Mechanism of the Electric Response of Lipid Bilayers to Bitter Substances

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ABSTRACT In order to clarify by what mechanism the lipid bilayer membrane changes its potential under the stimulation of bitter substances, a microscopic model for the effects of the substances on the membrane is presented and studied theoretically. It is assumed that the substances are adsorbed on the membrane and change the partition coefficients of ions between the membrane and the stimulation solution, the dipole orientation in the polar head, and the diffusion constants of ions in the membrane. It is shown, based on the comparison of the calculated results with the experimental ones, that the response arises mainly from a change in the partition coefficients. Protons play an essential role in the membrane potential variation due to the change in their partition coefficients. The present model reproduces the following observed unique properties in the response of lipid bilayers to bitter substances, which cannot be accounted for by the usual channel model for the membrane potential: 1) the response of the membrane potential appears even under the condition that there is no ion gradient across the membrane, 2) the response remains even when the salt in the stimulating solution is replaced with the salt made of an impermeable cation, and 3) the direction of the polarization of the potential is not reversed, even when the ion gradient across the bilayer is reversed.

INTRODUCTION

There are a large diversity of chemicals that have a bitter taste, including artificial ones. Therefore, more than one transduction pathway would be expected for the bitter taste. Ozeki (1971) attributed the depolarization of the receptor potential by quinine to a blockage of K^+ channels in the taste cells. Okada et al. (1988) proposed that quinine activated an active secretion of Cl^- through the apical membrane. Voltage-gated Na^+ channels and K^+ channels were found in the cells, and it has been shown that quinine blocks, at least in part, these channels producing the depolarization of cell potential (Avenet and Lindemann, 1987; Kinnamon and Roper, 1988). Akabas et al. (1988) found that denatonium, which is a nonpermeant bitter agent, caused the release of Ca^{2+} from intracellular stores in some cells and suggested a receptor-second messenger mechanism.

Whereas all of the above pathways are related to the receptor or channel proteins, it often has been suggested that the lipid bilayer in the apical membrane takes part in the bitter transduction (Kurihara, 1973; Kumazawa et al., 1985; Kurihara et al., 1986; Kumazawa et al., 1988). The membrane potential of planar lipid bilayers and liposomes are depolarized by the application of various bitter substances (Kumazawa et al., 1988). It also has been shown that the potential across a synthetic lipid multibilayer is hyperpolarized by bitter substances (Okahata and En-na, 1987). There exists a good correlation between the minimum concentrations of various bitter substances required to depolarize the membrane potential of lipid bilayers and those concentrations required to elicit bitter taste in humans (Kumazawa et al., 1988).

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In this paper, we study theoretically the mechanism of the electric response of lipid bilayers to bitter substances. To obtain a comprehensive understanding of the taste transduction mechanism, a system model that includes various possible pathways is necessary. Then, it is quite important to investigate the characteristics of each of the main elements in the transduction system in detail to make the system model a practical one. The lipid bilayer is one such element.

In addition, the lipid bilayer has interesting properties by itself. It has been found experimentally that the responses of the lipid bilayers to the bitter stimuli have the following unique properties, which cannot be accounted for by the usual ion channel model for membrane potential: 1) the response of the membrane potential appears even under the condition that there is no ion gradient across the membrane (Kumazawa et al., 1988), 2) the response remains even when the salt in the stimulating solution is replaced with the salt made of an impermeable cation, and then the response becomes rather larger (Kumazawa et al., 1988), and 3) the direction of the polarization of the potential is not reversed, even when the ion gradient across the multibilayer is reversed (Okahata and En-na, 1987). The membrane potential usually is described by the ion permeation, as in the widely accepted Goldman-Hodgkin-Katz model (Goldman, 1943; Hodgkin and Katz, 1949), for the potential induced by ion channels. All the above properties conflict with the predictions based on the channel model.

The purpose of this paper is to show by what mechanism these unique properties of the responses of lipid membranes to bitter stimuli are realized. Some phenomenological predictions have been made already for the mechanism. Kumazawa et al. (1988) proposed that the adsorption of bitter substances on the lipid membranes induced the conformation change in the membranes and, as a result, changed the phase boundary potential. Okahata and En-na (1987) suggested,

based on the analysis of their experimental results, that the bitter substances were adsorbed near the surface of the membrane, rather than penetrating the membrane. However, the microscopic mechanism through which the adsorption of the substances induces a change in the membrane potential has not yet been clarified.

To obtain a better understanding of the microscopic mechanism of the potential change due to the bitter substances, we investigated theoretically the effect of the adsorption on the potential. The bitter substances may be adsorbed on the surface region of the membrane or, may be adsorbed on the deep, inner region of the membrane. We have calculated the potential changes induced by the adsorption under various conditions. We assume, in the present model, that the adsorption of the bitter substances on the surface region induces both a change in the magnitude of the partition coefficient of ions between the membrane and the stimulation solution, and a change in the orientation of electric dipoles of the membrane surface at the side where the stimuli are applied. Furthermore, we assume that the adsorption of the substances on the inner region changes the magnitude of diffusion constants of ions in the membrane.

We show, based on the comparison of the calculated results with the experimental ones, that the response of the lipid bilayers to the bitter stimuli arises mainly from a change in the partition coefficients. Protons play an essential role in the potential variation because of the change in their partition coefficients. The present model has reproduced definitively the observed unique properties of the responses of lipid membranes to the bitter stimuli. The model also explains consistently other properties, such as the hyperpolarization of uncharged membranes by some bitter substances.

THEORETICAL MODEL

Model for membrane potential change

We consider a model system in which a lipid bilayer membrane is in aqueous solution divided into two regions, as shown in Fig. 1. We refer to the region where stimuli are applied as the *cis* side, and to the other region as the *trans* side. The lipid bilayers considered are a mixture of twitterionic and ionizable lipid molecules, and their mixing ratio in the monolayer on the *cis* side is equal to that in the monolayer on the *trans* side. We consider a model bilayer membrane whose surface layers include both electric charges and dipoles, and in which their distributions are smoothed over the entire membrane surface.

We investigate how the membrane potential of this model system is varied by the adsorption of bitter substances on the hydrophobic region near the membrane surface on the *cis* side and/or by the penetration of bitter substances into the membrane. We assume that the substances adsorbed on the surface region change the magnitude of ion partition coefficients between the membrane and the solution on the *cis* side and the orientation of dipoles, as shown in Fig. 1. We also assume that the substances penetrated and adsorbed on the inner region of the membrane change the magnitude of diffusion constants of ions in the membrane. We neglect the slight influence of the adsorption on the properties of the opposite (*trans*) layer.

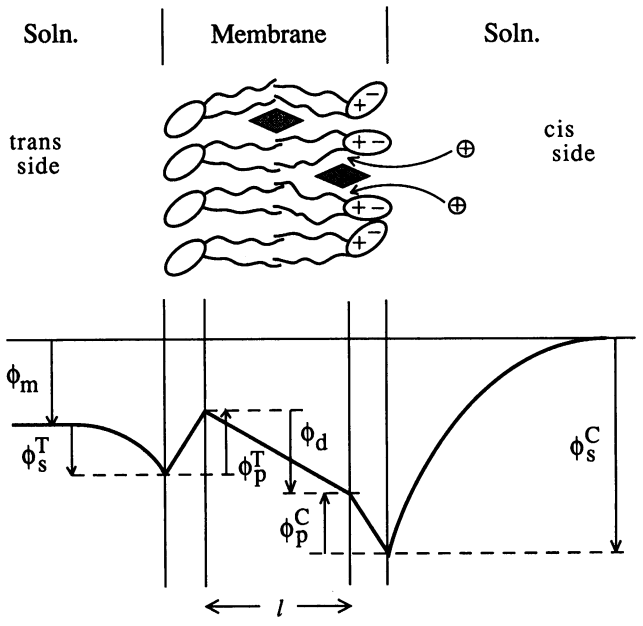

FIGURE 1 Model for the reception of bitter substances on a lipid bilayer membrane and the diagram of membrane potential. l, thickness of alkyl chain region; ϕ_m, membrane potential; ϕ_s^T and ϕ_s^C, surface potentials; ϕ_p^T and ϕ_p^C, potential differences due to dipoles in polar heads.

The membrane potential ϕ_m is defined as the potential difference between the two bulk solutions across the membrane, and is obtained by

$$\phi_m = \phi_s^C + \phi_p^C - \phi_d - \phi_p^T - \phi_s^T \qquad (1)$$

as shown in Fig. 1. Here, ϕ_s^C and ϕ_s^T are the surface potentials on the *cis* and *trans* sides, respectively, and are produced by the membrane surface charges. The potentials ϕ_p^C and ϕ_p^T arise from the dipoles at the relevant surfaces. The diffusion potential ϕ_d is the potential difference across the alkyl chain region due to the permeation of ions through the region.

The ion species considered in the solutions are a single kind of alkaline ion M^+ and a single kind of halogen ion A^-, besides proton H^+ and hydroxide ion OH^-. In the *cis* side solution, a single kind of impermeable cation I^+, such as choline$^+$ and Bis-Tris$^+$, is also considered when we investigate the effects of ionic environments. The cations are adsorbed on the negatively charged heads of ionizable lipids and modify the surface charge densities of the membrane. The ions hardly are adsorbed on the heads of the lipids with electric dipole.

Basic equations for each component potential

We derive basic equations that determine the five potentials in Eq. 1. We consider the potentials only in stationary states of the lipid bilayer system, because we can assume that the observed values of the potential are those for the stationary states, as discussed in the Appendix.

The dipole potential on the *cis* side, ϕ_p^C, changes when the orientation of dipoles is changed by the adsorption of

bitter substances in the surface region, whereas the dipole potential on the *trans* side, ϕ_p^T, does not. These potentials are expressed as

$$\phi_p^T = \phi_{p0} \tag{2}$$

and

$$\phi_p^C = \phi_{p0} + \psi_p, \tag{3}$$

where ϕ_{p0} is the dipole potential in the case of no adsorption, and ψ_p denotes the change in dipole potential on the *cis* side because of the adsorption. When there is no adsorption, both ϕ_p^T and ϕ_p^C have the same value, ϕ_{p0}, because we consider only the case where the mixing ratio of twitterionic and ionizable lipid molecules in the monolayer on the *cis* side is equal to that in the monolayer on the *trans* side.

The diffusion potential ϕ_d is derived from the condition that the electric current flowing through the membrane is zero for an open circuit condition:

$$F \sum_\nu z_\nu \Phi_\nu = 0, \tag{4}$$

where the symbol ν stands for permeable ion species (ν = H for proton, OH for hydroxide ion, M for alkaline ion, and A for halogen ion), Φ_ν is the flux of the ion ν, z_ν is its valency, and F is the Faraday constant. Using Goldman's approximation, we express the flux Φ_ν as

$$\Phi_\nu = -P_\nu(1 + \theta_\nu) z_\nu \beta \phi_d \times \frac{C_\nu^{ST} - (1 + \delta_\nu)\exp(-z_\nu\beta\psi_p)\exp(z_\nu\beta\phi_d)C_\nu^{SC}}{1 - \exp(z_\nu\beta\phi_d)}, \tag{5}$$

where

$$P_\nu = \alpha_{\nu0}(D_{\nu0}/l)\exp(-z_\nu\beta\phi_{p0}). \tag{6}$$

Here, C_ν^{SX} (X = T or C) is the surface concentration of ion ν in the solution on the X side, β denotes F/RT, P_ν corresponds to the permeability of ion ν (Naito et al., 1991), $D_{\nu0}$ is the diffusion constant of ion ν in the alkyl chain region in the case where there is no penetration of bitter substances into the region, l is the thickness of alkyl chain region, and $\alpha_{\nu0}$ is the partition coefficient of ion ν between the solution and the alkyl chain region, in the case where the surface potentials ϕ_s^X and the dipole potentials ϕ_p^X (X = T, C) are zero and there is no bitter stimulation. The partition coefficient $\alpha_{\nu0}$ on both sides of the membrane are equivalent. The quantity θ_ν is the relative change in the diffusion constant due to the adsorption of the bitter substances on the inner region of the membrane. The diffusion constant is changed by the stimulation from $D_{\nu0}$ to

$$D_\nu = D_{\nu0}(1 + \theta_\nu). \tag{7}$$

The quantity δ_ν is the relative change in the partition coefficient due to the adsorption of bitter substances on the hydrophobic region near the surface. The partition coefficient on the *cis* side is changed from $\alpha_{\nu0}$ to

$$\alpha_\nu^C = \alpha_{\nu0}(1 + \delta_\nu), \tag{8}$$

when the bitter stimulation is applied to the membrane surface on the *cis* side. The partition coefficient on the *trans* side is not changed by the stimulation.

Substituting Eq. 5 into Eq. 4, we obtain the diffusion potential ϕ_d as

$$\phi_d = \frac{1}{\beta}\ln\frac{\Sigma_\xi(1 + \theta_\xi)P_\xi C_\xi^{ST} + \Sigma_\eta(1 + \theta_\eta)(1 + \delta_\eta)\exp(\beta\psi_p)P_\eta C_\eta^{SC}}{\Sigma_\xi(1 + \theta_\xi)(1 + \delta_\xi)\exp(-\beta\psi_p)P_\xi C_\xi^{SC} + \Sigma_\eta(1 + \theta_\eta)P_\eta C_\eta^{ST}}. \tag{9}$$

Here, ξ denotes cation species and η denotes anion species. The surface concentrations, C_ν^{SX}, are expressed in terms of the ion concentrations, C_ν^{BX}, in the bulk solution based on the Boltzmann distribution, which is determined by the surface potentials, ϕ_s^X, as

$$C_\nu^{SX} = C_\nu^{BX}\exp(-z_\nu\beta\phi_s^X). \tag{10}$$

The equation determining the surface potential is obtained using Gauss' law in the polar head region. The contribution from the electric field in the alkyl chain region can be neglected because the dielectric constant of the alkyl chain region is much smaller than that of the aqueous solution (Naito et al., 1991). The electric field in the solution at the membrane surface is expressed in terms of the surface potential by using the Gouy-Chapman theory, that is, by solving the Poisson-Boltzmann equation in the electric double layer. Gauss' law leads to the equation for the surface potential.

$$\left\{2\epsilon_w RT \sum_\nu C_\nu^{BX}[\exp(-z_\nu\beta\phi_s^X) - 1]\right\}^{1/2} = -\sigma^X \tag{11}$$

Here, σ^X is the charge density in the polar head region on the X side, and is expressed in terms of ϕ_s^X, by using the Langmuir isotherm for the adsorption probabilities of cations on the ionized polar heads as

$$\sigma^X = \frac{-eN_0\rho}{1 + \Sigma_\xi K_\xi \exp(-\beta\phi_s^X)C_\xi^{BX}/10^3}, \tag{12}$$

where N_0 is the areal density of lipid molecules in the membrane, ρ is the fraction of ionizable lipids, K_ξ (ξ = H, M, or I) is the association constant (in M^{-1}) between the ion ξ and the lipid in the adsorption reaction. The symbol I stands for impermeable cation I^+. The summation with ξ is taken over the cation species. The fractions of ionizable lipids are the same on both sides of the bilayer.

The membrane potential ϕ_m is obtained from Eqs. 1, 2, 3, 9, and 10 as

$$\phi_m = \frac{1}{\beta}\ln\frac{\Sigma'_\xi(1 + \theta_\xi)(1 + \delta_\xi)P_\xi C_\xi^{BC} + b\exp(\beta\psi_p)\Sigma_\eta(1 + \theta_\eta)P_\eta C_\eta^{BT}}{\Sigma'_\xi(1 + \theta_\xi)P_\xi C_\xi^{BT} + b\exp(\beta\psi_p)\Sigma_\eta(1 + \theta_\eta)(1 + \delta_\eta)P_\eta C_\eta^{BC}}, \tag{13}$$

where b denotes $\exp[\beta(\phi_s^T + \phi_s^C)]$. The summation with ξ is taken over permeable cations (ξ = H, M) and the summation with η is taken over permeable anions (η = OH, A). The membrane potential in the case where the *cis* side solution contains the salt made of impermeable cation also is calculated by using Eq. 13 for $C_M^{BC} = 0$.

Membrane resistance

The electric resistance of the membrane is well approximated with the slope resistance $\partial\phi_m/\partial J$, where $J = F\sum_\nu z_\nu\Phi_\nu$ is the ionic current (Naito et al., 1991). In the present model, the resistance of the membrane is expressed by

$$R = \frac{R_1 - R_2}{\ln(R_1/R_2)}, \tag{14}$$

where

$$R_1 = \frac{1}{\beta}\frac{1}{F[P_H^C C_H^{SC} + P_M^C C_M^{SC} + P_{OH}^T C_{OH}^{ST} + P_A^T C_A^{ST}]} \tag{15}$$

$$R_2 = \frac{1}{\beta}\frac{1}{F[P_H^T C_H^{ST} + P_M^T C_M^{ST} + P_{OH}^C C_{OH}^{SC} + P_A^C C_A^{SC}]} \tag{16}$$

and

$$P_\nu^C = (1 + \theta_\nu)(1 + \delta_\nu)\exp(-z_\nu\beta\psi_p)P_\nu \tag{17}$$

$$P_\nu^T = (1 + \theta_\nu)P_\nu \tag{18}$$

The surface concentrations of ions in Eqs. 15 and 16 are given by Eq. 10. When the *cis* side solution contains the salt made of impermeable cation, the membrane resistance is calculated by setting $C_M^{SC} = 0$ in Eq. 15.

Values of the parameters

We adopted the following values for the parameters in the present model. The area density of lipids is $N_0 = 2 \times 10^{18}$ m^{-2}. The fraction ρ of the ionizable lipids is taken in the range 0 to 0.2. The dielectric constants of the solution is $\epsilon_w = 78$ ϵ_0, where ϵ_0 is that of a vacuum. The values of association constants of proton and alkaline ion with the ionizable lipids are $K_H = 2000$ M^{-1} and $K_M = 0.6$ M^{-1}, respectively, where the value of K_M corresponds to that for Na^+ binding to phosphatidylserine (Ohki and Kurland, 1981). We assumed that impermeable cations were not adsorbed on the lipid molecules and set $K_I = 0$. The values of permeabilities for permeable ions have been published in a variety of sources for lipid liposomes. The values vary in the range from 10^{-12} to 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; Nicols 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 to 100 times the value for alkaline ions. We took the values of P_M and P_H in the above ranges, and set $P_{OH} = P_H$ and $P_A = 10\,P_M$. We specially considered the following three cases: 1) $P_H = 3 \times 10^{-9}$ m/s, $P_M = 3 \times 10^{-14}$ m/s ($P_M/P_H = 10^{-5}$), 2) $P_H = 2 \times 10^{-8}$ m/s, $P_M = 2 \times 10^{-14}$ m/s ($P_M/P_H = 10^{-6}$), and 3) $P_H = 4 \times 10^{-8}$ m/s, $P_M = 4 \times 10^{-16}$ m/s ($P_M/P_H = 10^{-8}$). The resistances calculated using Eq. 14 were 9.2 $K\Omega m^2$ in case 1), 8.1 $K\Omega m^2$ in case 2), and 8.6 $K\Omega m^2$ in case 3), for the fraction ρ of 0.2 and the salt concentration of 0.1 M in both *trans* and *cis* side solutions. These values are comparable to the resistance obtained in the experiment on soybean phospholipid (azolectin) bilayer membranes (Kumazawa et al., 1988).

The following standard values of the parameters are used, if not stated otherwise. The fraction ρ of the ionizable lipids is 0.2, and the pH of the bulk solution is 7 on both *trans* and *cis* sides. Permeable ions M^+ and A^- are used in the calculation, if not stated otherwise. All of the calculations were carried out at 25°C.

CALCULATED RESULTS

We have considered the three effects that the bitter substances may have on the properties of lipid bilayer membranes: 1) change in the partition coefficients of ions, 2) change in the dipole potential, and 3) change in the diffusion constants of ions. We investigated which change or changes are essential for the realization of the response properties mentioned in the Introduction.

Response in the symmetric condition

The magnitude V_m of the response of membrane potential to the bitter stimuli is defined as the difference of the potential under stimulation from the resting potential. The resting potential is obtained by setting $\theta_\nu = 0$, $\delta_\nu = 0$, and $\psi_p = 0$ in Eq. 13. When the solutions on both *trans* and *cis* sides are symmetric, V_m is given by the right-hand side of Eq. 13 with $C_\nu^{BT} = C_\nu^{BC}$ because the resting potential is zero. If all of δ_ν values are zero, that is, if there are no changes in the partition coefficients of ions, it is clear from Eq. 13 that the response V_m is zero; that is, no response appears irrespective of the values of θ_ν and ψ_p. Therefore, changes in the partition coefficients are necessary to obtain the finite response of membrane potential in the symmetric condition.

The physical reason why the response does not appear due to the changes in diffusion constants of ions and/or dipole potential is as follows. First, the variation in diffusion constant of ion ν changes the flux of the ion from *trans* to *cis* sides, and from *cis* to *trans* sides by the same ratio. Then, the diffusion potential does not change if the ion concentrations in *trans* and *cis* side solutions are equivalent. Note that Eq. 13 essentially is equivalent to the Goldman-Hodgkin-Katz equation when only the diffusion constants are varied ($\theta_\nu \neq 0$; $\delta_\nu = 0$; $\psi_p = 0$). Second, when the dipole potential ϕ_p^C is changed, the potential at the interface between the polar head region and the hydrophobic region of the membrane changes at the *cis* side. The ion concentration at the interface then changes and, as a result, the diffusion potential also changes.

The change in the diffusion potential tends to cancel the change in the dipole potential, and under the symmetric condition, the canceling is complete.

The condition under which the membrane potential depolarizes is obtained from Eq. 13. In the case where the membrane has net negative surface charges, the contribution from the anion flux is neglected, and we obtain the condition for the depolarization,

$$\delta_H(1 + \theta_H)P_H C_H^B + \delta_M(1 + \theta_M)P_M C_M^B > 0 \quad (19)$$

where $C_H^B = C_H^{BC} = C_H^{BT}$ and $C_M^B = C_M^{BC} = C_M^{BT}$. It is seen from this equation that the membrane depolarizes when δ_H and/or δ_M is positive. This arises from the fact that the amount of cations penetrating the membrane from the *cis* side is increased, and the cation flux from the *cis* side to the *trans* side also increases.

As seen from Eq. 13, the depolarization increases monotonically with δ_H and δ_M. The magnitude of the depolarization is affected by the relative changes, θ_ν, of the diffusion constants when the constants change with the partition coefficients. The dependence of the response V_m on θ_H is represented, by using Eq. 13, in the case where the contribution of anions is negligible as a functional form

$$\frac{\partial V_m}{\partial \theta_H} = A(\theta_H, \theta_M, \delta_H, \delta_M) \cdot (\delta_H - \delta_M), \quad (20)$$

where $A(\theta_H, \theta_M, \delta_H, \delta_M)$ is a positive function of θ_H, θ_M, δ_H, and δ_M. When $\delta_H > \delta_M$, V_m increases with θ_H ($\partial V_m/\partial\theta_H > 0$). When $\delta_H < \delta_M$, V_m decreases with increasing θ_H ($\partial V_m/\partial\theta_H < 0$). The dependence of V_m on θ_M also is estimated from the function

$$\frac{\partial V_m}{\partial \theta_M} = -B(\theta_H, \theta_M, \delta_H, \delta_M) \cdot (\delta_H - \delta_M), \quad (21)$$

where $B(\theta_H, \theta_M, \delta_H, \delta_M)$ is a positive function. V_M increases with θ_M when $\delta_H < \delta_M$ and decreases for $\delta_H > \delta_M$. We will show the calculated results relevant to these considerations in the next subsection.

Effects of ion permeation

We discuss here the effects of ion permeation on the lipid bilayer response, comparing the case where the salt in the *cis* side solution is made of impermeable cation, with the case where it is made of permeable cation.

We consider the cases where the membrane has a net negative surface charge. We set $\psi_p = 0$, $\theta_\eta = 0$, and $\delta_\eta = 0$ in Eq. 13 because the electric potential of negatively charged membranes is quite insensitive to the values of ψ_p, θ_η, and δ_η, as long as they are not very large. This comes from the factor $b = \exp[\beta(\phi_s^T + \phi_s^C)]$ in Eq. 13, which is much smaller than 1.

Fig. 2 shows the calculated response magnitude of the membrane potential as functions of the relative changes in the partition coefficients, δ_H and δ_M, of H^+ and M^+, and the relative change θ_H in the diffusion coefficient of the proton. The relative change θ_M for M^+ is −0.5, and the ratio P_M/P_H of permeabilities is 10^{-6}. Both *trans* and *cis* side solutions contain the same concentration of 0.1 M of salt. Fig. 3 shows the response potential as functions of the relative changes δ_H and δ_M and the permeability ratio P_M/P_H for the cases where there are no relative changes ($\theta_\xi = 0$) in the diffusion constants of ions. As seen in the figures, the response of the membrane potential appears even in the cases where the salt in the *cis* side solution is made of the impermeable cation, except for case *b* shown in Fig. 3. Here, the values of δ_ξ and θ_ξ were chosen such that the calculated potential response was around 10 mV, as in the experiment on lipid bilayers (Kumazawa et al., 1988). Incidentally, the depolarization of the receptor potential induced by the bitter stimulation is also around 10 mV (Okada et al., 1988). Although the receptor potential is measured across the basolateral membrane, the magnitude of the potential response is nearly the same, both in the basolateral and in the apical membranes (Kashimori et al., manuscript in preparation).

In the case where the salt in the *cis* side solution is IA, the condition for the depolarization is derived from the difference of ϕ_m, with $C_M^{BC} = 0$, between the stimulating state and

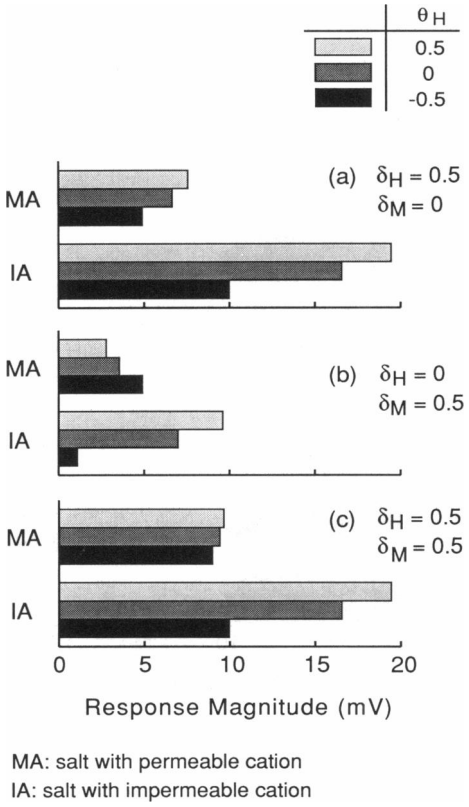

FIGURE 2 Calculated response of the membrane potential as a function of the relative changes δ_H and δ_M in the cation partition coefficients and the relative change θ_H in the diffusion constant of the proton. Solutions are symmetric and contain 0.1 M of salt. pH is 7. $\rho = 0.2$, $\psi_p = 0$, $\delta_{OH} = \delta_A = 0$, $\theta_M = -0.5$, $\theta_{OH} = \theta_A = 0$, and $P_M/P_H = 10^{-6}$.

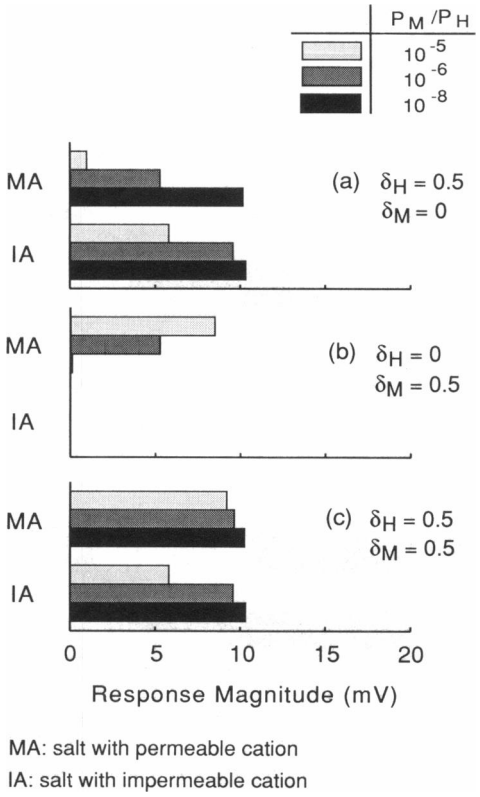

FIGURE 3 Calculated magnitude of the change in membrane potential induced by the bitter stimulation as a function of the relative changes δ_H and δ_M in partition coefficients of cations and the permeability ratio P_M/P_H. Solutions are symmetric and contain 0.1 M of salt. pH is 7. $\rho = 0.2$, $\psi_p = 0$, $\delta_{OH} = \delta_A = 0$, $\theta_H = \theta_M = \theta_{OH} = \theta_A = 0$.

the resting state ($\theta_\xi = \delta_\xi = 0$) as

$$\delta_H(1 + \theta_H)P_H C_H^B + [\theta_H - \theta_M + \delta_H(1 + \theta_H)]P_M C_M^{BT} > 0. \quad (22)$$

No response in the membrane potential appears in case *b* in Fig. 3, where only the partition coefficient of M^+ is changed because the inequality (Eq. 22) is not satisfied for $\delta_H = \theta_H = \theta_M = 0$. The physical reason is that there are very few M^+ ions responsive to the change δ_M in the partition coefficient of M^+ on the *cis* side. On the other hand, the response appears for $\delta_H \neq 0$ in the aqueous solution with pH 7, as shown in Fig. 3, because there are enough protons responsive to the change δ_H in the partition coefficient.

The depolarization response increases with θ_H in the case where the salt is IA, as shown in Fig. 2. This comes from the fact that the increasing rate of the total ion influx, because of the increase in θ_H, is larger than that of the total ion efflux when the salt is IA. The influx of cations consists only of the H^+ flux, whereas the M^+ flux, as well as the H^+ flux, contributes to the efflux. As a result, the increase in θ_H affects the whole influx, but affects only part of the efflux. The effect of the change θ_M in the diffusion constant of M^+ also can be estimated based on a similar consideration. The depolarization response decreases with increasing θ_M because the influx is not changed with θ_M; however, the efflux increases with θ_M.

In the case where the salt in the *cis* side solution is MA, the dependence of the response on θ_H is a little more complex because the influx also consists both of H^+ flux and M^+ flux. Two types of dependence appear: that is, the response increasing with θ_H (*a* in Fig. 2), and that which decreases with increasing θ_H (*b* in Fig. 2). This dependence is equivalent to that estimated from Eq. 20.

We consider here the dependence of the potential response on the permeability ratio P_M/P_H. In the case where the salt is MA, it is seen from *a* and *b* in Fig. 3 that the response due to δ_H increases, and the response due to δ_M decreases, as the ratio is decreased. This is reasonable because the smaller P_M/P_H is, the larger the contribution of H^+ to the membrane potential becomes, and the contribution of M^+ becomes smaller.

It has been shown (Kumazawa et al., 1988) that the potential responses of azolectin bilayers to bitter substances are larger by 20–30% for impermeable cations, such as bis-Tris$^+$ and choline$^+$ added in the *cis* side solution, than the responses for permeable cations, such as Na^+ and K^+. It is seen in Figs. 2 and 3 that there are several cases in the present calculation where the response of the membrane system containing the salt IA is larger than that in the system containing MA. We explain the reason by considering case *a* in Fig. 3 as an example. When the salt is IA, the cation influx contributing to the potential consists dominantly of the proton flux. Then, almost the entire flux is changed by the variation δ_H in the partition coefficient of H^+ induced by the bitter stimulation. On the other hand, when the salt is MA, there are two kinds of cation influx (M^+ and H^+). Only the partial flux (H^+ flux) is changed by the stimulation. As a result, the response in the case for IA becomes larger than that in the case for MA. However, the difference of the response between the two cases decreases as the permeability ratio P_M/P_H is decreased because the H^+ flux becomes dominant as the ratio is decreased, even in the case of MA.

We obtain the condition that the depolarization becomes larger for IA than for MA from Eq. 13. Because the anion flux is negligibly small in the negatively charged membranes, the condition becomes

$$(1 + \theta_H)(1 + \delta_H) > (1 + \theta_M)(1 + \delta_M). \quad (23)$$

This condition means that the change in the influx of H^+ is larger than that of M^+.

Effect of reversing the ion gradient

Okahata and En-na (1987) found in their synthetic lipid multibilayer that the direction of the polarization of the membrane potential is not reversed even when the concentrations of salt in *trans* and *cis* side solutions are reversed. We calculated the potential response for the reversed concentration of salt, and Fig. 4 shows the results. We set the values of θ_η,

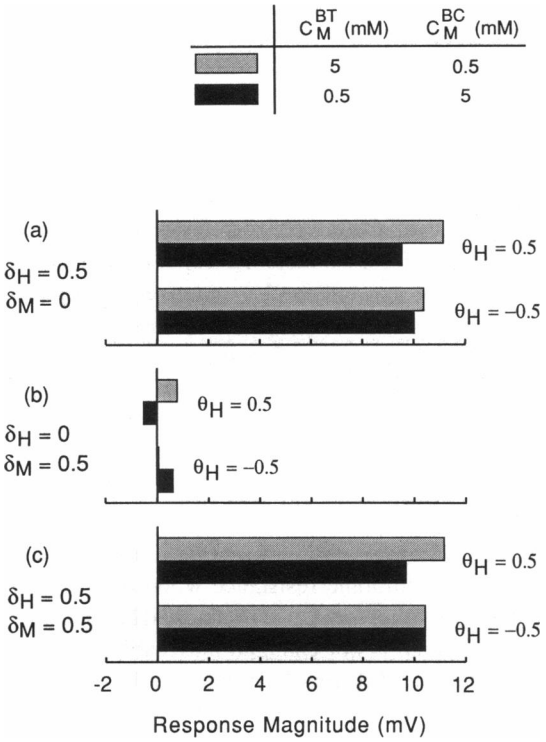

FIGURE 4 Comparison of the direction of polarization in the case where the salt concentration in *cis* side solution is higher than that in *trans* side solution, with the polarization direction in the case where the salt concentration in *trans* side is higher than that in *cis* side. pH is 7 in both *trans* and *cis* sides. $\rho = 0.2$, $P_M/P_H = 10^{-6}$, $\psi_p = 0$, $\delta_{OH} = \delta_A = 0$, $\theta_M = -0.5$, and $\theta_{OH} = \theta_A = 0$.

δ_η, and ψ_p as zero because we treat the negatively charged membranes and the potential as not sensitive to the values. As seen from the figure, the response is the depolarization for both directions of ion gradient when the partition coefficient of H^+ is changed; that is, $\delta_H > 0$ (*a* and *c* in Fig. 4). When $\delta_H = 0$ and $\delta_M > 0$, on the other hand, the membrane potential may hyperpolarize in the case where the salt concentration in the *trans* side solution is lower than that in the *cis* side solution (*b* in Fig. 4).

From Eq. 13 we obtain the condition that the potential of the negatively charged membrane depolarizes. When the salt concentration in the *cis* side solution is very low, that is, $C_M^{BC} \ll C_M^{BT}$ and $P_M C_M^{BC} \ll P_H C_H^B$, the condition becomes the same as the condition (Eq. 22) for the salt IA ($C_M^{BC} = 0$). The condition always should hold because, experimentally, the membrane depolarizes in the cases where the salt is IA. When the salt concentration in the *trans* side solution is very low, that is, $C_M^{BT} \ll C_M^{BC}$ and $P_M C_M^{BT} \ll P_H C_H^B$, the condition is given by

$$\delta_H(1 + \theta_H)P_H C_B^H + [(1 + \theta_M)(1 + \delta_M) - (1 + \theta_H)]P_M C_M^{BC} > 0 \quad (24)$$

This condition is satisfied for the case of $\theta_H = -0.5$ in Fig. 4*b*, while it is not satisfied for $\theta_H = 0.5$. The condition (Eq. 24) becomes more acceptable as δ_H increases. Then, the membrane depolarizes regardless of the direction of the ion gradient. The physical reason is that the effect of an increase in δ_H on the proton current is equivalent to that of increase in the proton concentration in the *cis* side solution for both directions of salt ion gradient. Incidentally, Okahata and En-na (1987) observed the effect of reversing the ion gradient in the positively charged synthetic lipid layers. The essential factor is the change δ_{OH} in the partition coefficient of OH^-.

If one observes both of the following two kinds of response properties for a single membrane sample, the change in the partition coefficient of H^+ should be induced in the membrane by the bitter stimulation. One response is that the depolarization due to the stimulation is larger for the IA salt than for MA, and the other is that the depolarization occurs regardless of the direction of salt ion gradient. If the change is not induced, that is, $\delta_H = 0$, the condition (Eq. 24) for the latter reduces to $1 + \theta_H < (1 + \theta_M)(1 + \delta_M)$, but this is inconsistent with the condition (Eq. 23) for the former.

Effects of surface charges

Some kinds of bitter substances, such as N—C=S substances, hyperpolarize the electrically neutral membranes, such as phosphatidylcholine (PC) bilayer, whereas the bitter substances depolarize the negatively charged membranes (Kumazawa et al., 1988). To know how the potential response to the bitter stimuli depends on the surface charges of membranes, we calculated the dependence using Eq. 13 for various values of θ_ν and δ_ν, where the salt concentration was fixed at 0.1 M both in *trans* and *cis* sides for simplicity.

We show the calculated results for several tentative cases in Fig. 5. The dependence on the surface charge has the following characteristics: 1) the depolarization of the electrically neutral membrane is, in general, smaller than that of the negatively charged membrane, and 2) when the partition coefficients of anions are increased, that is, when $\delta_{OH} > 0$, $\delta_A > 0$, the potential of the neutral membrane is hyperpolarized as seen in *b* of Fig. 5. Table 1 shows the calculated results for the neutral membrane. Whether the potential of the neutral membrane depolarizes or hyperpolarizes, depends on the values of ψ_p and P_M/P_H, as seen in the table. A similar tendency of the response also is obtained in the case where the salt in the *cis* side solution is IA.

We derive the condition for the hyperpolarization of the potential of the neutral membrane from the inequality of $\phi_m < 0$ in the symmetric case where the salt concentration and pH in the *cis* side solution are the same as those in the *trans* side solution. The condition is represented as

$$\sum_\xi \delta_\xi(1 + \theta_\xi)P_\xi C_\xi^B < \exp(\beta\psi_p) \sum_\eta \delta_\eta(1 + \theta_\eta)P_\eta C_\eta^B \quad (25)$$

where ξ denotes permeable cation species and η denotes permeable anion species. It can be seen from Eq. 25 that an

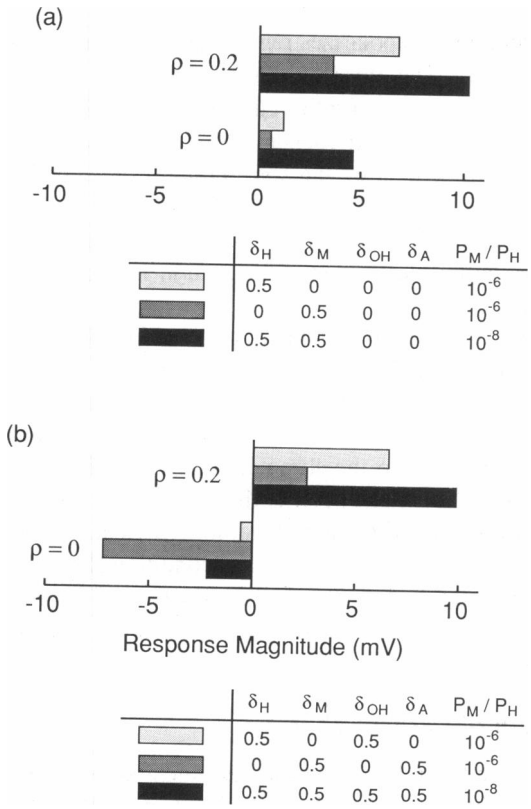

FIGURE 5 Response magnitude of the membrane potential as a function of the fraction ρ of the ionizable lipids in the membrane. Solutions are symmetric and contain 0.1 M of salt. pH is 7. ψ_p = 10 mV, θ_H = 0, θ_M = −0.5, θ_{OH} = 0, and θ_A = −0.5.

increase in partition coefficient of anions is essential to the hyperpolarization.

The magnitude of the hyperpolarization seems to depend sensitively on the cation permeability ratio P_M/P_H, as seen in Fig. 5 and Table 1. This dependency comes from two kinds of origins. One of them is the direct effect of the cation permeabilities P_ξ to the hyperpolarization, as shown in Eq. 25. The other is the effect of the anion permeabilities, P_η, which are determined through the relations of $P_A = 10\,P_M$ and $P_{OH} = P_H$ in the present calculation. When the values of P_M and P_A are large ($P_M/P_H = 10^{-6}$), the hyperpolarization is induced mainly through the change δ_A in the partition coefficient of A^-. The result for $\delta_A = 0.5$ and $P_M/P_H = 10^{-6}$ corresponds to this case. When the values of P_M and P_A are small ($P_M/P_H = 10^{-8}$), the condition (Eq. 25) is reduced approximately to $\delta_H(1 + \theta_H) < \exp(\beta\psi_p)\,\delta_{OH}(1 + \theta_{OH})$ because of $C_H^B = C_{OH}^B$. The results for $P_M/P_H = 10^{-8}$, shown in Table 1, can be estimated by using this reduced condition.

N—C═S substances, such as phenylthiourea, thiourea, and thiouracil, hyperpolarize the potential across the membrane made of electrically neutral lipids. Although the other bitter substances, such as lincomycine, brucine, quinine, and papaverine, depolarize the potential of neutral membranes, the magnitude of the depolarization is noticeably smaller in the neutral membranes than in the negatively charged membranes (Kumazawa et al., 1988). The difference of the response between neutral and negatively charged membranes comes essentially from the difference in the contribution of anions to the response. The contribution of anions is comparable with that of cations in the neutral membranes. It is highly possible that the main origin for the hyperpolarization is the increase in partition coefficients of anions due to the N—C═S substances. On the other hand, the contribution of anion flux is very small in the negatively charged membranes. It is seen by comparing the responses for $\rho = 0.2$ in Fig. 5*a* with those for $\rho = 0.2$ in Fig. 5*b* that the magnitude of depolarization hardly depends on the changes δ_{OH} and δ_A in the anion partition coefficients.

Change in membrane resistance

It is interesting to note how the present model induces the change in the membrane resistance when the membrane is stimulated with bitter substances because it has been reported that the resistance is not changed noticeably by the stimulation in lipid multibilayers (Okahata and En-na, 1987) and in taste cells (Akaike et al., 1976; Kumazawa et al., 1985). In this section, we consider only negatively charged membranes, where the contributions from cations H^+ and M^+ are dominant in determining the resistance. As seen from Eqs. 14–18, the membrane resistance decreases with an increase in the diffusion constants and the partition coefficients of H^+ and M^+, whereas the resistance increases with an increase in ψ_p.

Table 2 shows the calculated results for the ratio R_s/R_a of the resistance R_s under the stimulation to the resistance R_a at the resting state, as well as the response magnitude V_m of the membrane potential. The salt concentration used is 0.1 *M*, both in the *trans* and *cis* side solutions. In the calculation we set $\theta_\eta = 0$ and $\delta_\eta = 0$, where η denotes permeable anion species, because the contributions of anions to the resistance are small in the negatively charged membranes. The values of δ_H and δ_M are chosen such that the potential response is around 10 mV or less, as in the experiments. As seen from the table, the ratio R_s/R_a is near unity if the bitter stimulation does not induce a large change in the diffusion constant of H^+ or M^+. On the other hand, the stimulation induces a large change in the resistance if at least one of the diffusion constants of H^+ or M^+ is increased noticeably, or both of them are decreased noticeably. The membrane resistance of the synthetic lipid multibilayer is not changed noticeably by the bitter stimulation (Okahata and En-na, 1987). It seems that the bitter substances do not induce large changes in the diffusion constants.

Dependence of response magnitude on salt concentration

The present model has shown that the changes δ_ν in the partition coefficients of ions, especially δ_H of proton, play an

TABLE 1 Response magnitude (in millivolts) of the potential of electrically neutral ($\rho = 0$) membrane to bitter stimulation for various values of the relative changes δ_ν (ν = H, M, OH, and A) in partition coefficients of ions, as functions of the change ψ_p in the dipole potential on the *cis* side, and the permeability ratio P_M/P_H

				$P_M/P_H = 10^{-6}$			$P_M/P_H = 10^{-8}$		
δ_H	δ_M	δ_{OH}	δ_A	ψ_p = 10 mV	$\psi_p = 0$	ψ_p = −10 mV	ψ_p = 10 mV	$\psi_p = 0$	ψ_p = −10 mV
0.5	0	0	0	0.7	1.0	1.3	4.5	5.5	6.4
0	0.5	0	0	0.7	1.0	1.3	0.1	0.1	0.1
0.5	0.5	0	0	1.4	1.9	2.6	4.5	5.5	6.5
0.5	0	0.5	0	−0.3	0	0.4	−1.9	0	1.9
0	0.5	0	0.5	−8.0	−7.4	−6.6	−0.7	−0.5	−0.4
0.5	0.5	0.5	0.5	−8.1	−7.2	−6.0	−2.4	−0.4	1.6

$\theta_H = \theta_M = \theta_{OH} = \theta_A = 0$, $C_M^{BC} = C_M^{BT} = 100$ mol/m^3 (0.1 M), pH 7.

TABLE 2 Ratio R_s/R_a of the membrane resistance under the stimulation to that of the resting state, and response magnitude V_m of the membrane potential

Parameters						R_s/R_a	V_m
δ_H	δ_M	θ_H	θ_M	ψ_p	P_M/P_H		
				mV			mV
0.5	0	0	0	0	10^{-5}	0.98	1.0
0.5	0	0	0	5	10^{-6}	0.98	5.2
0.5	0	0	0	10	10^{-8}	0.99	10.1
0	0.5	0	−0.5	0	10^{-6}	1.21	3.5
0.5	0.5	0.5	−0.5	15	10^{-6}	1.05	9.1
0.5	0.5	0	−0.5	0	10^{-6}	1.08	9.4
0.5	0.5	−0.5	−0.5	−30	10^{-6}	1.05	9.9
0.5	0.5	0	0	0	10^{-6}	0.83	9.7
0.5	0.5	0	−0.9	0	10^{-6}	1.43	9.1
0.5	0.5	−0.9	−0.9	0	10^{-6}	4.98	5.8
0.5	0.5	10	0	0	10^{-6}	0.15	10.3

$\delta_{OH} = 0$, $\delta_A = 0$, $\theta_{OH} = 0$, $\theta_A = 0$, $\rho = 0.2$, $C_M^{BC} = C_M^{BT} = 100$ mol/m^3 (0.1 M), pH 7.

essential role in the potential response of lipid bilayers to bitter stimuli. We consider how the magnitude of the response depends on pH and the salt concentration in the solutions through the change δ_ν. In this section, we investigate the salt concentration dependence.

Fig. 6 shows several calculated results for the dependence on the salt concentration in the *cis* side solution in the case where the membrane has net negative charges. The salt concentration in the *trans* side solution is fixed at 0.1 M. There are two types of dependence: 1) one of which shows an almost constant response (cases *a* and *b* in Fig. 6), and 2) the other of which shows a decrease for high salt concentrations (cases *c* and *d* in Fig. 6).

In order to make clear the dependence of the response magnitude on the salt concentration, we derive the magnitude V_m in the limits of low and high salt concentrations from Eq. 13. When the salt concentration is low enough and $P_M C_M^{BC} \ll P_H C_H^B$, V_m is given by

$$V_m = \frac{1}{\beta}\ln(1 + \delta_H) + \frac{1}{\beta}\ln\left\{\frac{1 + (P_M C_M^{BT})/(P_H C_H^B)}{1 + [(1 + \theta_M)P_M C_M^{BT}]/[(1 + \theta_H)P_H C_H^B]}\right\}. \quad (26)$$

When the concentration is high enough, $P_M C_M^{BC} \gg P_H C_H^B$ and $P_M C_M^{BC} \gg P_A C_A^{BT}$, the response magnitude is

$$V_m = -\psi_p + \frac{1}{\beta}\ln\left[\frac{(1 + \theta_M)(1 + \delta_M)}{(1 + \theta_A)(1 + \delta_A)}\right]. \quad (27)$$

There are two kinds of conditions in which V_m hardly depends on the salt concentration. In one condition, the permeability ratio P_M/P_H is sufficiently small. The fluxes of M^+ and A^- are negligibly small and, therefore, do not contribute to the response. This corresponds to case *a* in Fig. 6. The other condition is that the effects of δ_ν, θ_ν, and ψ_p balance each other such that V_m in the low concentration region (Eq. 26) is almost the same value as V_m in the high concentration region (Eq. 27). Case *b* in Fig. 6 corresponds to this case. There are also two different conditions in which V_m decreases with an increase in the salt concentration. One condition is that only the partition coefficient of H^+ is changed with the bitter stimulation. Then, it is seen from Eqs. 26 and 27 that V_m does not depend on the salt concentration in the low concentration region and approaches zero in the high concentration limit (case *c* in Fig. 6). The other condition is where the response in the low salt concentration comes mainly from the second term of the right hand side of Eq. 26.

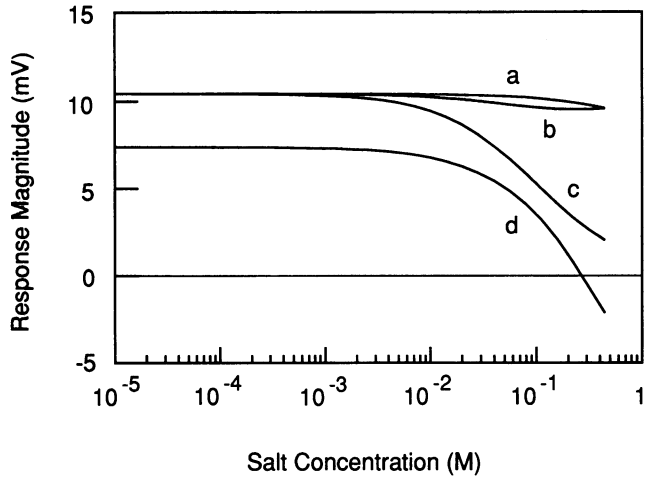

FIGURE 6 Dependence of the response magnitude of the membrane potential on the salt concentration in *cis* side solution. (*a*) $\delta_H = 0.5$, $\delta_M = 0$, $\theta_M = 0$, $P_M/P_H = 10^{-8}$; (*b*) $\delta_H = 0.5$, $\delta_M = 0.5$, $\theta_M = 0$, $P_M/P_H = 10^{-6}$; (*c*) $\delta_H = 0.5$, $\delta_M = 0$, $\theta_M = 0$, $P_M/P_H = 10^{-6}$; (*d*) $\delta_H = 0$, $\delta_M = 0.5$, $\theta_M = -0.5$, $P_M/P_H = 10^{-6}$. In every case, $\psi_p = 0$, $\delta_{OH} = \delta_A = 0$, and $\theta_H = \theta_{OH} = \theta_A = 0$. The salt concentration in the *trans* side solution is fixed at 0.1 M. pH is 7 in both *trans* and *cis* side solutions. $\rho = 0.2$. $\psi_p = 0$.

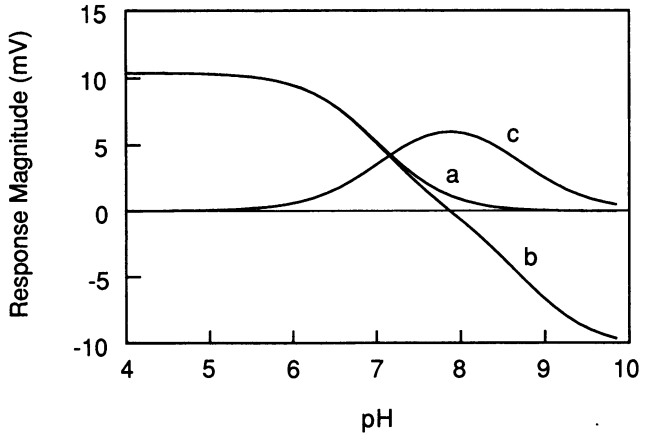

FIGURE 7 pH dependence of the response for the cases (*a*) $\delta_H = 0.5$, $\delta_M = 0$, $\delta_{OH} = 0$, $\theta_M = 0$; (*b*) $\delta_H = 0.5$, $\delta_M = 0$, $\delta_{OH} = 0.5$, $\theta_M = 0$; and (*c*) $\delta_H = 0$, $\delta_M = 0.5$, $\delta_{OH} = 0$, $\theta_M = -0.5$. In every case, $\delta_A = 0$, $\theta_H = \theta_{OH} = \theta_A = 0$. Solutions are symmetric and contain 0.1 M salt. $\rho = 0.2$. $\psi_p = 0$. $P_M/P_H = 10^{-6}$.

This comes from the reduction of the M^+ efflux due to the decrease ($\theta_M < 0$) in the diffusion constant of M^+ under the stimulation. Then, in the high concentration region, the response may be a hyperpolarization ($V_m < 0$), as seen in Eq. 27 for $(1 + \theta_M)(1 + \delta_M) < (1 + \theta_A)(1 + \delta_A)$. This is case *d* in Fig. 6.

Finally, we propose a method to check whether the partition coefficients of protons are changed by bitter stimulation. When one lowers the salt concentration in the *trans* side solution, as well as that in the *cis* side solution, and realizes the condition $P_M C_M^{BT} \ll P_H C_H^B$, the magnitude V_m of the response given by Eq. 26 is reduced to the simple form of $(1/\beta)\ln(1 + \delta_H)$. Thus, the value of δ_H can be estimated from the measurement of V_m under the condition mentioned above.

pH dependence of the response

Fig. 7 shows three calculated results for the dependence of the response on pH in the case where the solutions are symmetric; both the *trans* and *cis* side solutions contain 0.1 M of salt, and the membrane has net negative charges. For quite low pH (pH < 5), the response magnitude is determined solely by the change in the partition coefficient of the proton, whereas the response is determined by the change in the partition coefficient of OH^- for very high pH (pH > 10). The change in the diffusion constant of the proton does not induce the membrane potential change because the solutions are symmetric. We obtain the response magnitude V_m from Eq. 13. When pH is low and the condition $P_H C_H^B \gg P_A C_A^B$ holds, V_m is represented as

$$V_m = (1/\beta)\ln(1 + \delta_H). \quad (28)$$

When pH is high, $P_{OH} C_{OH}^B \gg P_A C_A^B$ and $P_{OH} C_{OH}^B \gg b^{-1} P_H C_H^B$, V_m is given by

$$V_m = -(1/\beta)\ln(1 + \delta_{OH}). \quad (29)$$

Thus, if one observes the depolarization at low pH in the symmetric solutions, it is highly expected that the partition coefficient of the proton is increased by the bitter stimulation. If the bitter stimuli induce the hyperpolarization at high pH, it means that the partition coefficient of OH^- is increased.

However, the response V_m does not depend on pH in the case where the salt concentration is very low because one of the conditions, $P_{OH} C_{OH}^B \gg b^{-1} P_H C_H^B$, in which OH^- contributes noticeably to the potential, is not satisfied. This is because *b* becomes quite small because the large negative surface potentials are hardly screened by the dilute salt ions.

DISCUSSION

Kumazawa et al. (1988) proposed that the observed response of the lipid bilayers to bitter stimuli was for a nonequilibrium (transient) state, and the response was induced by the change in the phase boundary potential. This proposal is based on the consideration that when the solutions on both sides of the membrane are symmetric, the response does not appear in an equilibrium (stationary) state. However, we have shown in the present paper that the response may indeed appear in the stationary state, even for the symmetric condition, if the partition coefficients of ions are changed by the stimulation.

Although ion channels that respond to bitter substances have been found in taste cells (Avenet and Lindemann, 1987; Kinnamon and Roper, 1988), there is a hypothesis that lipid bilayers provide a prominent transduction pathway for taste (Kumazawa et al., 1985; Kurihara et al., 1986; Kumazawa et al., 1988). The mechanism presented here may explain the bitter taste transduction in such cases. Then, we can further discuss the mechanism of the bitter response of lipid bilayers

with the help of experimental results on taste cells. The electric resistance of taste cells hardly changes in the bitter response of the cells (Akaike et al., 1976; Kumazawa et al., 1985), likewise, the resistance of bilayers (Okahata and Enna, 1987). The present result for the change in resistance of lipid bilayers suggests that the bitter substances do not induce large changes in the diffusion constants of ions in the membrane. In the case of cell membranes, it has been reported, based on the change in membrane fluidity, that the bitter substances are adsorbed on the hydrophobic region near the surface of the membrane of mouse neuroblastoma cells and are not adsorbed on the inner region of the membrane (Kumazawa et al., 1986). If this is also the case for lipid bilayers, it is reasonable that the bitter substances do not induce large changes in the diffusion constants of ions.

The responses of the frog taste nerve to bitter stimuli do not depend on the salt concentration in the solution that contains the electrically neutral bitter stimuli (Kumazawa et al., 1986). If the responses also do not depend on the salt concentration in the lipid bilayers, the following two cases are probable, as seen from Fig. 6. One is that the permeability ratio P_M/P_H is sufficiently small, and the membrane potential is determined mainly by the flux of H^+ and/or OH^-, and the other is that the changes δ_ν, θ_ν and ψ_p, due to the stimulation, are balanced such that the dependence is small. Although it might seem difficult for many quantities to balance accidentally, the balancing can be realized by quite plausible changes such that $\delta_H \simeq \delta_M$ with $\delta_A \simeq 0$, $\psi_p \simeq 0$, and $\theta_\nu \simeq 0$, as seen from Eqs. 26 and 27.

In the present calculation, we have not considered the conformation change of the model membrane that may be induced by salt. Because the monovalent salts within the usual concentration range hardly change the temperature of gel-liquid crystal phase transition in the lipid bilayer (Träuble and Eibl, 1974; MacDonald et al., 1976), it seems reasonable that we neglect the effects of conformation change due to these salts. It is not the case for divalent salts such as $CaCl_2$, because the phase transition temperature increases with the adsorption of divalent cations.

We assumed that the permeation of the permeable bitter substances is fast enough and the concentration of the substances in the membrane reach a stationary equilibrium state rapidly. When the bitter substances that are permeable but have quite low permeabilities are involved, transient phenomena associated with the gradual change in the concentration may be induced. For the substances that are adsorbed on the deep, inner region of the membrane, the diffusion constants of ions may change gradually, and a gradual change in the membrane potential due to the changes in the diffusion constants may be induced, in addition to a rapid change in the potential caused by the changes in the partition coefficients of ions.

The changes in the partition coefficients are induced at the surface of the membrane and hardly will be affected by the permeability of the bitter substances. The nonpermeant bitter substances will have an effect only on the partition coefficients and the dipole moment.

APPENDIX

Assumption of the stationary response

We assumed in the present paper that the transient response of the membrane system to bitter stimulation ended shortly after the stimulation, and we calculated the stationary response of the membrane potential. We estimate here the time period required to reach the stationary state after the stimulation. The transient response of the membrane potential ϕ_m appears as a result of a gradual change in the diffusion potential ϕ_d. The gradual change in ϕ_d is caused by the delay of the response of ϕ_d to the stimulation, which arises from the charging up of membrane capacitance (Naito et al., 1991). The time constant τ_d of the charging, that is, the delay time of the change in ϕ_d, is given by $C_m R_m$, where C_m is the capacitance of the membrane and R_m is the resistance. Using $R_m = 10^4\ \Omega m^2$ (Kumazawa et al., 1988) and $C_m = 10^{-2}\ Fm^{-2}$ (Nomura and Kurihara, 1987) for planar lipid bilayers, we obtain $\tau_d = 100$ s, which is of the order of one min. Because the response potential can be measured experimentally within several min after the onset of the stimulation (Kumazawa et al., 1988), we can safely assume that the measured potential is the stationary one.

When the permeation of membrane permeable bitter substances is noticeably slow, it may be necessary to consider the transient phenomena associated with the permeation. This is because it would take a long time for the concentration of the substances to equilibrate in the membrane. Then, the stimulation process itself may occur gradually with the change in the concentration of the substances. However, we assumed that the equilibration occurred very rapidly and did not consider the transient phenomena induced by it. Okahata and En-na (1987) showed that the responses of the membrane potential of lipid multibilayers to bitter substances reached the stationary state within 30 s. Because the thickness of their membranes was about 100 μm, the response is expected to be much faster in the bilayer of the thickness of 5 nm. Kumazawa et al. (1988) also reported a quick response of the membrane potential of lipid bilayers to bitter substances. They showed that a faster stirring of the solution led to a rapid change in the potential, indicating the rate limiting step in the potential response is the diffusion of the substances in the solution, rather than into the membrane.

The result of Okahata and En-na (1987) and Kumazawa et al. (1988) that the response is quick also suggests that the adsorption of the bitter substances on the membrane, and the resulting conformation change of the membrane, occur rapidly. We therefore assumed that the transient phenomena associated with these reactions can be ignored.

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