thin biological membranes (MacGillivray and Hare, 1969). The field E_m is then related to the diffusion potential ϕ_d as

$$E_m = -\phi_d / l, \tag{4}$$

where l is the thickness of alkyl chain region. We assume the current J to be zero according to the usual setup for measuring the membrane potential. Setting $J = 0$ and using Eqs. 3 and 4, we obtain

$$\frac{d\phi_d}{dt} = \frac{l}{\epsilon_m} F \sum_\nu z_\nu \Phi_\nu. \tag{5}$$

The ion flux Φ_ν is represented as

$$\Phi_\nu = -D_\nu \frac{\partial C_\nu}{\partial x} - \frac{F}{RT} z_\nu D_\nu C_\nu \frac{\phi_d}{l}, \tag{6}$$

where C_ν and D_ν are the concentration (mol/m^3) and the diffusion constant (m^2/s), respectively, for the ion ν in the alkyl chain region, and x is the spatial coordinate in the direction perpendicular to the membrane. It is shown in Appendix A that Φ_ν is safely treated as spatially constant in such thin layers as biomembranes. Thus, Eq. 6 is easily solved and we obtain Goldman's expression for the flux

$$\Phi_\nu = -P_\nu \frac{z_\nu F \phi_d}{RT} \frac{C_\nu^{SI} - \exp(z_\nu F \phi_d / RT) C_\nu^{SE}}{1 - \exp(z_\nu F \phi_d / RT)}, \tag{7}$$

where C_ν^{SX} ($X = I$ or E) is the surface concentration of ion ν in the solution on side X, and

$$P_\nu = \alpha_\nu \frac{D_\nu}{l} \exp\left(-\frac{z_\nu F}{RT} \phi_p\right). \tag{8}$$

Here the constant α_ν is the partition coefficient for ion ν between water and alkyl chain region on condition that there are no surface charges and dipole moments. The surface concentration C_ν^{SX} is expressed in terms of ϕ_s^X as

$$C_\nu^{SX} = \exp\left(-\frac{z_\nu F}{RT} \phi_s^X\right) C_\nu^{BX}, \tag{9}$$

where C_ν^{BX} is the concentration of ion ν in the bulk solution on the side of X. In Eqs. 8 and 9, we assumed that the ion distribution is in a thermal equilibrium state in the diffused electric double layer as well as in the polar head region. The reliability of this assumption is discussed in Appendix B. Substituting Eqs. 7 and 9 into Eq. 5, we obtain the equation for ϕ_d in which surface potentials ϕ_s^I and ϕ_s^E are included as unknown quantities.

We obtain the equations determining ϕ_s^I and ϕ_s^E using Gauss' law in the polar head regions:

$$\epsilon_m E_m - \epsilon_w E_w^{SI} = \sigma^I, \tag{10}$$

$$\epsilon_w E_w^{SE} - \epsilon_m E_m = \sigma^E, \tag{11}$$

where ϵ_w is the dielectric constant of the solution, E_w^{SX} ($X = I$, E) the electric field in the solution at the membrane surface on side X, and σ^X the charge density in the X polar head region. The surface fields are given as

$$E_w^{SI} = \left\{\frac{2RT}{\epsilon_w} \sum_\nu C_\nu^{BI}\left[\exp\left(-\frac{z_\nu F}{RT} \phi_s^I\right) - 1\right]\right\}^{1/2}, \tag{12}$$

$$E_w^{SE} = -\left\{\frac{2RT}{\epsilon_w} \sum_\nu C_\nu^{BE}\left[\exp\left(-\frac{z_\nu F}{RT} \phi_s^E\right) - 1\right]\right\}^{1/2}. \tag{13}$$

We derived these equations using the Gouy–Chapman theory, that is, by solving the Poisson–Boltzmann equation in the electric double layer. We express the surface charges σ^I and σ^E in terms of the surface potentials, assuming adsorption equilibrium between cations (H^+, M^+) and ionized polar heads (L^-) such as $H^+ + L^- \rightleftharpoons HL$ and $M^+ + L^- \rightleftharpoons ML$. It is a reasonable assumption that the adsorption equilibrium is attained every moment during the stimulation because the adsorption process is much faster than the rate of the onset of chemical stimulation. Assuming the Langmuir isotherm

for the adsorption probability, we obtain σ^X ($X = I, E$) as

$$\sigma^X = \frac{-eN_0\rho^X}{1 + \sum_\nu {}' K_\nu \exp(-F\phi_s^X/RT) C_\nu^{BX}/10^3}, \quad (14)$$

where N_0 is the areal density of lipid molecules in the membrane, ρ^X is the fraction of ionizable lipids on the X side of the bilayer, K_ν ($\nu = H$ or M) is the association constant (M^{-1}) in the adsorption reaction and the summation with ν is taken over the cation species H^+ and M^+.

Thus, we obtain a set of equations determining the diffusion potential ϕ_d and the surface potentials ϕ_s^I and ϕ_s^E from Eqs. 5, 7, and 9, and also from Eqs. 4 and 10–13.

$$\frac{d\phi_d}{dt} = \frac{lF}{\epsilon_m} \sum_\nu P_\nu \frac{z_\nu F\phi_d/RT}{\exp(z_\nu F\phi_d/RT) - 1} \times \left[\exp\left(-\frac{z_\nu F}{RT}\phi_s^I\right) C_\nu^{BI} - \exp\left(\frac{z_\nu F}{RT}\phi_d\right) \exp\left(-\frac{z_\nu F}{RT}\phi_s^E\right) C_\nu^{BE}\right], \quad (15)$$

$$\epsilon_m \frac{\phi_d}{l} + \left\{2\epsilon_w RT \sum_\nu C_\nu^{BI}\left[\exp\left(-\frac{z_\nu F}{RT}\phi_s^I\right) - 1\right]\right\}^{1/2} = -\sigma^I, \quad (16)$$

$$\epsilon_m \frac{\phi_d}{l} - \left\{2\epsilon_w RT \sum_\nu C_\nu^{BE}\left[\exp\left(-\frac{z_\nu F}{RT}\phi_s^E\right) - 1\right]\right\}^{1/2} = \sigma^E, \quad (17)$$

where σ^X ($X = I, E$) is given by Eq. 14. The quantity P_ν defined by Eq. 8 is essentially equivalent to the ion permeability observed for lipid bilayer membranes (see Appendix C). We hereafter call it ion permeability.

When the ionic concentrations, C_ν^{BX}s, in the bulk solutions are given, we are able to know how the membrane potential ϕ_m varies every second using Eq. 1 and Eqs. 15–17.

Expression of chemical stimuli

We consider dynamic responses of membrane potential to the following processes simulating usual experimental setups. The membrane is first adapted to external solution with fixed ionic concentrations for adequate durations. Second, chemical stimuli are applied in an external solution at $t = 0$. Finally, the external surface of the membrane is rinsed off at $t = t_s$ with a rinsing solution with an ionic constituent the same as that of the adapting solution. Concentrations C_ν^{BI}s in the internal bulk solution are fixed to the values corresponding to physiological values in the chemoreceptor cells, and C_ν^{BE}s in the external bulk solutions are expressed by functions of time simulating the chemical stimulation of membranes.

We fixed the ionic concentrations in the internal bulk solution as follows. The solution is neutral (pH 7) and contains 150 mM monovalent salt;

$$C_H^{BI} = C_{OH}^{BI} = 1 \times 10^{-4}\ \text{mol/m}^3,$$
$$C_M^{BI} = C_A^{BI} = 150\ \text{mol/m}^3. \quad (18)$$

In the usual experimental setup, the stimulating solutions are applied to the membrane by a flow through the nozzle of a micropipette. The replacement of the adapting solution by the stimulating solution does not occur instantaneously but over various durations depending on the rate of flow (Sato, 1976). We thus simulated the stimulation and rinse processes by considering the following time dependence of the concentration C_ν^{BE} of cation species in the external bulk solution.

$$C_\nu^{BE}(t) = \begin{cases} C_{\nu a}: & t < 0 \\ C_{\nu a}[1 - f(t)] + C_{\nu s} f(t): & 0 \le t \le t_s \\ C_\nu^{BE}(t_s)[1 - f(t - t_s)] + C_{\nu a} f(t - t_s): & t > t_s, \end{cases} \quad (19)$$

where t_s is the duration of the stimulation, $C_{\nu a}$ is the concentration in the adapting solution, $C_{\nu s}$ is the concentration in the stimulating solution, and $f(t)$ is the function simulating the replacement process of the solutions at the outside region of the electric double layer and varying between 0 and 1. For $\nu = H$, C_{Hs} was set equal to C_{Ha} because the proton concentration in the external bulk solution is held constant in the salt stimulation. We used the following type of time dependence for $f(t)$.

$$f(t) = \frac{(t/\tau_s)^n}{1 + (t/\tau_s)^n}, \quad (20)$$

where τ_s is a time constant and n is a positive integer. The time spent for the replacement of solutions decreases with increasing the flow rates of the solutions (Sato, 1976). The flow rates correspond qualitatively to $1/\tau_s$.

The anion concentrations C_{OH}^{BE} and C_A^{BE} are given in the solution with pH $\le$ 7 by

$$C_{OH}^{BE} = K_w/C_H^{BE}, \quad (21)$$

$$C_A^{BE} = C_H^{BE} + C_M^{BE} - K_w/C_H^{BE}, \quad (22)$$

where K_w is the ion product of water which takes the value 1×10^{-8} $(\text{mol/m}^3)^2$ at 25°C.

Values of system parameters

We adopted the following values for the parameters included in the present model. Those relevant to the structures of membranes are $l = 50$ Å for the thickness of the membrane, $N_0 = 1/50$ Å^{-2} for the area densities of lipids. We considered the case where fractions of ioniz-

able lipids are the same on both sides of the bilayer; that is, $\rho^I = \rho^E$. Their values are fixed as $\rho = \rho^I = \rho^E = 0.15$, if not stated otherwise. When we examined the dependence of membrane potential response on ρ, we also used the values of 0.05 and 0.25 for ρ ($= \rho^I = \rho^E$). The dielectric constants of the alkyl chain region and of solution are $\epsilon_m = 2.4\epsilon_0$ and $\epsilon_w = 78\epsilon_0$, respectively, where ϵ_0 is that of vacuum. The values of the association constants of ions with the ionizable lipids are $K_H = 2{,}000$ M^{-1} and $K_M = 0.6\ M^{-1}$, if not stated otherwise, where this value of K_M corresponds to that for Na^+ binding to phosphatidylserine (Ohki and Kurland, 1981). We also used the values $K_M = 0.06\ M^{-1}$ and $6\ M^{-1}$ to examine the dependence of the potential response on the ion species through their adsorption. The values of ion permeabilities have been obtained for various lipid liposomes. The values, however, scatter in the range 10^{-12}–10^{-16} m/s for monovalent cations such as Na^+, K^+, or Rb^+ (Johnson and Bangham, 1969; Papahadjopoulos et al., 1971; Hauser et al., 1973; Nichols et al., 1980; Pike et al., 1982; El-Mashak and Tsong, 1985), and also from 10^{-5} to 10^{-9} m/s for H^+/OH^- (Nichols and Deamer, 1980; Kell and Morris, 1980; Nozaki and Tanford, 1981; Deamer and Nichols, 1983; Cafiso and Hubbell, 1983; Krishnamoorthy and Hinkle, 1984; Grzesiek and Dencher, 1986). The permeability of Cl^- has been estimated to be 10–100 times the value for monovalent cations (Papahadjopoulos et al., 1971; Hauser et al., 1973). In view of these observed results, we took values in the range 10^{-5}–10^{-8} m/s for P_H and in the range 10^{-11}–10^{-14} m/s for P_M, and set $P_{OH} = P_H$ and $P_A = P_M$, $10\,P_M$, $100\,P_M$ for anion permeabilities. All of the calculations were carried out at 25°C.

We solved the set of Eqs. 15–17 with the following procedure. The differential Eq. 15 with respect to time was approximated by a difference equation derived using the implicit Euler method (Stoer and Bulirsch, 1980*a*). Then, Eqs. 15–17 are represented at every time step by a set of nonlinear algebraic equations. The set was solved using the Newton–Raphson iteration (Stoer and Bulirsch, 1980*b*).

CALCULATED RESULTS

Initial phasic depolarization

We calculated the responses of the membrane potential ϕ_m to the stimulation with 0.1 M monovalent salt such as NaCl for various adapting conditions. The results are shown in Fig. 2. When the membrane is adapted to water, an initial depolarization peak (phasic response) appears just after the stimulation at $t = 0$ and a depolarization plateau (tonic response) after the peak exists during the stimulation. When salt is added to the adapting solution, the phasic response decreases as shown by pattern *b* (1 mM salt adaptation) and disappears from the adapting solutions containing high enough concentrations of salt (≥ 30 mM). The potential level of the tonic response, however, does not depend on the salt concentration in the adapting solution, though it does depend on the salt concentration in the stimulating solution. This behavior corresponds to the observed results of the receptor potential in frog taste cells. When the cells were stimulated with salts after adaptation to water, phasic depolarization occurs (Sato, 1977), whereas it was not observed in the cells adapted to Ringer solution (Sato, 1976). The preferential suppression of phasic response by the preadaptation is also observed in nerve responses (Kashiwagura et al., 1976).

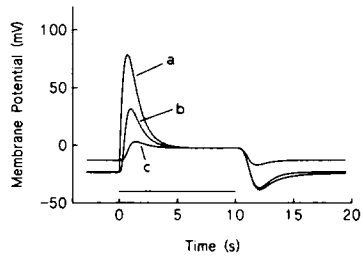

FIGURE 2 Calculated responses of membrane potential to the stimulation with 0.1 M monovalent salt for various adapting conditions. Adapting and rinsing solutions are (*a*) water, (*b*) 1 mM monovalent salt solution, and (*c*) 30 mM monovalent salt solution. Underbar denotes the stimulating period. Ion permeabilities used in the calculations are $P_H = P_{OH} = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s, and $P_A = 10\,P_M$. The calculations are performed as follows. In Eq. 19, we set C_{H_s} and C_{H_B} as 10^{-4} mol/m^3 ($=10^{-7}$ M), $C_{M_s} = 100$ mol/m^3, and $C_{M_B} = 0$ for the water adaptation case; $C_{M_B} = 1$ mol/m^3 for the 1 mM adaptation; and $C_{M_B} = 30$ mol/m^3 for the 30 mM adaptation. The duration of the stimulation, t_s, is 10 s. The values of τ_s and n in the function $f(t)$ (Eq. 20) are $\tau_s = 1$ s and $n = 4$.

We consider here the reason why the phasic response occurs and why it depends on the adapting condition. The membrane potential ϕ_m consists of the internal and external surface potentials ϕ_s^I and ϕ_s^E, the internal and external dipolar potentials ϕ_p^I and ϕ_p^E, and the diffusion potential ϕ_d as shown in Eq. 1. The dipolar potentials are fixed in the present calculation. We show the time variations of the other potentials ϕ_s^I, ϕ_s^E, and ϕ_d in Fig. 3 for both the water-adapted and salt-adapted cases. The value of ϕ_s^I is almost independent of time, and the dynamic response of ϕ_m to stimulation is determined through the responses of ϕ_s^E and ϕ_d as seen in the figure. When the external surface of the membrane is preadapted to water, ϕ_s^E quickly rises high with the application of salt at $t = 0$ as shown in Fig. 3 *a* because of the abrupt increase in ionic strength in the external solution, but the change in diffusion potential ϕ_d is not as

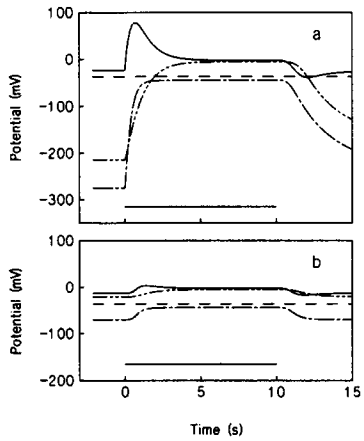

FIGURE 3 Time variations of interior surface potential ϕ_s^I (*broken lines*), exterior surface potential ϕ_s^E (*single-dot chain lines*), diffusion potential ϕ_d (*double-dot chain lines*), and membrane potential ϕ_m (*solid lines*) for (*a*) the water adaptation and (*b*) the 30 mM salt adaptation cases described in Fig. 2.

fast as that of ϕ_s^E. The membrane potential ϕ_m rises sharply at first due to the rapid rising of ϕ_s^E and then the slow increase in ϕ_d induces a decrease in ϕ_m resulting in an overshoot of ϕ_m, i.e., the phasic response. When the external membrane surface is preadapted to salt solution, the change in ionic strength due to the stimulus application is minor compared with the water adaptation case. The changes in potentials ϕ_s^E and ϕ_d are minor as seen in Fig. 3 *b*. Then, the overshoot of ϕ_m is suppressed. It is concluded that the initial phasic response occurs as a result of the association of large rapid change in the surface potential with large slow change in the diffusion potential.

It is seen in Fig. 2 that the membrane potential also shows a transient response (hyperpolarization) after the stimulation is switched off. The origin of this after-hyperpolarization is equivalent to that of the initial phasic depolarization. It arises from a combination of the rapid fall of the surface potential ϕ_s^E with the slow decay of the diffusion potential ϕ_d.

Dynamic properties of surface and diffusion potentials

Here we show the detailed mechanisms inducing dynamic responses of the component potentials ϕ_s^I, ϕ_s^E, and ϕ_d. The calculations show that the internal surface potential ϕ_s^I is almost constant regardless of the stimulation as seen in Fig. 3. This is due to the fact that the dielectric constant ϵ_m of the alkyl chain region is much lower than that of water, ϵ_w. The term $\epsilon_m E_m$ in Eq. 10 which determines ϕ_s^I is negligibly small as compared with the term $\epsilon_w E_w^{SI}$. The potential ϕ_s^I is then well approximated by the solution of the equation $\epsilon_w E_w^{SI} = -\sigma^I$ which is constant and takes -36.5 mV for the present values of ionic concentrations $C_\nu^{BI}s$ in the internal solution, density $N_0\rho$ of the ionizable lipids and association constants $K_\nu s$ of the cations.

The external surface potential ϕ_s^E is also determined practically by the ionic concentrations $C_\nu^{BE}s$ in the external bulk solution, $N_0\rho$ and $K_\nu s$ through the well-approximated equation $\epsilon_w E_w^{SE} = \sigma^E$ for Eq. 11. We can obtain the value of ϕ_s^E at a time t as a function of the values of $C_\nu^{BE}s$ at t using Eqs. 13 and 14. This means that the change of ϕ_s^E follows that of $C_\nu^{BE}s$ almost without delay; that is, a fast (slow) rise in stimulus concentration may induce a fast (slow) rise of ϕ_s^E. The result is derived on condition that the ion adsorption process is quite fast compared with the chemical stimulation process. If the rates of the two processes become comparable, we cannot use Eq. 14 and need to treat the adsorption process dynamically.

The dynamic property of the diffusion potential ϕ_d is determined from Eq. 5. To see how ϕ_d responds to the stimulation represented by $C_\nu^{BE}(t)$, we rewrite Eq. 5 as

$$\frac{d\phi_d}{dt} = -\frac{1}{\tau_d}(\phi_d - \phi_{d,0}), \tag{23}$$

where $\phi_{d,0}$ is the value of ϕ_d for the stationary state ($\Sigma_\nu z_\nu \Phi_\nu = 0$), and

$$\tau_d \equiv -\frac{\epsilon_m}{l}\frac{\phi_d - \phi_{d,0}}{F\sum_\nu z_\nu \Phi_\nu}. \tag{24}$$

The potential $\phi_{d,0}$ is expressed using Eq. 7 as

$$\phi_{d,0} = \frac{RT}{F}\ln\left[\frac{P_H C_H^{SI} + P_M C_M^{SI} + P_{OH} C_{OH}^{SE}(t) + P_A C_A^{SE}(t)}{P_H C_H^{SE}(t) + P_M C_M^{SE}(t) + P_{OH} C_{OH}^{SI} + P_A C_A^{SI}}\right], \tag{25}$$

where this is the well-known Hodgkin–Katz equation. Because $\phi_{d,0}$ is the stationary diffusion potential and is represented every second as a function of $C_\nu^{BE}(t)s$ using Eq. 9, it corresponds to the diffusion potential that can change as $C_\nu^{BE}s$ change, without delay, as ϕ_s^E can. The time constant τ_d in Eq. 23 for the relaxation process $\phi_d \to \phi_{d,0}$ thus means the delay time in the response of ϕ_d to the stimulation. To estimate the value of τ_d, we expand the denominator $\Sigma_\nu z_\nu \Phi_\nu$ in Eq. 24 with respect to $(\phi_d - \phi_{d,0})$. Considering up to the linear term, we obtain τ_d as

$$\tau_d = \frac{\epsilon_m}{l}\frac{R_{in} - R_{out}}{\ln(R_{in}/R_{out})}, \tag{26}$$

where

$$R_{in} = \frac{RT}{F}\frac{1}{F[P_H C_H^{SE}(t) + P_M C_M^{SE}(t) + P_{OH} C_{OH}^{SI} + P_A C_A^{SI}}, \quad (27)$$

$$R_{out} = \frac{RT}{F}\frac{1}{F[P_H C_H^{SI} + P_M C_M^{SI} + P_{OH} C_{OH}^{SE}(t) + P_A C_A^{SE}(t)]}. \quad (28)$$

The surface concentrations $C_\nu^{SE}(t)s$ are obtained from $C_\nu^{BE}(t)s$ using Eq. 9. The values of τ_d calculated from Eq. 26 are of the order of the stimulation time constant τ_s ($\sim$1 s). Hence the change of ϕ_d due to the stimulation lags noticeably behind the change of ϕ_s^E as shown in Fig. 3.

Such large relaxation times, τ_d, result physically from the high electric resistance of the membranes because the dynamic response of ϕ_d comes mainly from the charging of membrane capacitance. In the expression 24 for τ_d, ϵ_m/l is the specific membrane capacitance C_m. $-(\phi_d - \phi_{d,0})/(F\Sigma_\nu z_\nu \Phi_\nu)$ corresponds to the specific membrane resistance R_m, because $-(\phi_d - \phi_{d,0})$ is the deviation $\Delta\phi_m$ of the membrane potential ϕ_m from the value $\phi_{m,0}$ for the stationary state ($\Sigma_\nu z_\nu \Phi_\nu = 0$) and $F\Sigma_\nu z_\nu \Phi_\nu$ is the density of ionic electric current across the membrane induced by $\Delta\phi_m$. Thus, τ_d equals $C_m R_m$ which is the time constant for the charging of membrane capacitance. Whether the response of ϕ_d becomes slower than that of ϕ_s^E depends on the relative magnitude of the stimulation flow rate $1/\tau_s$ to $1/(C_m R_m)$.

We note a method by which one may estimate the values of τ_d from observed phasic response patterns. After the stimulation becomes stationary ($t > \tau_s$), transient depolarization (phasic response) is determined mostly by the change of ϕ_d given by Eq. 23. Then the membrane potential ϕ_m may decay exponentially into its stationary value as seen in Fig. 2. The decay constant corresponds to τ_d. It is 0.89 s for the phasic response curves in Fig. 2 and does not depend on the adapting condition. It is noted that the value of τ_d calculated from Eq. 26 is 0.83 s.

Effect of stimulant flow rate

The magnitude of the gustatory phasic response has been known to depend on the flow rate of stimulus solution (Sato, 1977; Smith and Bealer, 1975; Kashiwagura et al., 1980). We investigated how and why the depolarization pattern of lipid bilayer depends on the stimulation pattern using the present model. In Fig. 4 it is shown how the calculated patterns of membrane potential ϕ_m vary depending on the applied patterns of salt concentration $C_M^{BE}(t)$. The peak height of phasic depolarization increases with decreases in the rise time τ_s of stimulation or increases in the flow rate of stimulus

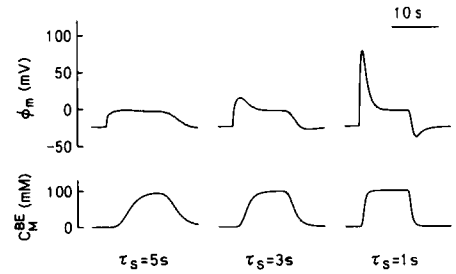

FIGURE 4 Dependences of response patterns of membrane potential ϕ_m upon various stimulation patterns determined by the stimulus flow rate. $1/\tau_s$ corresponds to the stimulus flow rate. C_M^{BE} denotes the concentration of monovalent salt in the bulk external solution. The adapting and rinsing solutions are water. The parameter values used are $P_H = P_{OH} = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s, $P_A = 10\,P_M$, and $n = 4$.

solution. Tonic depolarization does not depend on the flow rate.

The reason why the phasic response depends on the stimulus flow rate comes from the dynamic response by the diffusion potential ϕ_d described in the previous section. When the application of stimulus is very fast ($\tau_s < \tau_d$), the change in ϕ_d cannot follow immediately that of the surface potential ϕ_s^E which reflects the stimulation pattern $C_M^{BE}(t)$. Then, the membrane potential ϕ_m shows a transient phasic depolarization pattern. On the other hand, because the quite gradual application of stimulus makes the rise of ϕ_s^E slow enough, ϕ_d can easily catch up with the change in ϕ_s^E. Then, ϕ_m shows only the stationary depolarization (tonic response) pattern.

To see how the peak amplitude V_p of the phasic response depends on the stimulant flow rate $1/\tau_s$, we calculated the dependence for various values of ion permeabilities P_H, P_M, and stimulus concentrations. Fig. 5 shows the results. The dependence of V_p on $1/\tau_s$ is represented by a power law; that is, V_p is proportional to $(1/\tau_s)^\alpha$ in a definite range of $1/\tau_s$. The exponents α of the power law are almost independent of the stimulus concentration and also of the values of P_H and P_M for a fixed value of n in Eq. 20, though the magnitudes of V_p depend on them. The exponent seems to be determined mainly by the pattern of the stimulus application given by the value of n. The values of α in Fig. 5 are 0.58 in the case of $n = 2$ and nearly equal to 1.0 in the case where $n = 4$. The slope of the curves gradually levels with increasing V_p because V_p is saturated by increasing the stimulant flow rate $1/\tau_s$. It is seen in Fig. 3 that the saturated value of V_p corresponds to the magnitude of the discrete change in ϕ_s^E due to the stimulation.

Smith and Bealer (1975) studied the sensitivity of the rat gustatory system to the rate of stimulus onset by

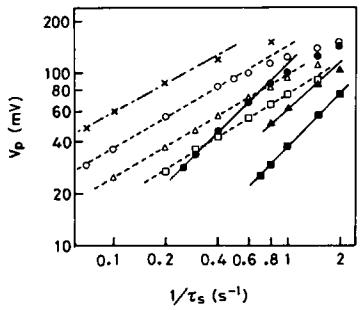

FIGURE 5 Dependences of the peak height V_p of phasic response on the stimulus flow rate $1/\tau_s$ in various cases. V_p is measured from the resting potential. The adapting solution is water. (○): the applied salt concentration C_{Ms} is 0.1 M, $n = 2$ in Eq. 20, $P_H = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s; (△): $C_{Ms} = 0.1$ M, $n = 2$, $P_H = 10^{-6}$ m/s, $P_M = 3 \times 10^{-12}$ m/s; (□): $C_{Ms} = 0.1$ M, $n = 2$, $P_H = 3 \times 10^{-6}$ m/s, $P_M = 3 \times 10^{-12}$ m/s; (×): $C_{Ms} = 0.5$ M, $n = 2$, $P_H = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s; (●): $C_{Ms} = 0.1$ M, $n = 4$, $P_H = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s; (▲): $C_{Ms} = 0.1$ M, $n = 4$, $P_H = 10^{-6}$ m/s, $P_M = 3 \times 10^{-12}$ m/s; (■): $C_{Ms} = 0.1$ M, $n = 4$, $P_H = 3 \times 10^{-6}$ m/s, $P_M = 3 \times 10^{-12}$ m/s. In all cases, the anion permeabilities are taken as $P_{OH} = P_H$ and $P_A = 10 P_M$.

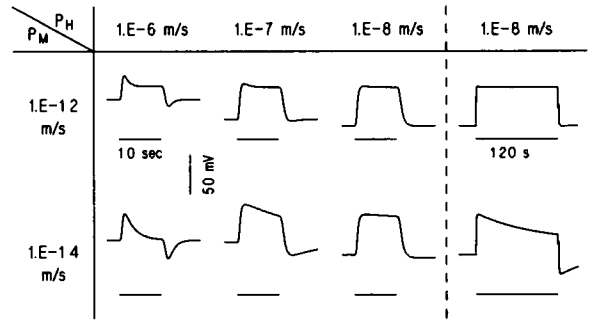

FIGURE 6 Response patterns of the membrane potential for various values of cation permeabilities. The applied salt concentration is 0.1 M. The adapting and rinsing solutions are 10 mM salt solution. The permeabilities of anions are taken as $P_{OH} = P_H$ and $P_A = 10 P_M$. The stimulus flows are given by $\tau_s = 1$ s and $n = 4$.

observing responses of the chorda tympani nerve to stimulation with both linearly rising anodal current and NaCl presented at different rates of flow. They showed that the relationship between the amplitude of the phasic response and the rate of increasing stimulation is well described by a power function where the exponent is ~0.5. It is suggested based on the calculations that the power law observed in the nerve response may hold in the responses of receptor membranes.

Dependence of response pattern on ion permeability

It is well known that ion permeabilities affect the magnitude of static (tonic) response to stimulation. For example, when the permeability of some cation is increased, the tonic response to the cation increases. It is seen from Eqs. 23–28 that they also affect the dynamics of the diffusion potential ϕ_d and, thereby, the response pattern of the membrane potential ϕ_m changes with the values of the permeabilities. It has been shown in Fig. 5 that the magnitude of the phasic response V_p varies with the permeabilities.

To see how the response pattern changes with permeabilities, we calculated the variation of ϕ_m due to 0.1 M salt stimulation after the adaptation with 10 mM salt for various values of P_H and P_M. The permeabilities of anions are taken as $P_{OH} = P_H$ and $P_A = 10 P_M$. Fig. 6 shows the results. The response pattern varies qualitatively with the ion permeability. The patterns in Fig. 6 are classified into the three types. The first type consists of an initial phasic depolarization, a tonic depolarization, and an after hyperpolarization. The second type consists of a gradually decreasing depolarization and the third type of pattern consists of a simple tonic depolarization. The effect of the proton permeability P_H to the response pattern is qualitatively different from that of the alkaline ion permeability P_M. As P_H is decreased from 10^{-6} m/s to 10^{-8} m/s, the response pattern becomes simpler. On the other hand, as P_M is decreased, the pattern tends to become more complex.

The dependence of response pattern on the permeabilities arises from two factors. One is the relative magnitude of the decay time τ_d of ϕ_d to the stimulation period t_s (10 s in Fig. 6). The other is the difference $\Delta\phi_d^0$ in the steady diffusion potential before and after the stimulation. The decay time τ_d increases with the decreasing values of P_H and P_M, and $\Delta\phi_d^0$ increases with increasing P_H and decreasing P_M, as will be explained later. First, we show how and why the response pattern changes depending on τ_d/t_s and $\Delta\phi_d^0$.

When τ_d is shorter than t_s, the transient response of the membrane potential ϕ_m induced by the change in ϕ_d finishes within the stimulation period t_s. This corresponds to the cases where the values of P_H and/or P_M are relatively large. Then, the phasic response occurs if there is a noticeable change $\Delta\phi_d^0$ in ϕ_d. The response pattern of ϕ_m belongs to the first type as shown in the cases where $P_H = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s, and $P_H = 10^{-6}$ m/s, $P_M = 10^{-14}$ m/s. Even when τ_d is shorter than t_s, the phasic response is not observed if $\Delta\phi_d^0$ is quite small because the response of ϕ_m is determined primarily by the response of ϕ_s^E. The simplification of the response pattern from the first type to the third type is seen in the case of $P_M = 10^{-12}$ m/s when P_H is decreased. This is induced because $\Delta\phi_d^0$ decreases with P_H.

When τ_d is comparable with t_s, the changing of ϕ_d with

time does not finish within the period t_s. The membrane potential ϕ_m then decreases gradually without showing a sharp phasic response peak; that is, the response pattern is the second type. This corresponds to the cases of relatively low values of P_H and P_M such as $P_H = 10^{-7}$ m/s and $P_M = 10^{-14}$ m/s.

When τ_d is much longer than t_s, the change in ϕ_d with time is quite small in the time period t_s even when $\Delta\phi_d^0$ is noticeable. This corresponds to the cases of very low values of P_H and P_M. Then, because the response of ϕ_m is mostly determined by the response of ϕ_s^E, it becomes a simple tonic pattern of the third type. The simplification of the response pattern of $P_M = 10^{-14}$ m/s due to the decrease in P_H is induced by this mechanism. In the case of $P_M = 10^{-14}$ m/s and $P_H = 10^{-8}$ m/s, τ_d is 83 s which is much longer than the stimulation period of 10 s. Although the response pattern is quite similar to that of $P_M = 10^{-12}$ m/s and $P_H = 10^{-8}$ m/s, the mechanism of the latter simplification is quite different. The latter response pattern corresponds to the usual tonic response arising in the steady state, whereas the former pattern corresponds to a transient response with a decay time much longer than the scale of observation time. This can be seen by comparing the patterns of a sufficiently prolonged stimulation period as shown in the case of t_s = 120 s in Fig. 6. In the former case, the transient change in ϕ_m becomes observable in the prolonged stimulation with t_s comparable to τ_d.

Now we consider how τ_d and $\Delta\phi_d^0$ depends on the values of the permeabilities P_H and P_M. The decay time τ_d of the diffusion potential ϕ_d depends on the permeabilities as shown in Eqs. 26–28. τ_d increases with decreasing the values of permeabilities because the membrane resistance increases. The difference $\Delta\phi_d^0$ comes mostly of the differences in the external surface cation concentrations C_H^{SE} and C_M^{SE}, before and after the stimulation, when the stimulus salt concentration is not high enough for the acidic lipid molecules to be neutralized. The surface anion concentrations C_{OH}^{SE} and C_A^{SE} are quite low because of the electrostatic repulsion due to the negatively charged surface. Then, neglecting the contributions from the anions in Eq. 25, we obtain the approximated $\Delta\phi_d^0$ as

$$\Delta\phi_d^0 \cong \phi_{d,0}(\tau_d) - \phi_{d,0}(0) \cong \frac{RT}{F} \ln\left[\frac{P_H C_H^{SE}(0) + P_M C_M^{SE}(0)}{P_H C_H^{SE}(\tau_d) + P_M C_M^{SE}(\tau_d)}\right], \quad (29)$$

where $C_\nu^{SE}(0)$ and $C_\nu^{SE}(\tau_d)$ are the concentrations of ν ion before and after the stimulation with the salt MA. The difference $\Delta\phi_d^0$ depends sensitively on the values of P_H and P_M when the magnitude of $P_H C_H^{SE}$ is comparable to that of $P_M C_M^{SE}$. Then, $\Delta\phi_d^0$ increases with increasing P_H and decreasing P_M because the relations $C_H^{SE}(0) > C_H^{SE}(\tau_d)$ and $C_M^{SE}(0) < C_M^{SE}(\tau_d)$ are satisfied for the salt stimulation.

It has been observed (Ozeki, 1971) that when gustatory cells are stimulated over long durations (a few tens of seconds) by concentrated NaCl solutions, the receptor potentials show gradual decreases after their depolarizations peak. This is the so-called adaptation of response. This type of time dependence of ϕ_m is also obtained in the present model for comparatively low values of ion permeabilities. Fig. 7 shows the calculated response patterns of $P_H = P_{OH} = 10^{-7}$ m/s, $P_M = 10^{-13}$ m/s, and $P_A = 10\,P_M$ as functions of salt concentration C_M^{BE}. The adaptation rate of the response increases with the concentration. A similar phenomenon has been observed by Ozeki (1971) using a rat taste cell.

These adaptation properties arise from the dynamic feature of the diffusion potential ϕ_d as described above. The decay pattern of ϕ_m is expressed reasonably as $\phi_m = A \exp(-t/\tau_d) + B$, where A and B are constants. It is seen from Eqs. 26–28 and 9 that the relaxation time τ_d decreases by increasing the salt concentration C_M^{BE} in the external solution. The surface concentrations C_M^{SE} and C_A^{SE} increase, and then the ion concentrations in the membrane increase resulting in a decrease in membrane resistance. In the case of Fig. 7 the values of τ_d are 8.3, 4.9, and 3.1 s for the application of 0.1, 0.5, and 1 M salts, respectively. It should be noted that the anion A^- such as Cl^- plays a quite important role in the decrease of τ_d in the case of concentrated stimulus solutions. When the salt concentration C_M^{BE} in the external bulk solution is increased, the surface concentration C_M^{SE} of alkaline ion does not increase markedly, but the concentration C_A^{SE} of halogen ion does. In case of Fig. 7, C_A^{SE} increases from 0.018 to 0.65 M with increasing C_M^{BE} from 0.1 to 1 M, whereas the increase in C_M^{SE} is from 0.56 to 1.53 M. The reason why C_M^{SE} does not change markedly is that when

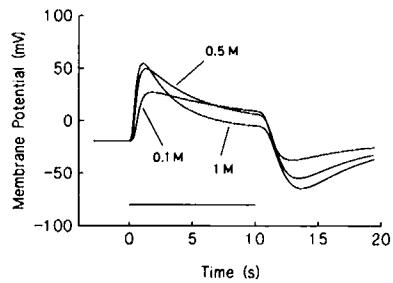

FIGURE 7 Response patterns of membrane potentials showing slow adaptations to stimulation. The value of salt concentration in the stimulating solution is shown for each pattern. The adapting and rinsing solutions are both 10 mM salt solution. The parameter values used are $P_H = P_{OH} = 10^{-7}$ m/s, $P_M = 10^{-13}$ m/s, $P_A = 10\,P_M$, $n = 4$, and $\tau_s = 1$ s.

the concentration C_M^{BE} is increased, C_M^{SE} given by Eq. 9 does not increase at the same rate as C_M^{BE} because of the factor exp $(-F\phi_s^E/RT)$. Because ϕ_s^E is negative and its absolute value decreases with increasing ionic strength, the value of exp $(-F\phi_s^E/RT)$ decreases with increasing C_M^{BE}. On the other hand, because of the factor exp $(+F\phi_s^E/RT)$ for anions, the surface concentration C_A^{SE} increases markedly with increasing C_A^{BE} $(=C_M^{BE})$.

The anion contributes essentially to the concentration dependence of adaptation through the marked increasing rate of C_A^{SE} together with the large permeability of halogen ion A^-.

Concentration dependence of response magnitudes

To know how the phasic and tonic responses depend on the concentration of stimulus salt, we calculated the dependences for various values of parameters P_A, K_M, and ρ. The concentration dependences of the responses vary with the species of both cations M and anions A in the stimulus salt and also with the features of the receptor membranes. In the present model, the cation specificity arises from the cation permeability P_M and the association constant K_M for the cation adsorption on the ionized lipids. The anion specificity comes from the anion permeability P_A. The membrane specificity arises from the fraction ρ of ionizable lipids as well as P_M, P_A, and K_M. Because the P_M dependence of the responses has been shown previously, we show the results for the effects of P_A, K_M, and ρ in Fig. 8. The values of the other parameters are fixed at $P_H = P_{OH} = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s, and $K_H = 2{,}000\ M^{-1}$.

The magnitude of the phasic response has a logarithmic dependence on the stimulus concentration in a definite range of the concentration as seen in the figure. The logarithmic dependence of the response is known as the Weber-Fechner rule in various sensory systems. It seems to support the importance of the dynamic response in gustatory recognition that the phasic component satisfies this rule. The concentration dependence of the tonic response does not obey the Weber-Fechner rule. When the salt concentration is high enough, the tonic response decreases with increased concentration.

Fig. 8 *a* shows the response curves for the three values of permeability P_A of the halogen ion A^-. The peak amplitude of the phasic response is quite insensitive to the permeability of halogen ion, whereas the tonic response varies noticeably with it. The reason why the phasic response is insensitive to halogen permeability P_A is as follows. The peak amplitude of the phasic response depends on the decay constant τ_d of the diffusion potential. When the membrane is stimulated, the membrane potential ϕ_m usually reaches its phasic peak before the surface potential rises fully as seen in Fig. 3. Because the surface potential ϕ_s^E has relatively large hyperpolarization $(\phi_s^E < 0)$ until ϕ_m reaches the phasic peak, the anion concentration C_A^{SE} at the membrane surface keeps quite low values as seen from Eq. 9. Then, the value of τ_d does not depend on P_A at the early stage of the stimulation in which the phasic response occurs.

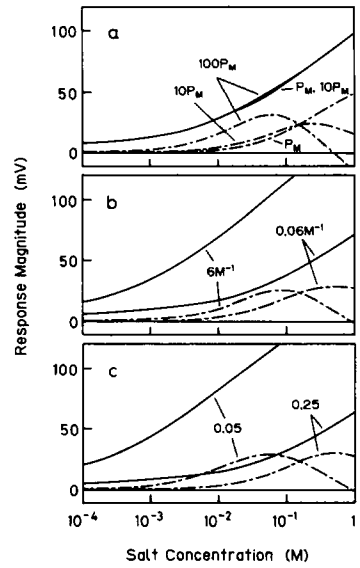

FIGURE 8 Stimulus concentration dependence of phasic response peak (*solid lines*) and tonic response (*chain lines*) as functions of P_A, K_M, and ρ. Magnitudes of the responses are measured from the resting level. The adapting solution is water. The parameter values commonly used are $P_H = P_{OH} = 10^{-6}$ m/s, $P_M = 10^{-12}$ m/s, $n = 4$, and $\tau_s = 2$ s. *a* shows the dependence as a function of P_A with its value shown at each curve. The values of K_M and ρ are fixed at 0.6 M^{-1} and 0.15, respectively. *b* shows the dependence as a function of K_M with its value shown on each line. $P_A = 10\,P_M$ and ρ = 0.15. *c* shows the dependence as a function of ρ with its value shown on each line. $P_A = 10\,P_M$ and $K_M = 0.6\ M^{-1}$.

The noticeable dependence of the tonic response curves on P_A, shown in Fig. 8 *a*, shows the definite contribution of the halogen ion to the static membrane potential. When the salt concentration in the external solution is less than that (150 mM) in the internal solution, an outflux of halogen ion is induced and this tends to depolarize the membrane potential. Because the contribution of this flux becomes more prominent for larger P_A, the tonic depolarization increases with P_A. On the other hand, when the salt concentration in the external solution exceeds 150 mM, the flux of the halogen ion is an influx tending to hyperpolarize the membrane potential. The tonic response becomes lower for larger P_A. For large P_A and high salt concentrations, the magnitude of tonic response decreases with in-

creased concentration, as shown in Fig. 8 *a*, because the main contribution to ϕ_d comes from the anion flux.

In Figs. 8, *b* and *c*, both the phasic and tonic responses depend on the value of the association constant K_M of alkaline ion and on the fraction ρ of ionizable lipids. The dependence of the phasic response on these parameters arises from the dependence of the degree of surface charges on them. When K_M becomes larger or ρ becomes smaller, the absolute value of the surface charge density decreases (see Eq. 14). The decrease in the surface charge increases both the response of the surface potential ϕ_s^E to the stimulation and the decay time τ_d of the diffusion potential ϕ_d as in the following. The large response of ϕ_s^E directly induces large phasic response and the large τ_d indirectly induces large phasic response through the increased delay in the response of ϕ_d. A larger response of ϕ_s^E for decreased surface charge is because ϕ_s^E under the stimulation is shallower (i.e., larger). The value of τ_d becomes higher for a decreased surface charge because the surface concentrations C_H^{SX} and C_M^{SX} of cations become smaller.

The dependence of the tonic response on K_M and ρ arises from an origin similar to that of the P_A dependence. The contribution of the halogen ion flux to the tonic response increases with increasing K_M or decreasing ρ because of decreasing the surface charge. Thus the concentration dependence of the tonic response shows qualitatively similar variation under the three kinds of conditions that P_A increases, K_M increases, and ρ decreases. The surface charge and surface potential do not directly affect the membrane potential ϕ_m in the steady state but they indirectly change it through the change in the portion of anion flux in the total ion flux.

DISCUSSION

The model in the present paper considers the electrochemical diffusion of H^+, OH^-, alkaline ions, and halogen ions across lipid bilayer. The transient response of the membrane potential in the model arises from the time lag between the change in diffusion potential and that in surface potential. The displacement current to charge the membrane capacitance induces a delay in the response of diffusion potential. The model shows that bare lipid bilayer has the functions of reproducing various observed properties of the dynamic responses of receptor cells to salts. Lipid bilayer would contribute to the taste transduction directly as transduction pathways and/or indirectly through their surface charges which modify the ion transport through channels. Recently, Jordan (1987) calculated the electric potential in various kinds of cylindrical pores spanning a lipid membrane to investigate the effects of the membrane surface charges on the ion permeability of transmembrane ionic channels. He has shown that changing the lipid surface potential influences noticeably the channel conductance in the following cases: the ion channel protein is encapsulated by the lipid bilayer, or even if the protein protrudes through the bilayer, the rate-limiting step in ion permeation takes place around the center of the channel. The present study indicates that the contributions of lipid bilayer should be appropriately incorporated in a unified model of the lingual epithelium.

Lipid bilayer membranes do not, of course, account for every aspect of the taste responses of real cells. For example, the present model will not explain the difference in the amiloride-sensitivities of tonic responses between halogenated and nonhalogenated sodium salts. The tonic responses for halogenated sodium salts such as NaCl, NaBr, NaI are not inhibited completely by amiloride, whereas those for nonhalogenated sodium salts such as sodium acetate and $NaHCO_3$ are inhibited (Formaker and Hill, 1988). They suggested that sodium transduction occurred exclusively through an amiloride-sensitive pathway and the residual response after amiloride application was halogen related. On the other hand, the tonic responses are not inhibited completely by amiloride for other nonhalogenated salts such as Na_2SO_4, NaSCN and $NaNO_3$ (Nakamura and Kurihara, 1988).

In real cells, there are channels such as amiloride-blockable passive Na^+ channels and active K^+ channels, pumps, and shunt pathway besides lipid bilayers (Kinnamon, 1988). These different transport systems are activated under varying ionic conditions and several of these simultaneously participate in the taste transduction for salts. However, there is some evidence that the lipid bilayer plays an important role in the phasic response. The phasic responses are only partially inhibited by amiloride both for halogenated and nonhalogenated sodium salts (Formaker and Hill, 1988). This is expected if the main pathway for the phasic response is the lipid bilayer which is insensitive to amiloride. The effects of anesthetics on phasic response also support the importance of lipid bilayers. The phasic response to NaCl is preferentially suppressed by the action of anesthetics (Kashiwagura et al., 1976). Anesthetics increase the fluidity of lipid membranes (Vanderkooi et al., 1977; Janoff et al., 1981; Yoshida et al., 1983; Ueda et al., 1986). The increase in fluidity results in the increase in ion permeabilities that induces the diminution of phasic response as in Fig. 5. The present calculation showed that lipid bilayer produced the phasic response with the observed time scale of the order of seconds. Other transduction pathways give much larger or much smaller time constants. Heck et al. (1989) and Fidelman and Mierson (1989) have developed channel-pump models

based on simultaneous measurements of current across epithelium and the nerve response. The models simulated an intracellular depolarization and a subsequent repolarization in response to hyperosmotic NaCl observed by Ozeki (1971). The transient responses in their models arise from the accumulation and depletion of ions in taste cells due to the operation of channels and pumps. The time constants of the potential variation are of the order of several tens of seconds. The channel-pump models account for appropriately the gradual adaptation of tonic responses rather than the initial phasic response. Shunt pathway in the epithelium consists of tight junctions. Transient responses of tight junctions are estimated to be too fast to produce the phasic responses with the observed time scale. The permeabilities of alkaline and halogen ions across tight junctions are roughly 5×10^{-9} m/s per unit area of epithelial membrane (Fidelman and Mierson, 1989). Then the electric relaxation time is estimated using the method shown in Appendix A. The time obtained is of the order of milliseconds.

In most epithelia the paracellular pathway is the major route for ion transport. The highly permeable pathway may mask the effects of lipid bilayers in the apical membranes. The present study suggests, however, that the lipid bilayers operate in the taste response. The lipid bilayers participate in the taste transduction when the apparent permeability across lipid bilayer per unit area of epithelium is not too much smaller than that across the tight junction. This may occur when the area of the apical membrane is large enough. The situation is quite possible considering such complicated structures of the receptive part of taste cells as microvilli. Little is known about specific ion transport properties of lingual epithelia (Fidelman and Mierson, 1989). There may be still other possibilities that make the lipid bilayer functional. The observed magnitude of phasic response is much smaller than the calculated one. The latter is from several tens of millivolts to a hundred millivolts whereas the former is of the order of ten millivolts. This reduction of the magnitude would be due to the shunting effect of paracellular pathway.

The present model will also apply as a base for studying the functions of artificial gustatory sensors made of lipid bilayers. It was once a leading hypothesis (Price and DeSimone, 1977) that phospholipids were the primary salt receptors. A model system, consisting of artificial porous membranes impregnated with lipids, simulated the responses of taste cells to salt and acid (Kamo et al., 1974*a*,*b*), and to bitter substances (Okahata and En-na, 1987). The development of a taste sensing system utilizing the lipid membranes has been suggested as promising (Toko et al., 1981, 1986; Yoshikawa et al., 1984, 1985; Ishii et al., 1986).

The response patterns of the cells to salty stimuli depend on both species of cation and anion in the salt (Beidler, 1967; Akaike and Sato, 1976). In the present model, the cation dependence comes from the differences in both the association constant with the ionizable lipids and the permeability between cation species. It has been shown in the previous sections that dynamic response patterns vary quantitatively and in some cases qualitatively with the changes of cation permeability P_M and of the association constant K_M. Because we do not know the detailed values of P_M and K_M for each cation, the quantitative comparison of the calculated results with experiments remains as a future task.

The anion dependence of the response in the present model may be expected to arise from the difference in the anion permeability P_A. However, the magnitude of phasic response is insensitive to differences in P_A because the anion concentration at the membrane surface is usually quite low at the early stages of the stimulation. Although the anion dependence of the phasic depolarization of the potential has not been established experimentally, it may be expected because the observed neural bursts depend on anion species. DeSimone and Price (1976) reproduced, theoretically, the observed anion effect on the tonic response by taking into account the effect of recombination of anions with cations in the membrane surface region. This recombination effect may also induce the anion dependence of the phasic depolarization because the recombination occurs in a region a short distance from the membrane surface where there are appreciable amounts of anions. A large halogen flux may be expected if the halogen ions, transported to the membrane surface as electrically neutral molecules, permeate the membrane through their respective channels.

Kamo et al. (1980) considered that the dynamic response pattern reflected noticeably the time courses of the ion adsorption process and the conformation changes in the adsorption sites. We do not consider explicitly the effect of the time dependence of the adsorption process in the present model because the adsorption processes may occur more rapidly than the processes of stimulant flow and ion permeation across membranes. We assume that the adsorption reaction very quickly reaches a thermal equilibrium state before the latter processes finish.

We have not considered the conformation change of the model membrane which may be induced by the salt stimulation. Because the monovalent salts in the usual concentration range hardly change the temperature of the gel-liquid crystal phase transition in the lipid bilayer (Träuble and Eibl, 1974; MacDonald et al., 1976), it

seems reasonable that we neglect the effect of conformation change due to these salts. However, it is not the case for divalent salts such as $CaCl_2$ because the phase transition temperature increases with the adsorption of divalent cations. Changes in association constants of ions, permeabilities, and the dipole moment in polar heads may be induced by the conformation change. When the relaxation time of the conformation change becomes comparable with the time τ_s of the stimulating flow rate or the time τ_d of the charging process of membrane capacitance, the response pattern is affected in a qualitatively different manner by the process of conformation change.

The means by which we simulate the concentration change, i.e, Eq. 20, is artificial. A more realistic method would be a hydrodynamic approach (DeSimone and Heck, 1980) because ions are transported by convective diffusion toward the membrane surface. Increasing the flow rate (velocity) would decrease the thickness of the diffusion-boundary layer and would have the effect of more rapid stimulus delivery onto the surface. However, the treatment of hydrodynamics for general geometries in experimental setups is quite complicated. For this reason, we used an equation having the expected features of stimulus delivery to simulate the concentration change. Eq. 20 with $n \geq 2$ is a function of time which smoothly rises from zero and approaches its saturated value monotonically and smoothly similar to the general solution to the diffusion equation. The qualitative features of the results obtained using Eq. 20 would not differ from those obtained using the hydrodynamic approach.

APPENDIX A

Assumption of constant flux

Although the fluxes of ions are not always spatially constant in transient phenomena, they are reasonably assumed to be constant in membranes under the following conditions. When the diffusion of ions through a membrane is fast enough for the redistribution of ions to finish within a short time after a disturbance compared with the time scale of observation, the ion distribution in the membrane could be regarded as quasistationary and the fluxes could be regarded as spatially constant in transient phenomena in that time scale.

To see the condition explicitly, we rewrite the continuity equation for the ionic flow in a dimensionless form using the following scalings of variables.

$$\tilde{t} = t/t_e \tag{A1}$$

$$\tilde{x} = x/l \tag{A2}$$

$$\tilde{C}_\nu = C_\nu/C_\nu^{MI} \tag{A3}$$

$$\tilde{\Phi}_\nu = \Phi_\nu l/(D_\nu C_\nu^{MI}), \tag{A4}$$

where C_ν^{MI} is the concentration of the ion ν in the alkyl chain region at the boundary between the region and the polar head region and t_e is the electric relaxation time given by

$$t_e = \frac{\epsilon_m}{F \sum_\nu (F/RT) D_\nu C_\nu^{MI}}. \tag{A5}$$

The continuity equation in the membrane is then written as

$$\frac{\partial \tilde{\Phi}_\nu}{\partial \tilde{x}} = -\lambda_\nu \frac{\partial \tilde{C}_\nu}{\partial \tilde{t}}, \tag{A6}$$

where

$$\lambda_\nu = \frac{l^2}{D_\nu}\frac{1}{t_e} = \frac{l^2 F^2}{\epsilon_m RT}\frac{\sum_\mu D_\mu C_\mu^{MI}}{D_\nu}. \tag{A7}$$

The normalized concentration $\tilde{C}_\nu$ is a smooth function of time $\tilde{t}$ and the magnitude of $\tilde{C}_\nu$ is of the order of 1. Because we consider the variation of $\tilde{C}_\nu$ in the time scale of t_e, $\tilde{t}$ is also of the order of 1. Then it comes from Eq. A6 that if $\lambda_\nu \ll 1$, $\partial\tilde{\Phi}_\nu/\partial\tilde{x}$ is near zero; that is, the fluxes are treated as spatially constant in the membrane for measurements in the time scale of t_e. Because the quantity λ_ν is the ratio of diffusion time l^2/D_ν to the time scale t_e, the condition of $\lambda_\nu \ll 1$ is equivalent to that of the diffusion time being much shorter than the relevant scaling time.

We estimate the order of magnitude of λ_ν for the lipid bilayer membranes. The permeability P_ν is of the order of $\beta_\nu(D_\nu/l)$, where $\beta_\nu \equiv C_\nu^{MI}/C_\nu^{BI}$ is the net partition coefficient of ion ν between the bulk solution and the alkyl chain region. Then the order of magnitude of $D_\nu C_\nu^{MI}$ is $lP_\nu C_\nu^{BI}$ and, hence, t_e is of the order of $\epsilon_m RT/(F^2 l \sum_\nu P_\nu C_\nu^{BI})$. For $\epsilon_m = 2.4\,\epsilon_0 = 2.1 \times 10^{-11}$ F/m, $l = 50$ Å, $P_H \simeq 10^{-6}$ m/s, $C_H^{BI} \simeq 10^{-7}$ M $= 10^{-4}$ mol/m^3, $P_M \simeq 10^{-12}$ m/s, and $C_M^{BI} \simeq 0.1$ M $= 100$ mol/m^3, t_e is of the order of seconds. This is the same order as the time scale of change in the membrane potential upon chemical reception (Sato, 1980). Substituting the above values into Eq. A7, we obtain $\lambda_\nu \sim 10^{-18}/D_\nu$. Then, λ_ν is much smaller than unity if $D_\nu \gg 10^{-18}$ m^2/s. The values of D_ν in the alkyl chain region have not been reported. However, the condition of $D_\nu \gg 10^{-18}$ m^2/s is highly probable from the following estimation. When we adopt the value 10^{-3} for the partition coefficient β_H of proton, which is the value of the partition coefficient of a water molecule between water and oil (Collander, 1949), we obtain 5×10^{-12} m^2/s for the value of D_H. D_H in water is of the order of 10^{-9} m^2/s.

The parameter λ_ν is equivalent to the parameter α^{-2} of MacGillivray and Hare (1969). They showed that the constant field assumption holds for $\alpha^{-2} \ll 1$. Because it has been shown (Ohki, 1985) that Goldman's constant field assumption holds in many biological membranes, the condition of $\alpha^{-2} \ll 1$, that is, $\lambda_\nu \ll 1$, may be reasonable for many biomembranes.

APPENDIX B

Assumption of equilibrium in the double layer and in the polar head region

We dealt with nonequilibrium phenomena in which each ion flux is not always zero. However, it is shown below that the ion distribution in the electric double layer is given in a good approximation by a thermal equilibrium state in the time scales of τ_s and/or τ_d. First, the leak of ions from the double layer region is negligible. This is because the ion concentration and the diffusion constant in the membrane are 1,000 times smaller than the relevant quantities in the solution region as

estimated in Appendix A. Second, the ion redistribution process in the double layer region occurs much more quickly than the disturbance of ion distribution in the region induced by the salt stimulation. The relevant times for the redistribution process are the ion diffusion time, $t_d = F\delta^2/(\mu RT)$, across the layer and the electric relaxation time, $t_e = \epsilon_w/(F\mu C_\nu)$, in the layer. Here, δ is the thickness of the double layer and is ~ 3 nm, μ is the mobility of ions in water and is of the order of 10^{-7} m^2/V · s. The values of t_d and t_e are estimated to be 3.5 and 7 ns for the ion concentration C_ν of 10 mM. These values are incomparably shorter than the time scale τ_s (~ 1 s) of the stimulation process.

Although we do not have any detailed information about the ion concentration and the ion mobility in the polar head region, it seems reasonable that the values of these quantities are between the values in the hydrophobic region and those in the solution. Then, the same consideration as for the double layer holds for the polar head region.

APPENDIX C

Meaning of the ion permeability P_ν

The ion permeability P_ν^{ex} usually defined in the experiments is obtained through the relation

$$\Phi_\nu = -P_\nu^{ex}(C_\nu^{BE} - C_\nu^{BI}) \qquad \text{(C1)}$$

under the condition that the electrostatic potential difference ϕ_m between the external and internal solutions is zero. When $\phi_m = 0$, the flux Φ_ν in the present formulation is expressed using Eqs. 7 and 9 and the relation of $\phi_d = \phi_s^E - \phi_s^I$ as

$$\Phi_\nu = -P_\nu \frac{z_\nu F(\phi_s^E - \phi_s^I)/RT}{\exp(z_\nu F\phi_s^E/RT) - \exp(z_\nu F\phi_s^I/RT)} \cdot (C_\nu^{BE} - C_\nu^{BI}). \qquad \text{(C2)}$$

It is seen comparing Eq. C2 with Eq. C1 that P_ν is almost equal to P_ν^{ex} when $|z_\nu F\phi_s^x/RT|$ ($X = I, E$) are less than unity, i.e., $|\phi_s^x|$ is < 10 mV. Then, we can estimate the values of P_ν from the observed values of P_ν^{ex}. The experimental values of the membranes of zwitterionic lipids or the membranes including acidic lipids with small ratios are useful in the estimation of P_ν.

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