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PHYSICOCHEMICAL STUDIES OF TASTE RECEPTION

II. POSSIBLE MECHANISM OF GENERATION OF TASTE RECEPTOR POTENTIAL INDUCED BY SALT STIMULI

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SUMMARY

This paper discusses the origin of the membrane potential in the model membrane which simulates taste receptor potentials in response to the electrolyte stimuli, on the basis of the results obtained with the model membrane and with the liposomes made of the same lipids as used for the model membrane.

1. The electric resistance of the model membrane was almost independent of the external salt concentration. A sandwiched membrane in which a platinum plate was placed between two sheets of the model membrane produced potential deflections similar to those observed with a sheet of the model membrane.

2. The ζ-potential of liposomes was measured under the presence of various concentrations of the salts (NaCl, KCl, $CaCl_2$ and $MgCl_2$). The relationship between the change in the ζ-potential and the NaCl concentration followed the taste equation proposed by Beidler (Beidler, L. M. (1954) J. Gen. Physiol. 38, 133–139). However, the surface charge density of liposomes calculated from the ζ-potential data did not increase with increase of salt concentration, which implied that the increase of the ζ-potential is not attributed to the Langmuir type adsorption of Na^+ on the anionic sites on the liposome surface. An application of 2 : 1 type electrolytes ($CaCl_2$ and $MgCl_2$) reversed the sign of the surface charge when the salt concentration exceeded about 30 mM.

3. $FeCl_3$ produced a large positive change in the ζ-potential due to the binding of Fe^{3+} to the liposome surface. An application of NaCl to Fe^{3+}-bound liposomes led to a decrease in the ζ-potential with an increase of the NaCl concentration.

On the basis of the above results, it was proposed that taste stimulation with salt stimuli is initiated by a change in the phase boundary potential at the microvilli membrane in taste cells.

INTRODUCTION

In 1954 Beidler [2] proposed a theory of taste reception stating that a chemical stimulation is initiated by the adsorption of taste substances onto specific sites of

microvilli membrane in taste cells. This theory was derived from his finding that the relationship between the taste response induced by sodium salts and their concentrations followed an equation similar to the Langmuir adsorption isotherm. The equation is generally referred to as the "taste equation" in the field of chemoreception. In the derivation of this equation, Beidler postulated that the taste nerve response is linearly related to the number of cations bound to the receptor sites. There is, however, no definite information about the interaction acting between cations and the sites. The adsorption of the cation onto the receptor sites induces a potential change in the taste cells, the so-called taste receptor potential. The mechanism of generation of the taste receptor potential is also not known at the present. These situations led us to the undertaking of the model analysis reported in this series of papers.

It was shown in Part I [1] of this series that a model membrane composed of a Millipore filter paper and the lipids extracted from bovine tongue epithelium produced a change in membrane potential in response to salts, acids and distilled water similar to the receptor potential observed with a living taste cell. In this paper, we present several data on the electric resistance and the membrane potential of the model membrane together with the electrokinetic potential (ζ-potential) of liposomes prepared from the same lipids as used for the model membrane. Taking these data into consideration, a plausible mechanism of the generation of potential in the model membrane is discussed. It becomes clear that the surface potential or the phase boundary potential at the interface of the membrane-stimulating solution is responsible for the salt stimulation. The implication of the taste equation proposed by Beidler [2] is discussed in the light of the phase boundary potential.

EXPERIMENTAL

Materials

The lipids used in the present study were extracted from bovine tongue epithelium and the model membrane was prepared as described in Part I [1]. Salt solutions were prepared by dissolving the analytical grade reagents in distilled water.

Measurements of the electric resistance of the membrane

The d.c. resistance of the membrane was evaluated from the relationship between the current applied to a membrane and the magnitude of the electric potential produced across the membrane. The induced voltage depended linearly on the current strength. The impedance of the membrane was measured by a dielectric-loss bridge (Ando Electric Co., Tokyo, Type TR-1B) in applied frequencies ranging between 10^2 and $3 \cdot 10^6$ Hz. The d.c. resistance was obtained by extrapolating to zero frequency, which agreed well with that obtained from the relationship between current and voltage. A pair of Pt–Pt plate electrodes were placed as close to the membrane surface as possible so as to eliminate the contribution of the resistance of the solution phases which are contiguous to the membrane.

Preparation of liposomes

The liposomes were prepared according to the essentially same method as employed by Bangham et al. [3]. The lipid was thinly coated on the inner surface of a round-bottom flask by the rotary evaporation of a chloroform solution under vacuum

and then distilled water was added. Dispersions were prepared by agitation of the flask with a Vortex mixer until all the lipids were freed from the wall of the flask. The lipid dispersions thus obtained were mixed with salt solution so as to give a desired salt concentration.

Measurement of ζ-potential

The ζ-potential was calculated from the electrophoretic mobility of the liposomes in accordance with the Helmholtz–Smoluchowski equation [4];

$$\zeta = 4\pi\eta u/D \tag{1}$$

Here u is the mobility of the liposome, and η and D are the viscosity and dielectric constant of the dispersion medium, respectively. The viscosity of the solution to be measured was taken from an appropriate table, while the dielectric constant of the salt solution was assumed to be the same as that of pure water. The electrophoretic mobilities were measured by a micro-electrophoretic apparatus (Karl Zwiss, West Germany, Cytopherometer). Measurements were made by the direct observation of the velocity of dispersed particles at the "stationary layer" [5] in a quartz flat cell regulated at 28 °C. The observed velocity of the liposome was proportional to the electric field applied externally and was independent of the size of vesicle observed. Under a given salt condition, 10 liposomes were timed in each direction of the field to eliminate the polarization of the electrodes and the average value was taken as the velocity of a particle.

Calculation of surface charge density

The surface charge density, σ, of the liposomes dispersed in an aqueous solution of 1 : 1 type salt was calculated from the observed ζ-potentials using the Gouy-Chapman relationship [6] for a flat double layer in the form

$$\sigma = \sqrt{\frac{2DRTI}{1000\pi}} \sinh\left(\frac{F\zeta}{2RT}\right) \tag{2}$$

where I is the ionic strength, R the gas constant, T the absolute temperature and F the Faraday constant. When the suspension medium was a solution of asymmetric salt such as $CaCl_2$ or $FeCl_3$, σ was evaluated from the following approximate equation [6]

$$\sigma = \sqrt{\frac{2DRTI}{1000\pi}} \left(\frac{F\zeta}{2RT}\right) \tag{3}$$

where the Debye–Hückel linearization approximation was introduced.

RESULTS AND DISCUSSION

The electric resistance of the model membrane was measured in the presence of various concentrations of NaCl, KCl and $CaCl_2$. The observed membrane resistance in a given salt solution varied from one membrane preparation to another, but the membrane resistance of a membrane was practically independent of salt composi-

tion and concentration in the bulk solution with most membrane preparations (see Curve 1 in Fig. 1). The membrane resistance only rarely decreased with increase of salt concentration as shown by Curve 2 in the figure, but the extent of decrease was

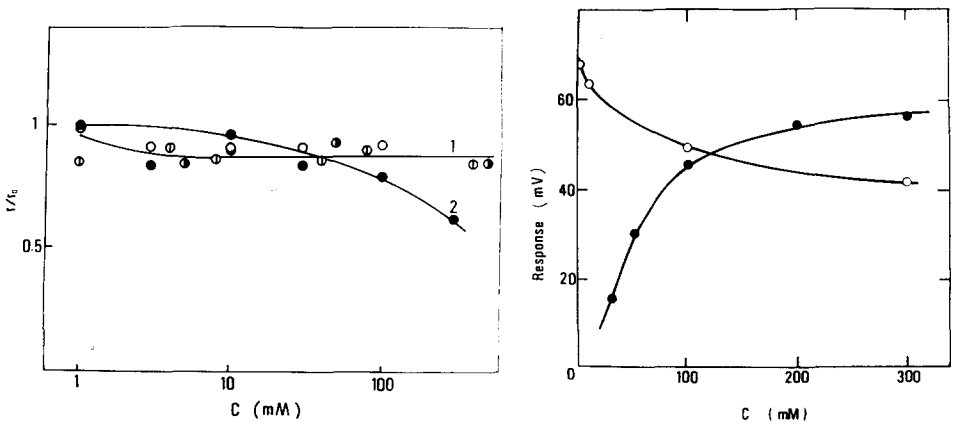

Fig. 1. Electric resistance of the model membrane in the presence of various concentrations of NaCl, KCl and $CaCl_2$. The resistance was measured with the model membrane interposed between two identical salt solutions. The ordinate represents the ratio of the membrane resistance (r) at any concentration over that at 1 mM NaCl (r_0). Data shown by Curve 1 were obtained from the membrane (Q = 20.2 mg/cm^2, r_0 = 25 M$\Omega \cdot$ cm^2) and those by Curve 2 with the membrane (Q = 14.0 mg/cm^2, r_0 = 110 M$\Omega \cdot$ cm^2). ○, NaCl; ⓘ, KCl; ◑, $CaCl_2$ for membrane 1. ●, NaCl for membrane 2.

Fig. 2. The potential responses of the sandwiched membrane interposing a platinum plate between two sheets of the model membrane (Q = 13.9 mg/cm^2, Q = 24.5 mg/cm^2) to various concentrations of NaCl (Curve 1, ●——●). The ordinate represents the magnitude of the potential deflections induced by the application of the salts to the membrane bathed in tap water. Curve 2 (○——○) shows the potential deflection induced by the application of NaCl to the membrane which had been treated with 60 mM $FeCl_3$ for 12 min.

very small. In a different series of papers [7, 8], we have shown that various transport processes, e.g. membrane potential, ion permeability, electric resistance, etc., across a partially permeable charged membrane are represented quantitatively in terms of the effective charge density of the membrane as a sole parameter. The effective fixed charge density calculated from the observed membrane potential for the membrane used above was 0.08 M and 0.25 M in 10 mM and 300 mM NaCl solutions, respectively. The theoretically calculated electric resistance of the same membrane with the use of these values for the fixed charge density decreased by a factor of about 10 when the salt concentration was changed from 10 to 300 mM. The observed value of the resistance of the membrane under the same conditions was much smaller than the predicted value even in the case of Curve 2 in Fig. 1. This implies that ions in the external solution scarcely permeate through the model membrane under study.

The above thought was also supported by the following result. When we denote the conductances at low and high frequency limits by G_0 and G_∞, respectively, the ratio of the porous leaky area to the total area of the membrane is approximately represented by $G_0/(G_\infty - G_0)$, provided that the specific conductance of the lipid region is negligibly small compared with that of an external solution. When the

conductance measured at 3 MHz, which was the high frequency limit accessible by our apparatus, was taken as G_∞, the value of $G_0/(G_\infty - G_0)$ was evaluated to be $7 \cdot 10^{-4}$. The real value of G_∞ must be much larger than that at 3 MHz and then, the ratio of the porous area is definitely less than 0.07 %.

As described in Part I [1], the function of the model membrane to respond to the electrolyte stimuli was practically independent of the quantity of adsorbed lipids if the amount of the adsorbed lipids exceeded about 8 mg/cm^2. Judging from the porosity of the filter paper and the amount of the adsorbed lipids, the void space in the filter paper must be fully filled up with the lipids. This fact, together with the results on the membrane resistance described above suggests that the membrane potential across the model membrane is not produced by the diffusion process of ions across the membrane.

The fact mentioned above has been confirmed further by the following experiments: a platinum plate was placed between two sheets of Millipore-lipids membranes, and the sandwiched membrane was subjected to the chemical stimulation. The membrane thus prepared should be impermeable to ions across the membrane. As shown in Fig. 2, the application of NaCl induced potential deflections similar to those observed in Part I [1] with a sheet of Millipore-lipids model membrane. In addition, the characteristic effect of $FeCl_3$ on the membrane potential [1] was reproduced with the sandwiched membrane; the application of NaCl to the membrane which was previously treated with $FeCl_3$ induced a potential change with a polarity opposite to that normally displayed. These results strongly support that the potential deflection observed with the model membrane is not produced by ion permeation through the membrane.

The origin of the potential across the membrane which is scarcely permeable to ions has been investigated by several authors by the use of various membranes [9–12]. For example, it was demonstrated that there was a drastic change of ionic specificity of a glass electrode when the external surface of the electrode was coated with a thin layer of collodion [9]. If the overall permeability of ions across the membrane is responsible for the ionic specificity of the membrane as postulated by Hodgkin and Huxley [13], there must be only one type of ionic specificity even in such a collodion-coated glass electrode; since the thin collodion coating possesses a relatively low resistance, the total resistance or ion permeability of the coated glass electrode should not be materially different from that of the parent glass electrode. From this reason together with other experiments, Ling [9] concluded that the adsorption of ions onto the surface of the electrode was responsible for the potential of the coated electrode. Clearer examples indicating that the specific adsorption of ions at a membrane surface plays an essential role for the generation of the overall potential difference across a membrane have been demonstrated by us in a previous paper [11]. For example, when a mercury layer was interposed between two KCl and NaCl solutions of different concentrations, the membrane potential changed as if the mercury membrane were an ideal cation permselective membrane. On the other hand, the membrane behaved as an ideal anion permselective membrane when it was contiguous with $(CH_3)_4N^+Cl^-$ solution. These phenomena virtually indicate that the membrane potential is determined by the specific adsorption of individual ion species at the mercury surface.

The experimental facts described above lead us to a notion that the potential

difference across the present model membrane stems from the difference in the phase boundary potentials at two interfaces of membrane and solution phases. The phase boundary potential of an oil membrane which scarcely dissolves ionic species can be represented by the same functional form as that of the Donnan potential (see Appendix). It is also known that the phase boundary potential represented in terms of the equilibrium is not very different from that of the solution of the Poisson–Boltzmann distribution of ions at a plain surface of a dielectric immersed in an electrolyte solution [14]. Therefore, it is desirable to measure the surface potential or surface charge density of the model membrane which is closely related to the phase boundary potential. The measurements of the surface potential or surface charge density are, however, not accessible experimentally with the present model membrane. Therefore, the ζ-potential of the liposomes prepared from the same lipids as used for the model membrane was measured as an experimental approximation to the phase boundary potential. In Fig. 3, the observed ζ-potential is plotted against the concentration of the 1 : 1 type (KCl, NaCl) and the 2 : 1 type ($CaCl_2$, $MgCl_2$) electrolytes. It is noted that the liposomes show a negative ζ-potential in the whole range of salt concentration of 1 : 1 electrolytes, which indicates that the relatively excess anionic groups exist on the liposome surface. These are considered to be mainly phosphate groups of the phospholipids exposed on the surface of the liposomes. An application of 1 : 1 type electrolytes brought about a positive-going potential change in the ζ-potential. The relationship between the ζ-potential and the concentration of 1 : 1 type electrolytes is quite similar to that between the membrane potentials across the model membrane and the

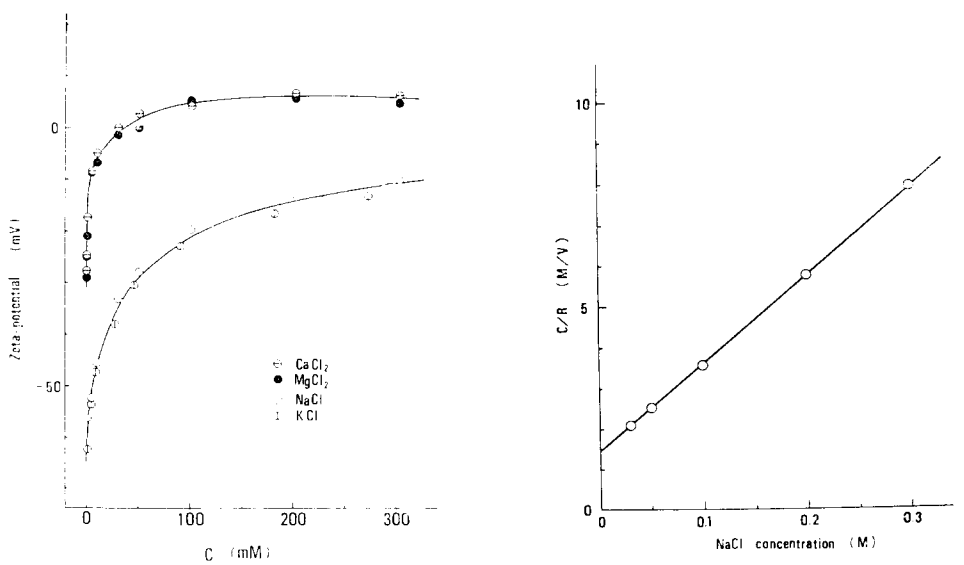

Fig. 3. The ζ-potential of the liposomes as a function of salt concentration. ⊖, $CaCl_2$; ●, $MgCl_2$; ○, NaCl; ⊕, KCl.

Fig. 4. Plot of the magnitude of the changes in the ζ-potential of the liposomes induced by NaCl according to the taste equation. R was obtained by subtracting the value of − 47.6 mV, which is the potential level for tap water, from the value of the ζ-potential shown by the curve for NaCl in Fig. 3.

salt concentration. As seen from the figure, an application of 1 : 1 type electrolytes never brought about a reversal of the polarity of the ζ-potential, while 2 : 1 type electrolytes caused the ζ-potential to become positive when the salt concentration exceeded about 30 mM. It is noted that there is no appreciable difference in the observed ζ-potential between NaCl and KCl which compared with the case of the membrane potentials observed with these salts.

It is important to note here that the relationship between the ζ-potential of the liposomes and the NaCl concentration given in Fig. 3 strictly follows the taste equation when R is defined by the magnitude of change in the ζ-potential measured from the potential level in tap water (see Fig. 4). The apparent equilibrium constant K calculated from the straight line in the figure was 14.8 $mole^{-1}$, which is close to that obtained from the electrophysiological data on taste stimulation with NaCl [15]. If the fact that the variation of the ζ-potential follows the equation means uniquely that Na^+ forms a complex with the anionic site at the surface of the liposomes, the negative charges at the liposome surface must be masked by the binding of Na^+ on the surface. However, the density of the negative charge at the liposome surface calculated from the observed ζ-potential increased with the concentration of salt in the dilute region, and approached an approximately constant level with a further increase of the salt concentration (see Fig. 5). Therefore, the change in the ζ-potential induced by NaCl is not attributable to the Langmuir-type adsorption of Na^+ on the anionic sites at the liposome surface. It is not unreasonable to consider that the cations of 1 : 1 type salts surround the anionic groups to form a diffused electrical double layer by the long range interaction of ions.

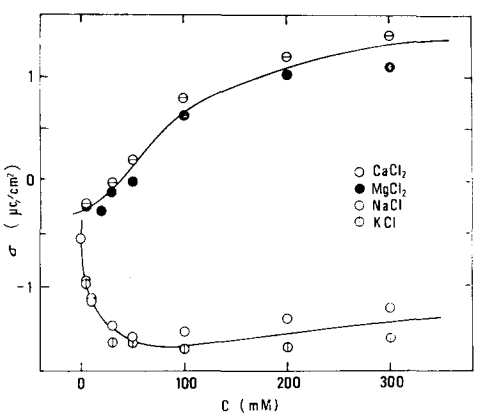

Fig. 5. The surface charge density of the liposomes as a function of salt concentration. The notations are the same as in Fig. 3.

Contrary to the case of 1 : 1 type electrolytes, the charge density of the liposome surface increased monotonously from negative to positive in sign by the application of 2 : 1 type salts (see Fig. 5). This is attributed to the binding of the divalent cations to the anionic groups of the lipids. In the case of $FeCl_3$, a more dilute solution is sufficient to reverse the sign of the surface charge of the liposomes as shown in Fig. 6. The ζ-potential decreased with the application of $FeCl_3$ in a higher than 0.5 mM

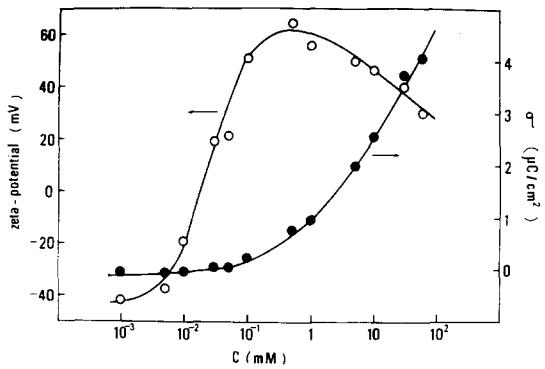

Fig. 6. The ζ-potential and surface charge density of the liposomes as a function of $FeCl_3$ concentration. ○, ζ-potential; ●, surface charge density.

concentration. In this concentration range of $FeCl_3$, the surface charge density increased with the increase of the $FeCl_3$ concentration, hence the decrease in the ζ-potential is attributed to an effect of ionic strength on the thickness of the electrical double layer with an increase of the $FeCl_3$ concentration.

When NaCl was applied to the liposomes carrying positive charge due to the binding of Fe^{3+}, the positive ζ-potential decreased with increase of NaCl concentration as illustrated in Fig. 7. This result closely compared to the potential deflection caused by the NaCl application against the $FeCl_3$-treated model and taste cell membranes [16]. As shown in the figure, the surface charge density of $FeCl_3$-treated liposome did not change by the application of NaCl of various concentrations. Therefore, the decrease of the ζ-potential shown in the figure is attributed to the effect of the ionic strength on the thickness of the double layer, which is the same reason as described in Fig. 5. Tateda and Beidler [16] reported that cocain also induces an effect similar to that of $FeCl_3$ on the taste cells. The following results obtained by Bangham et al. [17] seem to suggest that the mechanism of the action of cocain is

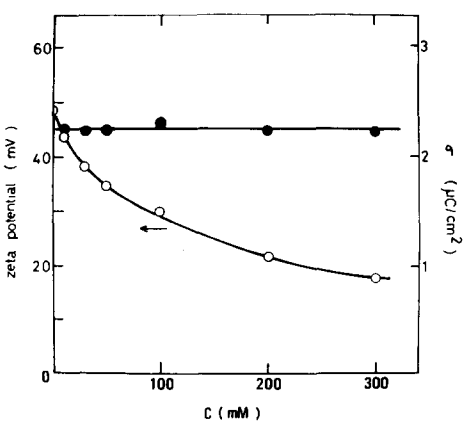

Fig. 7. The effect of various concentrations of NaCl on the ζ-potential and surface charge density of the liposomes in the presence of 10 mM $FeCl_3$. ○, ζ-potential; ●, surface charge density.

similar to that of $FeCl_3$; they measured the electrophoretic mobility of the liposome in the presence of cocain, and found that cocain, which is carrying a positive charge, adsorbed on the surface of the liposome and brought about a positive-going change in the ζ-potential.

From the results described above, the effect of $FeCl_3$ or cocain on the membrane potential in taste cells can be interpreted as follows; Fe^{3+} or cocain adsorbs strongly on the lipids in the microvilli membrane, which leads to a reversal of the sign of the surface potential at the microvilli membrane. The increase of ionic strength in the medium by the application of NaCl to $FeCl_3$-treated membrane leads to a decrease in the thickness of the electrical double layer at the surface of the membrane, which brings about a potential deflection with a polarity opposite to that normally displayed. At a certain $FeCl_3$ concentration, the negative charges carried by the phospholipids are concealed by the adsorption of Fe^{3+}, where no concentration of NaCl applied to the membrane produced a response, as demonstrated with taste cells [16] and the model membrane [1].

CONCLUDING REMARKS

Various results presented in this paper indicate that the potential across the model membrane composed of Millipore and lipids is attributed to the difference in the phase boundary potentials at two sides of the membrane. Since the model membrane gives a potential response quite similar to the receptor potential observed with the taste cells in response to the electrolyte stimuli, taste receptor potentials observed in living taste cells also seem to be generated by a similar mechanism. As pointed out in Part I [1], there are a number of reasons that the sodium theory proposed by Hodgkin and Huxley [13] is not applicable to the microvilli membrane. For example, as for the salt stimulation, the theory was not in accordance with the experimental facts that the microvilli membrane is scarcely permeable to ions and that both Na^+ and K^+ applied on the tongue surface induce, a more or less similar depolarization in the taste cells. On the other hand, the mechanism proposed here is in line with these facts.

Recently, it was reported that HCl, 2 : 1 type salts or distilled water produced either a slight change or no change in the electrical resistance of the taste cell membrane, when the receptor potential is elicited by these stimuli [18, 19]. An observable change in the membrane resistance is measured when a high concentration of 1 : 1 type salts is applied to the taste cell [18, 19]. Although the change in the membrane resistance is not always accompanied with the transports of ions, the results described above may imply that the diffusion process of ions in the membrane is not negligible. However, this is not inconsistent with our interpretation of the receptor potential discussed in this article, since the membrane potential inherently consists of two phase boundary potentials and an intramembrane diffusion potential (see Appendix).

The bovine taste buds bearing papillae contain five kinds of phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and sphingomyelin) [20]. Recent studies on the molecular arrangements in the lipid membrane or biological membrane indicated that the lipids in the membrane do not exist in a homogeneously mixed state, but each respective lipid or similar species of lipids forms clusters [21–24]. According to our preliminary studies (Miyake

et al., unpublished data), the model membrane containing only phosphatidylcholine did not respond to the salt stimuli, but did to distilled water. On the other hand, the membrane containing only phosphatidylethanolamine responded to the salt stimuli, but did not to distilled water. Therefore, it seems to be reasonable to consider that the microvilli membrane has small receptor domains carrying different functions.

At the present, we do not know how a change in the phase boundary potential at the microvilli membrane is transmitted to the endings of the taste nerve, but one of the hypothetical transmissive mechanisms is as follows: taste cells have a resting potential being positive on the outside with respect to the inside of the cell, and the application of cation species onto the microvilli membrane brings about a negative-going potential deflection in the phase boundary potential at the receptor domains. This change in the phase boundary potential at the domains creates an inwardly-directed circulating current, by which the nerve endings attached to the taste cells will be discharged. Depolarization induced by the stimuli which has been observed by inserting a microelectrode into a taste cell seems to be brought about by the potential drop caused by the circulating electric current. There is a possibility that the conformational change of the domain surface is induced by the variation of the phase boundary potential caused by taste stimuli, which in turn, regulates the circulating current. Although other explanations on the transmittance process may be possible, it can be safely concluded that the taste stimulation with the electrolyte stimuli is initiated by a change in the phase boundary potential at the surface of the microvilli membrane.

APPENDIX

The quantitative theory of membrane potentials across charged membrane may be said to have started in 1935 by the pioneering works of Teorell and of Meyer and Sievers [25, 26]. These authors obtained a mathematical expression for the membrane potential by integrating the Nernst-Planck equation for the diffusion of ions and subsequently adding the two-phase boundary potentials to the intramembrane diffusion potential. This method itself is applied even in the membrane made of non-aqueous lipid as illustrated below. For the sake of simplicity of discussion, we consider a system where two aqueous solutions of a 1 : 1 type electrolyte of different concentrations of C_1 and C_2 are separated by a membrane. The potential difference across the membrane is represented by the following equation;

$$\Delta\phi = -\frac{RT}{F}\int_{C_1}^{C_2}\frac{u_+ C_+ - u_- C_-}{u_+ C_+ + u_- C_-}\,\mathrm{d}\ln C \tag{A.1}$$

under the assumption that no mass movement occurs across the membrane. In Eqn (A.1), u_i and C_i are the mobility and concentration of the ion species i ($i = +, -$) in the membrane phase. The basic equation (A.1) is shown to be valid even in the case where the membrane is an oil membrane or a dense ion-exchanger membrane. If the concentration of cations and anions in the membrane are calculated by use of the Donnan equilibrium, the well-known expression of the Teorell, Meyer and Sievers theory (TMS theory, in abbreviation) is derived. The Donann equilibrium in the ordinary sense, however, is not applicable for the oil membrane as is encountered in

the present study. The electrochemical potential of species i in the membrane is expressed by

$$\tilde{\mu}_i = \mu_i^0(\mathrm{m}) + RT \ln C_i + z_i F\psi' \tag{A.2}$$

while that in the bulk aqueous solution which is contiguous with the membrane is represented by

$$\tilde{\mu}_i = \mu_i^0 + RT \ln C + z_i F\psi \tag{A.3}$$

Here z_i is the valence of ions, i.e. $+1$ or -1, and ψ is the electrical potential. Note that the standard chemical potential of an ion species in the membrane, $\mu_i^0(\mathrm{m})$, is different from that in the aqueous solution, μ_i^0. When the thermodynamic equilibrium holds between the membrane and solution phases, Eqns (A.2) and (A.3) must be equal to each other. Then we have

$$[\mu_i^0(\mathrm{m}) - \mu_i^0] + RT \ln (C_i/C) + z_i F(\psi' - \psi) = 0 \tag{A.4}$$

Elimination of $(\psi' - \psi)$ from the equations for the cation and anion species given by Eqn (A.4) leads to

$$C_+ C_- = C^2(K_m^0)^2 \tag{A.5}$$

where K_m^0 is defined by the following equation

$$\tfrac{1}{2}\{[\mu_+^0(\mathrm{m}) + \mu_-^0(\mathrm{m})] - (\mu_+^0 + \mu_-^0)\} = -RT \ln K_m^0 \tag{A.6}$$

and represents the difference in solubilities of the electrolyte component between the oil and the aqueous solution phases. The value of K_m^0 is a constant irrespective of the salt concentration of the external solution for a given combination of membrane and electrolyte components under consideration. Making use of the condition of electroneutrality in the membrane, i.e. $C_+ - C_- = X$, where X is the charge density fixed in the membrane, Eqn (A.5) is solved to give

$$C_i = \frac{X}{2}\left[1 + \frac{4C^2K_m^{02}}{X^2}\right]^{\frac{1}{2}} \pm \frac{X}{2} \qquad (i = +, -) \tag{A.7}$$

Introducing Eqn (A.7) into Eqn (A.1) and integrating between the two bulk solutions across the membrane, we obtain the following equation for the membrane potential under the assumption that X is constant irrespective of the salt concentration in the external solutions;

$$\Delta\phi = -\frac{RT}{F}\left[\ln\frac{C_2}{C_1} + U\ln\frac{(\theta^2 + 4C_2^2)^{\frac{1}{2}} + U\theta}{(\theta^2 + 4C_1^2)^{\frac{1}{2}} + U\theta} - \ln\frac{(\theta^2 + 4C_2^2)^{\frac{1}{2}} + \theta}{(\theta^2 + 4C_1^2)^{\frac{1}{2}} + \theta}\right] \tag{A.8}$$

where U and θ are defined as $(u_+ - u_-)/(u_+ + u_-)$ and $X/K_m{}^0$, respectively. This equation is identical to the TMS theory except that the fixed charge density X is replaced by θ. It is important to note that the TMS theory retains its original form even in the oil membrane, and that the effective fixed charge density in Eqn (A.8) includes a factor representing the difference in solubilities as given by Eqn (A.6).

When the membrane is scarcely permeable to the ion species as in the present system under study, the observed membrane potential mainly stems from the phase

boundary potential at the membrane surface. Thus the membrane potential given by Eqn (A.8) is simplified to give

$$\Delta\phi = -\frac{RT}{F} \ln \left[\frac{\sqrt{4(C/\theta)^2 + 1} - 1}{2(C/\theta)} \right] + \text{constant} \qquad \text{(A.9)}$$

when the concentration of salt in one side of the membrane is fixed. Eqn (A.9) together with Eqn (A-6) implies that the phase boundary potential is represented by the relative concentration, C/θ, with the effective fixed charge density θ of the membrane surface as the sole parameter. As pointed out above, the value of θ depends on the combination of the species of membrane and electrolyte components used. Thus the observed membrane potential must be affected not only by the cation species but by the anion species involved, although the effect of the anion species is much smaller than that of the cations and of the characteristics of the membrane used.

Although the contribution of the diffusion potential in the membrane phase, the first and second terms in Eqn (A.8), is neglected in the above discussion, this term should be taken into consideration in the more general cases, e.g. the membrane is immersed in an extremely high salt concentration medium.

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