Darszon, A., Strasser, R. J., & Montal, M. (1979) *Biochemistry 18*, 5205–5213.
Deese, A. J., Dratz, E. A., Dalquist, F. W., & Paddy, M. R. (1981) *Biochemistry 20*, 6420–6427.
De Grip, W. J., Drenthe, E. H. S., Van Etcheld, C. J. A., De Kruyff, B., & Verkley, A. J. (1979) *Biochim. Biophys. Acta 558*, 330–337.
Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
Ishimoto, M., & Wald, G. (1946) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 5*, 50–51.
Jost, P. C., McMillen, D. A., Morgan, W. D., & Stoeckenius, W. (1978) in *Light-Transducing Membranes* (Deamer, D., Ed.) pp 141–154, Academic, New York.
Kates, M., Joo, C. N., Palameta, B., & Shier, T. (1967) *Biochemistry 6*, 3329–3335.
Marsh, D., & Watts, A. (1982) in *Lipid–Protein Interactions* (Jost, P. C., & Griffith, O. H., Eds.) Vol. 2, pp 53–126, Wiley, New York.
Mason, W. T., & Fager, R. S. (1974) *Comp. Biochem. Physiol., Comp. Biochem. 47B*, 347–352.
Miljanich, G. P., Sklar, L. A., White, D. L., & Dratz, E. A. (1979) *Biochim. Biophys. Acta 552*, 294–306.
Mollenwanger, C. P. J., & De Grip, W. J. (1984) *FEBS Lett. 169*, 256–260.
O'Brien, D. O. (1982) *Methods Enzymol. 81*, 378–384.
Papermaster, D. (1982) *Methods Enzymol. 81*, 48–52.
Poincelot, R. P., & Abrahamson, E. W. (1970) *Biochemistry 9*, 1820–1825.
Pontus, M., & Delmelle, M. (1975) *Biochim. Biophys. Acta 401*, 221–230.
Rotstein, N. P., & Aveldaño, M. I. (1987) *Biochim. Biophys. Acta 921*, 221–234.
Rouser, G., Fleischer, A., & Yamamoto, A. (1970) *Lipids 5*, 494–496.
Sklar, L. A., Miljanich, P., Bursten, S. L., & Dratz, E. A. (1979) *J. Biol. Chem. 254*, 9583–9591.
Smith, A. D., & Litman, B. J. (1982) *Methods Enzymol. 81*, 57–61.
Stubbs, C. D., Kouyama, T. Kinosita, K., & Ikegami, A. (1981) *Biochemistry 20*, 4257–4262.
Stubbs, G. W., & Litman, B. J. (1978) *Biochemistry 17*, 215–219.
Tsuda, M., & Akino, T. (1981) *Biochim. Biophys. Acta 643*, 63–75.
Watts, A., Volotovski, I. D., & Marsh, D. (1979) *Biochemistry 18*, 5006–5013.
Wu, C.-S. C., Hachimori, A., & Yang, J. T. (1982) *Biochemistry 21*, 4556–4562.
Yoshizawa, T., & Shichida, Y. (1982) *Methods Enzymol. 81*, 333–354.
Zumbulyadis, N., & O'Brien, D. F. (1979) *Biochemistry 18*, 5427–5432.

Liposomes as Model for Taste Cells: Receptor Sites for Bitter Substances Including N–C=S Substances and Mechanism of Membrane Potential Changes†

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ABSTRACT: Various bitter substances were found to depolarize liposomes. The results obtained are as follows: (1) Changes in the membrane potential of azolectin liposomes in response to various bitter substances were monitored by measuring changes in the fluorescence intensity of 3,3′-dipropylthiocarbocyanine iodide [diS-C_3(5)]. All the bitter substances examined increased the fluorescence intensity of the liposome–dye suspension, which indicates that the substances depolarize the liposomes. There existed a good correlation between the minimum concentrations of the bitter substances to depolarize the liposomes and the taste thresholds in humans. (2) The effects of changed lipid composition of liposomes on the responses to various bitter substances vary greatly among bitter substances, suggesting that the receptor sites for bitter substances are multiple. The responses to N—C=S substances and sucrose octaacetate especially greatly depended on the lipid composition; these compounds depolarized only liposomes having certain lipid composition, while no or hyperpolarizing responses to these compounds were observed in other liposomes examined. This suggested that the difference in "taster" and "nontaster" for these substances can be explained in terms of difference in the lipid composition of taste receptor membranes. (3) It was confirmed that the membrane potential of the planar lipid bilayer is changed in response to bitter substances. The membrane potential changes in the planar lipid bilayer as well as in liposomes in response to the bitter substances occurred under the condition that there is no ion gradient across the membranes. These results suggested that the membrane potential changes in response to bitter substances stem from the phase boundary potential changes induced by adsorption of the substances on the hydrophobic region of the membranes.

Among various taste stimuli, substances that elicit bitter taste are most abundant. The structures of bitter substances are extremely diverse, and it is difficult to find a chemical structure common to bitter substances. An attempt to isolate the receptor protein for bitter substances was made (Dastoli et al., 1968), but it was pointed out that the protein isolated from the porcine tongue epithelium was not a true receptor molecule for bitter substances (Price, 1969; Koyama & Kurihara, 1971).

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In a previous paper (Kumazawa et al., 1985), we have reported that the mouse neuroblastoma (N-18 clone)[1] is depolarized by various bitter substances and that there is a good correlation between threshold concentrations to induce the depolarization in the cells and taste threshold concentrations in humans. These result suggest that a specific receptor protein unique to taste cells is not involved in the reception of bitter substances since the N-18 cell must not provide such protein. Koyama and Kurihara (1972) examined the interaction of various bitter substances with lipid monolayers and found that there is a good correlation between concentrations of bitter substances to give an increase in the surface pressure in the monolayers and their taste threshold concentrations. Hence, there is a possibility that bitter substances are adsorbed on the lipid layers of taste cell membranes and cause membrane potential changes of the cells. In the present study, we have used liposomes as model for taste cells and found that liposomes are depolarized by various bitter substances in similar concentration ranges to those where taste cells are depolarized.

It has been pointed out that the receptor sites for bitter substances in taste receptor membranes are not single. Multiplicity of bitter receptor sites was first implied by geneticists studying the bimodal distribution of taste thresholds for the N—C═S substances including phenylthiocarbamide (PTC) (Kalmus, 1971). The idea of multiple receptor sites for bitter substances in humans was further advanced by a number of investigators (McBurney et al., 1972; Hall et al., 1975). Data for multiple bitter receptor sites were also implied for rats (Stewart et al., 1983), mice (Lush, 1981; 1982 Harder et al., 1984), hamsters, and frogs. For example, one strain in mice showed a strong aversion to drinking sucrose octaacetate (SOA), while other strains tended to prefer the SOA solution. Electrophysiological studies revealed that the difference in such behavior of mice to SOA comes from that in their taste receptors (Shingai & Beidler, 1985). In addition, it was shown that the receptor sites for the bitter substances other than the N—C═S substances in hamsters (Herness & Pfaffmann, 1986) and frogs (Sugimoto & Sato, 1981, 1982) are also multiple. In the present study, the effects of changed lipid composition of liposomes on the response to various bitter substances are examined, and it is shown that the multiple receptor sites for bitter substances are explained in terms of difference in lipid composition of the taste receptor membranes.

The mechanism of generation of the taste receptor potential in response to bitter substances has been examined by inserting a microelectrode into taste cells. It was reported that the input resistance of taste cells in the frog (Akaike et al., 1976), mudpuppy (West & Bernard, 1978), and mouse (Tonosaki & Funakoshi, 1984) is little changed or slightly increased during depolarization by quinine. It is still unknown by what mechanism the depolarization by bitter substances is induced. In the present study, we examine the mechanism of depolarization of the lipid membranes by various bitter substances and show evidence indicating that the depolarization is induced by changes in the phase boundary potential.

Materials and Methods

Materials. The following chemicals were obtained from the companies indicated: soybean phospholipids (azolectin), cholesterol, quinine hydrochloride, theophylline, and thiouracil, Wako Pure Chemical Industries, Tokyo; sphingomyelin (SM) from egg yolks and lincomycin hydrochloride, Sigma Chemical Co., St. Louis; egg phosphatidylcholine (PC), Nihon Seika Co., Osaka; L-leucine, caffeine, thiourea, 1,1-diphenyl-2-thiourea, and 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol (bis-Tris), Nakarai Chemicals, Kyoto; phenylthiocarbamide (phenylthiourea) and brucine, Tokyo Kasei Industries, Tokyo; sucrose octaacetate, Kanto Chemical Co., Tokyo.

Soybean phospholipids purchased were washed 3 times with ice-cold acetone and extracted with ice-cold diethyl ether. The extract was evaporated to dryness and stored as a 10% (w/v) solution in chloroform at −20 °C until use. Egg phosphatidylethanolamine (PE) was extracted from fresh chicken egg yolks according to the method of Lea et al. (1955). The purity of PC, PE, and SM was checked by thin-layer chromatography on silica gel plates. Cholesterol purchased was recrystallized twice from methanol.

Preparation of Liposomes. Liposomes were prepared essentially as described previously (Nomura & Kurihara, 1987a,b). The dried lipid film in the flask was dispersed in 100 mM NaCl solution or 100 mM KCl solution containing 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes)–NaOH buffer (pH 7.3). The lipid suspension was sonicated in a bath type sonicator, followed by centrifugation of the sonicated suspension. The supernatant was used in this study. The phospholipid concentration was determined by measuring phosphorus content (Allen, 1940).

The bitter substances were dissolved in 100 mM NaCl solution or 100 mM solutions of various salts containing 5 mM Hepes buffer of pH 7.3; pH was adjusted to 7.3 when necessary.

Monitoring the Membrane Potential Change. Changes in the membrane potential of liposomes in response to bitter substances were monitored by measuring changes in the fluorescence intensity of 3,3′-dipropylthiocarbocyanine iodide [diS-C_3(5)]. The fluorescence intensity changes were measured essentially as described previously (Nomura & Kurihara, 1987). The fluorescence changes of diS-C_3(5) added to liposome suspension occurred immediately after addition of bitter substances and reached the maximal level within at most 2 s and remained constant for at least 30 min. The fluorescence intensity changes in response to bitter substances were measured within 10 min after addition of the substances. The fluorescence intensity change, ΔF, is defined as

$$\Delta F(\%) = (F - F_0)/F_0 \times 100 \quad (1)$$

where F and F_0 represent the fluorescence intensity in the presence and in the absence of bitter substances, respectively. The value of F_0 was unchanged for liposomes of different lipid composition used in the present study. Final concentration of phospholipids was 50 μM. The temperature was maintained at 30 °C by circulating water through the cuvette holder of the photometer.

Measurement of Changes in the Membrane Potential of the Planar Lipid Bilayer. The planar lipid bilayer was formed from monolayers of azolectin essentially according to the method of Montal and Mueller (1972). Detail for measurement of the membrane potential changes was described in previous papers (Nomura & Kurihara, 1987a,b). The cell for preparation of the bilayer consisted of two chambers that had a volume of about 2 cm^3 and an air interface of 1.2 cm in diameter. A Teflon sheet that had a hole of 0.2–0.4 mm in diameter was intercalated between the chambers. The bilayer was formed on the hole by raising the water phase on which monolayers of azolectin were formed by gentle application of

[1] Abbreviations: PC, egg phosphatidylcholine; PE, egg phosphatidylethanolamine; SM, egg sphingomyelin; diS-C_3(5), 3,3′-dipropylthiocarbocyanine iodide; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; bis-Tris, 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol; N-18 cell, mouse neuroblastoma cell (N-18 clone); PTC, phenylthiocarbamide; SOA, sucrose octaacetate.

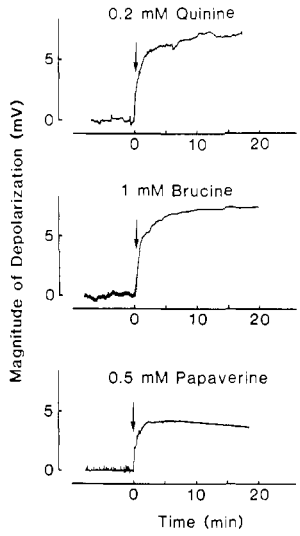

FIGURE 1: Time course of the membrane potential changes of the planar bilayer made of azolectin in response to 0.2 mM quinine, 1 mM brucine, and 0.5 mM papaverine. Arrow in the figure indicates injection of a bitter substance solution to the water phase at cis side.

5 μL of a hexane solution containing 2% azolectin. Formation of the bilayer was checked by measuring the electric resistance. The resistance of the bilayer was 100–200 GΩ. A small volume of a bitter substance solution was added under gentle stirring to the chamber where an electrode was connected to the ground. This side of the chamber is referred to as the cis side. Changes in the membrane potential of the bilayer in response to bitter substances were measured by using a Keithley 610C electrometer with Ag/AgCl electrodes. Both water phases contained a 100 mM NaCl solution containing 5 mM Hepes buffer (pH 7.3).

Culture of Neuroblastoma Cell. Clonal cell line N-18 derived from the mouse C-1300 neuroblastoma was used to examine the relationship between electrophysiological data and changes in the fluorescence intensity of diS-$C_3(5)$. The N-18 cell was cultured as described in previous papers (Miyake & Kurihara, 1983a,b; Kumazawa et al., 1985).

Changes in the fluorescence intensity of diS-$C_3(5)$ added to the N-18 cell suspensions were measured essentially according to the method for liposomes: 2 μL of 0.91 mM diS-$C_3(5)$ was added to 2 mL of Ringer solution containing the cells (5×10^5 cells/mL) in a cuvette. After the cell suspension containing diS-$C_3(5)$ was stirred for 15 min, the fluorescence intensity (F_0) was measured. A small volume of a bitter substance solution was then added to the cuvette. After the cell suspension containing the bitter substance was stirred for 10 min, the fluorescence intensity (F) was measured. The fluorescence intensity change, ΔF, was defined by eq 1.

RESULTS

Membrane Potential Changes of Planar Lipid Bilayer in Response to Bitter Substances. Figure 1 shows typical examples of membrane potential changes in planar lipid bilayer made of azolectin in response to quinine, brucine, and papaverine. The bitter substances were added to the water phase at cis side by injection of a small volume of solution containing the bitter substances of high concentration. The membrane potential across the bilayer starts to change immediately after application of the bitter substances and reaches a steady level. Here the potential at the trans side is changed to a positive direction (we refer to a potential change of this direction as depolarization in this paper). The time course of the membrane potential changes depended on the stirring speed with a magnetic bar of the water phase: a faster stirring led to a rapid change in the membrane potential. In the experiments for Figure 1, a 100 mM NaCl solution was placed in two chambers at both sides of the membrane. Essentially similar membrane potential changes to those shown in Figure 1 were observed in response to the bitter substances when 100 mM NaCl solution and 100 mM KCl solution were used for the solutions at the cis side and trans side, respectively (data not shown). This indicates that the ion gradient across the membrane does not contribute the potential changes in response to the bitter substances.

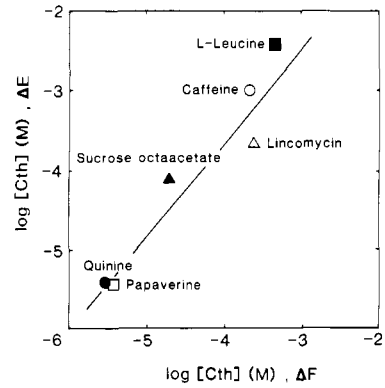

FIGURE 2: Comparison of threshold concentrations of various bitter substances to induce the membrane potential changes (ΔF) monitored with fluorescence changes of diS-$C_3(5)$ with respective thresholds (ΔE) determined electrophysiologically in the N-18 cell. The thresholds for the fluorescence changes were determined by extrapolating the concentration–response curves to control level. The thresholds determined electrophysiologically were taken from a previous paper (Kumazawa et al., 1985).

The changes of the planar lipid bilayer by the bitter substances were measured with quinine, brucine, and papaverine, but those by other bitter substances were not measured because addition of a large volume of saturated solutions of the bitter substances was needed to generate the membrane potential changes and hence led to unbalance of the water level in both chambers. Systematic studies on the membrane potential changes by bitter substances were carried out by using liposomes as shown below.

Membrane Potential Changes of Azolectin Liposomes by Various Bitter Substances. Changes in the membrane potential of liposomes in response to various bitter substances were monitored with a fluorescent dye, diS-$C_3(5)$, in the present study. In the previous paper (Kumazawa et al., 1985), we showed that the N-18 cell responds to various bitter substances. In order to confirm that the fluorescence changes of diS-$C_3(5)$ in response to bitter substances closely monitor the membrane potential changes, the fluorescence changes of the dye–N-18 cell suspension in response to bitter substances were compared with the membrane potential changes measured directly with a microelectrode. Figure 2 compares threshold concentrations of various bitter substances that induce the fluorescence changes of the dye–N-18 cell suspension with respective thresholds determined electrophysiologically. The thresholds were determined by extrapolating the concentration–response curves for various bitter substances to the control level. As seen from the figure, the two sets of data for each bitter substance closely correlate with each other, which suggests that the changes in the fluorescence intensity of diS-$C_3(5)$ in response to bitter substances closely correspond to the membrane potential changes.

Addition of bitter substances to diS-$C_3(5)$–liposome suspensions induced changes in the fluorescence intensity. As shown later, an ion gradient across the liposomal membrane

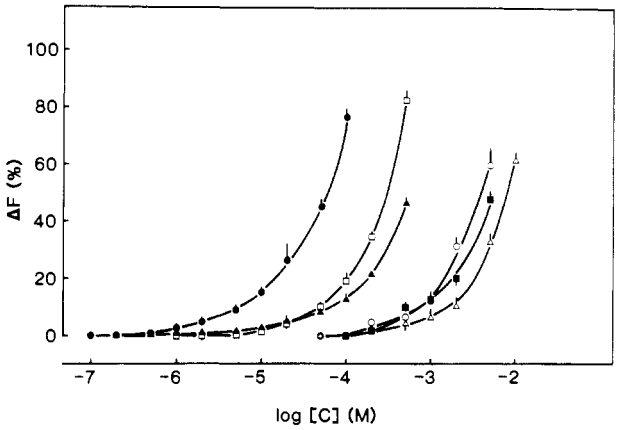

FIGURE 3: Changes in the fluorescence intensity of diS-$C_3(5)$ added to azolectin liposome suspension as a function of concentration of bitter substances. Internal and external solutions for liposomes were 100 mM NaCl. Each point is the mean value of data obtained from at least five preparations. (●) Quinine; (□) papaverine; (▲) brucine; (○) caffeine; (■) theophylline; (△) lincomycin.

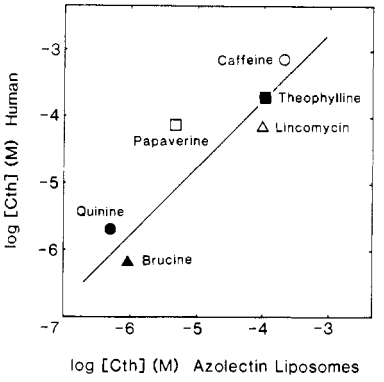

FIGURE 4: Threshold concentrations of various bitter substances to induce the fluorescence changes in diS-$C_3(5)$–azolectin liposome suspension with taste thresholds in humans. The thresholds in the liposomes were determined by extrapolating the curves in Figure 2 to zero level. The human taste thresholds were taken from a previous paper (Kumazawa et al., 1985).

was not necessary for generation of the fluorescence changes, and hence a 100 mM NaCl solution was used for both internal and external solutions for liposomes. Figure 3 shows changes in the fluorescence intensity of diS-$C_3(5)$ added to azolectin liposome suspensions as a function of concentrations of bitter substances. Various bitter substances increase the fluorescence intensity in a dose-dependent manner, which indicates that bitter substances change the potential at the inside of the liposomes to a positive direction. The potential change of this direction corresponds to depolarization. In taste systems, only depolarizing potential changes are effective to generate impulses in the taste nerves. Concentrations of bitter substances that depolarize the liposomes greatly vary among bitter substances. The threshold concentration for each bitter substance was obtained by extrapolating a concentration–response curve for the mean value of data obtained from at least five preparations to zero level. Figure 4 compares threshold concentrations of various bitter substances with taste thresholds in humans (Kumazawa et al., 1985). As seen from the figure, there is a good correlation between both thresholds.

Effects of Changed Lipid Composition of Liposomes on Response to Various Bitter Substances. Figure 5 shows the relative magnitude of the fluorescence change (ΔF value) in suspensions of liposomes having different lipid composition in response to various bitter substances. The liposomes made of azolectin or a mixture of azolectin and SM show relatively

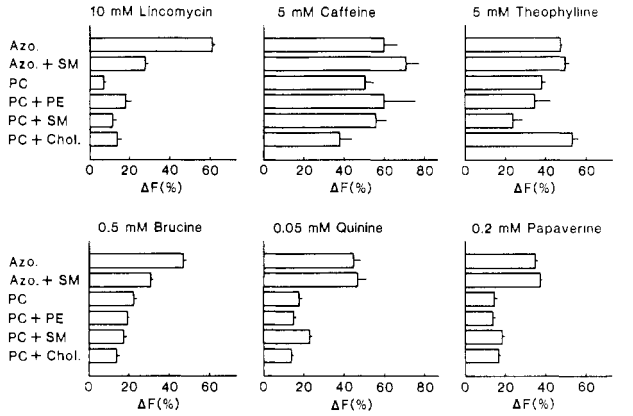

FIGURE 5: Effects of changed lipid composition of liposomes on the responses to various bitter substances. Each column represents magnitude of a fluorescence change (ΔF) ± SD. The lipid compositions of liposomes were as follows: Azo. (azolectin alone); Azo. + SM, 1:1 w/w; PC (PC alone); PC + PE, 4:1 w/w; PC + SM, 10:1 w/w; PC + chol. (cholesterol), 10:1 w/w. The internal and external solutions for liposomes were 100 mM NaCl. The ΔF value is the mean of data obtained from at least five preparations.

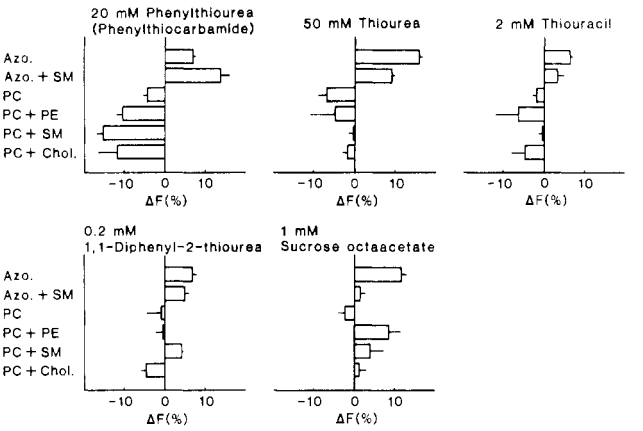

FIGURE 6: Effects of changed lipid composition of liposomes on the responses to N—C═S substances and sucrose octaacetate. The lipid compositions of liposomes were the same as those in Figure 4. The internal and external solutions for liposomes were 100 mM NaCl. The ΔF value is the mean of data obtained from at least five preparations.

large responses to various bitter substances. Liposomes containing PC show relatively small responses to lincomycin, brucine, quinine, and papaverine, while the liposomes show relatively large responses to caffeine and theophylline. Thus, effects of changed lipid composition on the responses greatly vary among bitter substances.

Figure 6 shows the effects of changed lipid composition on the response to thiourea derivatives [phenylthiourea (phenylthiocarbamide), thiourea, thiouracil, and 1,1-diphenyl-2-thiourea]. Only liposomes made of azolectin or a mixture of azolectin and SM show the fluorescence change in a direction corresponding to depolarization. On the other hand, liposomes of other lipid compositions show no fluorescence change or changes in a direction corresponding to hyperpolarization of these compounds except for the response of liposomes made of a mixture of PC and SM to 1,1-diphenyl-2-thiourea. The response of liposomes to sucrose octaacetate also greatly vary with the lipid composition.

Mechanism of Membrane Potential Changes. In order to explore the changes in the membrane potential in response to bitter substances, the effects of ions in external medium on the responses to the bitter substances were examined. Figure 7 compares the magnitude of the fluorescence change in response to 0.1 mM quinine and 0.5 mM brucine when external

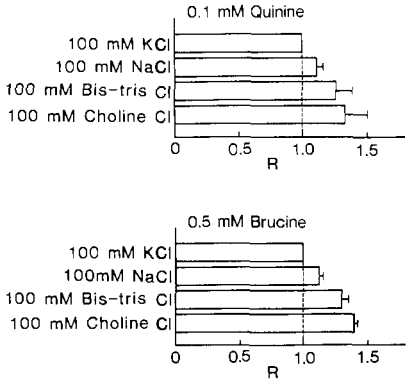

FIGURE 7: Relative magnitude of the fluorescence change of diS-$C_3(5)$ added to azolectin liposome suspension in various salt solutions in response to 0.1 mM quinine and 0.5 mM brucine. Solutions of 100 mM KCl, NaCl, bis-Tris chloride, and choline chloride were used for external solution. The magnitude of the fluorescence change (ΔF value) in 100 mM KCl solution is taken as unity. Internal solution for the liposome was 100 mM KCl. Each column represents data obtained from at least five preparations.

solutions containing various species of cations are used. Here a 100 mM KCl solution is used for the internal solution. The magnitudes of the fluorescence changes in response to the bitter substances in solutions of salts carrying K^+, Na^+, bis-$Tris^+$, and $choline^+$ are not greatly different, although the responses in the solutions containing the inorganic cations are a little larger. The fact that the magnitude of the responses in 100 mM NaCl solution is practically equal to that in 100 mM KCl solution indicates that the magnitudes of the responses to the bitter substances are independent of ion gradient across the liposomal membrane, similar to the responses in the planar lipid bilayer. These results suggest that permeability of ions across the membrane does not contribute to the membrane potential changes. This is consistent with the fact that replacement of inorganic catons by impermeable organic cations does not lead to a decrease in the responses to the bitter substances.

DISCUSSION

In the present study, the membrane potential changes of liposomes in response to bitter substances were monitored with a fluorescent dye, diS-$C_3(5)$. Comparison of fluorescence changes in the dye–N-18 cell suspension with electrophysiological data suggested that the fluorescence changes of diS-$C_3(5)$ in response to bitter substances closely correspond to the membrane potential change. In addition, the membrane potential changes of lipid bilayers in response to bitter substances were electrically measured by using the planar lipid bilayer.

As seen from the fact that many drugs elicit bitter taste, there are multifarious bitter substances. The structures of bitter substances are extremely diverse, and hence the idea that reception of the stimuli occurs via specific receptor protein may not be applicable a priori to the reception of bitter substances. The present results showed that bitter substances cause changes in the membrane potential of liposomes in similar concentration ranges to those where taste cells are depolarized. This strongly suggested that bitter substances are adsorbed on lipid layers of taste receptor membranes and cause the membrane potential changes. While there was a good correlation between the minimum concentrations of the bitter substances to depolarize azolectin liposomes and taste thresholds in humans, the correlation did not hold for PC liposomes (data not shown). This is probably because the lipid composition of azolectin (total lipids from soybean) resembles more closely the average lipid composition of taste cell membranes in humans than PC alone.

It is well-known that there is bimodal distribution of taste thresholds for the N—C=S substances such as PTC: there are "taster" and "nontaster" for certain concentrations of the substances. One may question how this phenomenon is explained by the idea that the bitter substances are perceived by lipid layers of the receptor membranes. The present study demonstrates that the difference in taster and nontaster for the N—C=S substances can be explained in terms of the difference in the lipid composition of taste receptor membranes. There may be a different gene controlling the lipid composition of taste cell membranes between taster and nontaster. It is interesting to note that the sensitivity of the N-18 cell to PTC greatly varies from cell to cell: the first and second types of cells are depolarized by PTC of above 3 and 0.2 mM, and the third type of cells is hyperpolarized by PTC, while the sensitivity to other bitter substances is constant from cell to cell (Kumazawa et al., 1985).

Genetic variation in taste receptor for SOA in mice (Lush, 1981; Shingai & Beidler, 1985) is also explained by the above mechanism. It has been pointed out that the receptor sites for bitter substances besides the N—C=S substances and SOA are also multiple (McBurney et al., 1972; Sugimoto & Sato, 1981; 1982; Herness & Lush, 1982; Steward et al., 1983; Pfaffmann, 1986). The present study showed that the effects of changed lipid composition of liposomes on the response to various bitter substances greatly vary among the substances, suggesting that the multiplicity of the receptor sites for the bitter substances besides the N—C=S substances and SOA can be also explained in terms of a difference in the lipid composition of the receptor membranes. Unfortunately, no information on lipid composition of taste cell membranes of taster and nontaster is available at the present.

In the taste systems, no specific ion in the solution perfusing the taste organ is concerned with generation of the response to bitter substances (Kumazawa et al., 1986). In addition, the membrane resistance is little changed or slightly increased during depolarization. The present results showed that the membrane potential changes of liposomes and the planar lipid bilayer in response to the bitter substances occur under the conditions that there is no ion gradient across the membranes. It is noted that the membrane potential of liposomes and the planar lipid bilayer in response to various odorants occurred under the conditions that there is no ion gradient across the membranes (Nomura & Kurihara, 1987a). In general, the membrane potential is composed of two phase boundary potentials at either side of the membrane and the diffusion potential of ions within the membrane. Under the above conditions, changes in the diffusion potential do not occur since there is no ion gradient across the membranes. Hence, the membrane potential changes in response to bitter substances seem to be brought about by changes in the phase boundary potential at cis side of the membrane.

In the membrane system having high permeability for ions, ion distribution within the membrane easily reaches an equilibrium state. In the equilibrium state, the phase boundary potential change does not lead to the membrane potential change (Kurihara et al., 1986). In the lipid bilayer membranes having a high membrane resistance, the ion distribution in the membrane does not easily reach an equilibrium state, and hence the phase boundary potential change leads to the membrane potential change. This type of membrane potential is called "asymmetric membrane potential" (Kamo & Kobatake, 1974), which is produced only in a nonequilibrium state.

It is noted that the membrane potential change of the planar lipid bilayer membrane induced by protons comes from the phase boundary potential change (Tien, 1974). Recent studies demonstrated that taste cells of various animals have high input membrane resistances [see a review by Kurihara et al. (1986)], suggesting that asymmetric membrane potential can be produced in taste cells. Changes in the phase boundary potential in response to bitter substances seem to be induced by the following mechanism. The bitter substances are adsorbed on the lipid membrane, which will induce a conformational change of the membrane. The conformational change will alter the orientation of the fixed charges and dipoles within the membrane and in turn lead to changes in the phase boundary potential.

Recently, possibilities that cyclic nucleotides may play a role in transduction in the chemosensory systems have been pointed out. Data in taste systems from different laboratories are not consistent with each other. Bitter substances suppressed (Kurihara, 1972) or activated (Price, 1973) the activity of phosphodiesterase in tongue epithelium. While a delivery of cyclic AMP through the frog lingual artery into taste cells led to suppression of the taste nerve responses to chemical stimuli (Nagahama & Kurihara, 1985), an injection of cyclic AMP into isolated taste cells led to a slow depolarization of the cells (Avenet & Lindemann, 1987). It is unknown at the present whether or not cyclic nucleotides play a role in transduction mechanism in taste cells under physiological conditions. The present study demonstrated that bitter substances depolarize the lipid bilayer membranes, suggesting that adsorption of bitter substances on lipid layers of taste receptor membranes leads to depolarization of the cells. The depolarization at the receptor membranes will spread electrotonically to the synaptic area of taste cells and activate the voltage-dependent Ca channels, which leads to a release of a chemical transmitter. There may be a possibility that cyclic nucleotides modulate the above mechanism.

Registry No. Quinine, 130-95-0; brucine, 357-57-3; papavarine, 58-74-2; caffeine, 58-08-2; theophylline, 58-55-9; lincomycin, 154-21-2; phenylthiourea, 103-85-5; thiourea, 62-56-6; thiouracil, 141-90-2; 1,1-diphenyl-2-thiourea, 3898-08-6; sucrose octaacetate, 126-14-7; cholesterol, 57-88-5.

REFERENCES

Akaike, N., Noma, A., & Sato, M. (1976) *J. Physiol. (London) 254*, 87–107.

Allen, R. J. L. (1940) *Biochem. J. 34*, 858–865.

Avenet, P., & Lindemann, B. (1987) *J. Membr. Biol. 97*, 223–240.

Dastoli, F. R., Lopiekes, D. V., & Doig, A. R. (1986) *Nature (London) 218*, 884–885.

Hall, M. J., Bartoshuk, L. M., Cain, W. S., & Stevens, J. C. (1975) *Nature (London) 253*, 442–443.

Harder, D. B., Whitney, G., Frye, P., Smith, J. C., & Rashotte, M. E. (1984) *Chem. Senses 9*, 311–323.

Herness, M. S., & Pfaffmann, C. (1986) *Chem. Senses 11*, 347–360.

Iwasaki, K., & Sato, M. (1981) *Chem. Senses 6*, 119–128.

Kalmus, H. (1971) *Handb. Sens. Physiol. 4 (Part 2)*, 165–179.

Kamo, N., & Kobatake, Y. (1974) *J. Colloid Interface Sci. 46*, 85–93.

Koyama, N., & Kurihara, K. (1971) *J. Gen. Physiol. 57*, 297–301.

Koyama, N., & Kurihara, K. (1972) *Biochim. Biophys. Acta 288*, 22–26.

Kumazawa, T., Kashiwayanagi, M., & Kurihara, K. (1985) *Brain Res. 333*, 27–33.

Kumazawa, T., Kashiwayanagi, M., & Kurihara, K. (1986) *Biochim. Biophys. Acta 888*, 62–69.

Kurihara, K. (1972) *FEBS Lett. 27*, 279–281.

Kurihara, K., & Koyama, N. (1972) *Biochem. Biophys. Res. Commun. 48*, 30–34.

Kurihara, K., Yoshii, K., & Kashiwayanagi, M. (1986) *Comp. Biochem. Physiol. A 85A*, 1–22.

Leach, E. J., & Noble, A. C. (1986) *Chem. Senses 11*, 339–345.

Lush, I. E. (1981) *Genet. Res. 38*, 93–95.

Lush, I. E. (1982) *Chem. Senses 7*, 93–98.

McBurney, D. H., Smith, D. V., & Schick, T. R. (1972) *Percept. Psychophysiol. 11*, 228–232.

Miyake, M., & Kurihara, K. (1983a) *Biochim. Biophys. Acta 762*, 248–255.

Miyake, M., & Kurihara, K. (1983b) *Biochim. Biophys. Acta 762*, 256–264.

Montal, M., & Mueller, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3561–3566.

Nagahama, S., & Kurihara, K. (1985) *J. Gen. Physiol. 85*, 431–442.

Nomura, T., & Kurihara, K. (1987a) *Biochemistry 26*, 6135–6140.

Nomura, T., & Kurihara, K. (1987b) *Biochemistry 26*, 6141–6145.

Price, S. (1969) *Nature (London) 221*, 779.

Price, S. (1973) *Nature (London) 241*, 54–55.

Shingai, T., & Beidler, L. M. (1985) *Chem. Senses 10*, 51–55.

Stewart, C. N., Reidinger, R. F., & Mason, J. R. (1983) *Vertebrate Pest Control and Management Materials: Fourth Symposium*, pp 155–164, American Society for Testing and Materials, Philadelphia, PA.

Sugimoto, K., & Sato, T. (1981) *Comp. Biochem. Physiol. A 69A*, 395–403.

Sugimoto, K., & Sato, T. (1982) *Comp. Biochem. Physiol. A 73A*, 361–372.

Tien, H. Ti (1974) *Bilayer Lipid Membranes; Theory and Practice*, pp 150–158, Marcel Dekker, New York.

Tonosaki, K., & Funakoshi, M. (1984) *Comp. Biochem. Physiol. A 78A*, 651–656.

West, C. H. K., & Bernard, R. A. (1978) *J. Gen. Physiol. 72*, 305–326.