Liposomes as a Model for Olfactory Cells: Changes in Membrane Potential in Response to Various Odorants†

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ABSTRACT: Various odorants were found to depolarize azolectin liposomes. The results obtained are as follows. (1) Changes in the membrane potential of azolectin liposomes in response to various odorants were monitored by measuring changes in the fluorescence intensity of 3,3′-dipropylthiocarbocyanine iodide [diS-C_3(5)]. Ten odorants examined increased the fluorescence intensity of the liposome–dye suspensions in a dose-dependent manner, which indicates that odorants depolarize the liposomes. Concentrations of odorants that depolarized the liposomes greatly varied among the odorants. There existed a good correlation between the minimum concentrations of odorants to depolarize the liposomes and the thresholds of respective odorants in the frog or porcine olfactory responses. (2) Addition of sphingomyelin (SM) to azolectin led to a large enhancement of depolarizations by nonanol, citral, and *n*-amyl acetate. The results indicate that lipid composition of liposomes is one of the factors that control the sensitivity to odorants. (3) Odorants changed the membrane fluidity of the liposomes, which was monitored by changes in the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH). The membrane fluidity was changed in concentration ranges of odorants similar to those where the membrane potential changes occurred, which suggests that changes in the membrane fluidity are related to generation of the membrane potential changes. (4) Changes in the membrane potential in response to odorants were electrically measured with the planar lipid bilayer made of an azolectin–SM (2:1 w/w) mixture. It was shown that odorants (nonanol, citral, and *n*-amyl acetate) depolarized the planar lipid bilayer. These results favor a conclusion that the odorant reception in olfactory systems is induced by adsorption of odorants on a hydrophobic region of the receptor membranes.

There are many types of internal receptors in our bodies for perceiving chemical substances such as hormones or neurotransmitters. These internal receptors provide specific receptor proteins and perceive only certain specified chemicals. On the other hand, the olfactory receptors, which are one of the external receptors, sense chemical substances in external environments, where there are numerous types of substances including artificial substances. How do the olfactory receptors perceive and discriminate such multifarious substances? The olfactory receptors must have unique receptor mechanisms different from those of internal receptors. A number of theories on olfactory reception (Amoore, 1970; Davies, 1971; Hornung & Mozell, 1981; Wright, 1964) have been proposed, but none of them have been verified yet.

Recently, the binding protein for anisol was isolated from dog olfactory epithelium (Price, 1981), but application of an antibody for the protein to the olfactory epithelium inhibited the olfactory responses to every odorant tested (Price, 1984). The binding protein for 2-isobutyl-3-methoxypyrazine was also isolated from the bovine and rat olfactory epithelium (Pelosi et al., 1982; Pevsner et al., 1985). However, the protein is water-soluble and exists in mucus covering the respiratory epithelium as well as in the olfactory epithelium (Pevsner et al., 1986). It was suggested that the protein does not play a direct role in odorant reception but plays a role for the odorant in transporting or concentrating odorants.

The responses to odorants are seen not only in olfactory systems but also in nonolfactory systems such as the vomeronasal organ (Tucker, 1971), the turtle trigeminal nerve (Tucker, 1971), the *Helix* ganglion (Arvanitaki et al., 1967), the *Nitella* internodal cell (Ueda et al., 1975), the fly taste nerve (Dethier, 1972), and the frog taste cell (Kashiwagura et al., 1977). Tucker (1971) stated that all the odorants that stimulate the turtle olfactory system also induce sensitively the responses in the turtle trigeminal nerve. In previous papers (Kashiwayanagi & Kurihara, 1984, 1985), we reported that the mouse neuroblastoma cell (N-18 clone, N-18 cell)[1] is depolarized by various odorants and that the receptor mechanism of odorants in the N-18 cell closely resembles that of the olfactory system. The nonolfactory systems are unrelated to olfactory systems, and hence, it is unlikely that the nonolfactory systems provide specific receptor proteins for odorants, which suggests that specific receptor proteins are not required for odor reception.

Koyama and Kurihara (1972) examined the interaction of various odorants with the lipid monolayers by measuring changes in the surface pressure and found that there is a good correlation between odorant concentrations to give an identical increase in the surface pressure and their olfactory threshold concentrations in humans. This suggested that an interaction between odorants and lipids in olfactory receptor membranes is important for olfactory reception.

Effects of odorants on planar lipid membranes were examined previously (Cherry et al., 1970; Fesenko et al., 1977), but none of the studies demonstrated that odorants cause changes in the membrane potential of the lipid membranes. In this

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[1] Abbreviations: N-18 cell, mouse neuroblastoma cell (N-18 clone); SM, egg sphingomyelin; diS-C_3(5), 3,3′-dipropylthiocarbocyanine iodide; DPH, 1,6-diphenyl-1,3,5-hexatriene; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid.

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study, we have measured the changes in the membrane potential of liposomes in response to various odorants with the use of a voltage-sensitive fluorescent dye. We found that liposomes made of azolectin are depolarized by various odorants and the responses in the liposomes are closely correlated to those in the olfactory systems. This study is the first one to demonstrate that odorants cause changes in the membrane potential of the lipid membranes.

Materials and Methods

Materials. Soybean phospholipids (azolectin; Wako Pure Chemicals, Tokyo) 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. Sphingomyelin (SM) from chicken egg yolk was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. 3,3′-Dipropylthiocarbocyanine iodide [diS-C_3(5); Nihon Sensitive Dye Laboratories Co., Okayama] was dissolved in ethanol and used for the experiments. 1,6-Diphenyl-1,3,5-hexatriene (DPH; Tokyo Chemical Industry, Tokyo) was dissolved in tetrahydrofuran. The odorants used were of the best grade available and dissolved in ethanol.

Preparation of Liposomes. Liposomes were prepared by evaporating 1.0 mL of chloroform solution containing 10% (w/v) azolectin to dryness in a round-bottom flask with a rotary vacuum evaporator. Final traces of volatile materials were removed by storing the flask in a vacuum desiccator for 2–3 h. Glass beads were added to the flask, and the dried lipid film in the flask was dispersed in 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes)–NaOH buffer (pH 7.3) containing 93 mM KCl and 7 mM NaCl by shaking the flask with a vortex mixer at room temperature. The lipid suspension was transferred into a tightly capped glass tube and sonicated at 15 °C for 1 h under argon atmosphere in a bath-type sonicator (W-375, Heat Systems-Ultrasonic Inc.). The sonicated suspension was centrifuged at 100000*g* for 60 min at 4 °C to eliminate undispersed lipids. The supernatant was used in this study. The phospholipid concentration was determined by measuring phosphorus content (Allen, 1940).

Measurement of Changes in the Membrane Potential of Liposomes. Changes in the membrane potential in response to odorants were monitored by measuring changes in the fluorescence intensity of diS-C_3(5). Changes in the fluorescence intensity were measured as follows: A small volume of azolectin liposome suspension was added to 2 mL of 5 mM Hepes–NaOH buffer (pH 7.3) containing 7 mM KCl and 93 mM NaCl in a cuvette, and then 4 μL of 0.91 mM diS-C_3(5) solution was added to the cuvette. After the liposome suspension was stirred for 30 min, the fluorescence intensity at 670 nm (F_0) was measured by exciting the probe at 622 nm. A small volume of an odorant solution in ethanol was then added to the cuvette. The fluorescence intensity (F) was measured at 10 min after the addition of odorants to the liposome suspension. The change in the fluorescence intensity 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 the absence of odorants, respectively. Final concentration of phospholipid in a cuvette was 50 μM. The concentration of ethanol was kept less than 1%. This concentration of ethanol did not affect the fluorescence of liposome–dye suspensions. The fluorescence was measured with a Hitachi spectrophotometer (MPF-2, Hitachi). The temperature was maintained at 30 °C by circulating water through the cuvette holder of the photometer.

Measurement of Fluorescence Polarization. The polarization of the DPH fluorescence in azolectin liposomes was measured as follows: 0.5 μL of 2 mM DPH solution was added to the liposome–suspension dispersed in 2 mL of 5 mM Hepes–NaOH (pH 7.3) containing 7 mM KCl and 93 mM NaCl in a cuvette. After the liposome–suspension containing DPH was stirred for 1 h, a small volume of an odorant solution was added to the cuvette followed by stirring of the solution for 10 min. The polarization of the DPH fluorescence was measured as described previously (Kashiwayanagi & Kurihara, 1985). The following relationship was employed to obtain the polarization (P) of the DPH fluorescence:

$$P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}) \quad (2)$$

Here $I_{\parallel}$ and $I_{\perp}$ stand for the fluorescence intensity parallel to and that perpendicular to the plane of polarization of the excitation beam, respectively. The emission of DPH was excited at 360 nm and monitored at 430 nm through a 390-nm cut-off filter. The molar ratio of DPH to phospholipids was 100, which was used for measurements of the fluorescence polarization in liposomes (O'Connor et al., 1985). Other conditions for the fluorescence measurements were the same as those for the membrane potential measurements.

Measurement of Changes in the Membrane Potential of Planar Lipid Bilayers. Planar lipid bilayers were formed from monolayers of a mixture of azolectin and SM (2:1 w/w) essentially according to the method of Montal et al. (1972; Benz et al., 1975): the cell for preparation of the planar lipid bilayers consisted of two chambers that had a volume of about 2 cm^3 and an air–water 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. Five microliters of 2% azolectin–SM solution in hexane–chloroform (2:1 v/v) was gently applied with a microsyringe onto both water surfaces adjusted below the hole. After evaporation of the solvent, the water surfaces were gradually raised above the hole. Formation of the bilayers was monitored by changes in the electrical capacity. A small volume of an odorant solution was added under a gentle stirring to the cis side chamber, where an electrode was connected to the ground. Changes in the membrane potential of the bilayer in response to odorants were measured on a Keithley 610C electrometer with Ag/AgCl electrodes. The electrical capacity of the bilayer used in this study was calculated to be 1.0 ± 0.1 μF cm^{-2} from the current records, and the effective dielectric thickness of the hydrocarbon region of the bilayer was estimated to be 19 Å (Montal et al., 1972). Both water phases contained 5 mM Hepes–NaOH buffer (pH 7.3), and ionic compositions were 7 mM KCl and 93 mM NaCl for cis side and 93 mM KCl and 7 mM NaCl for trans side.

Cell Culture. A 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 in Dulbecco's modified Eagle's medium containing 2% newborn calf serum and 2% horse serum and incubated in a CO_2 incubator at 37 °C as described in previously papers (Kashiwayanagi & Kurihara, 1984; Miyake & Kurihara, 1983a,b).

Changes in the membrane potential of the N-18 cell were measured by the method of intracellular recording as described previously (Kashiwayanagi & Kurihara, 1984; Miyake & Kurihara, 1983a,b).

Changes in the fluorescence intensity of diS-C_3(5) added to the N-18 cell suspensions were measured essentially ac-

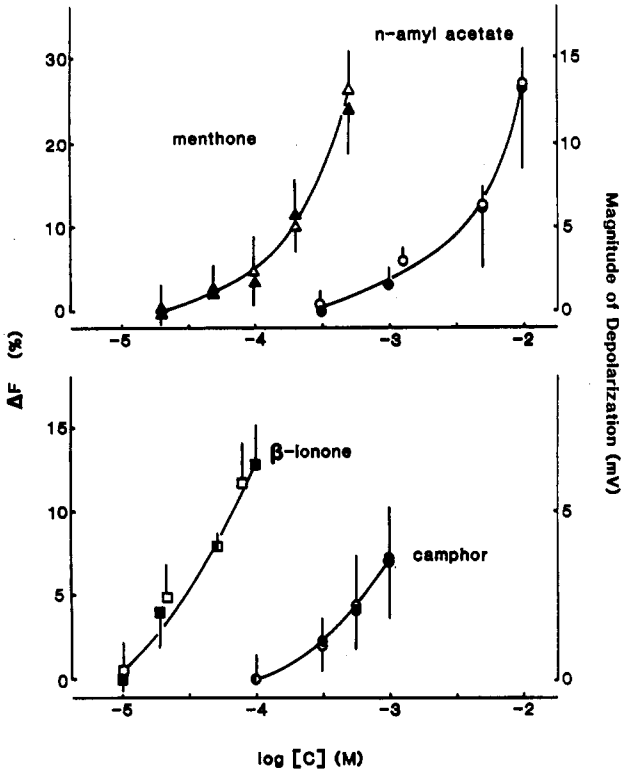

FIGURE 1: Comparisons of changes in the fluorescence intensity (ΔF) of diS-$C_3(5)$ (open mark) with those in the membrane potential (closed mark) of the N-18 cell in response to odorants which were measured with a microelectrode. The data for the membrane potential changes of the N-18 cell by *n*-amyl acetate, β-ionone, and camphor were taken from the previous paper (Kashiwayanagi & Kurihara, 1984). The membrane potential changes induced by menthone were measured in the present study. Each point is the mean value of data obtained from at least four preparations.

cording 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 an odorant solution was then added to the cuvette. After the cell suspension containing an odorant was stirred for 10 min, the fluorescence intensity (F) was measured. The changes in the fluorescence intensity, ΔF, are defined by eq 1. The composition of the Ringer solution was 150 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, and 0.8 mM $MgCl_2$. The pH of the solution was adjusted to 7.3 with 5 mM Hepes–NaOH buffer.

Results and Discussion

Membrane Potential Changes of Liposomes by Various Odorants. Changes in the membrane potential of liposomes in response to various odorants were monitored with the fluorescent dye diS-$C_3(5)$ in this study. In a previous paper (Kashiwayanagi & Kurihara, 1984), we showed that the N-18 cell responds to various odorants. In order to confirm that the fluorescence changes of diS-$C_3(5)$ in response to odorants closely monitor the membrane potential changes, the fluorescence changes of the dye in the N-18 cell suspension in response to odorants were compared with the membrane potential changes measured directly with a microelectrode. Figure 1 compares the fluorescence changes in response to menthone, *n*-amyl acetate, β-ionone, and camphor with respective electrophysiological data as a function of odorant concentrations. As seen from the figure, the two sets of data for each odorant closely correlate with each other, which suggests that the changes in the fluorescence intensity of

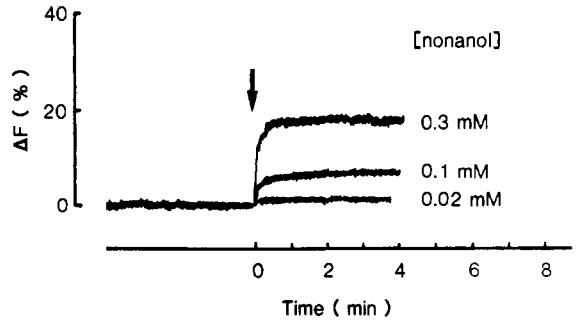

FIGURE 2: Time course of the fluorescence intensity changes (ΔF) of diS-$C_3(5)$ added to an azolectin liposome suspension when various concentrations of nonanol were added. The arrow in the figure indicates the injection of nonanol to the suspension.

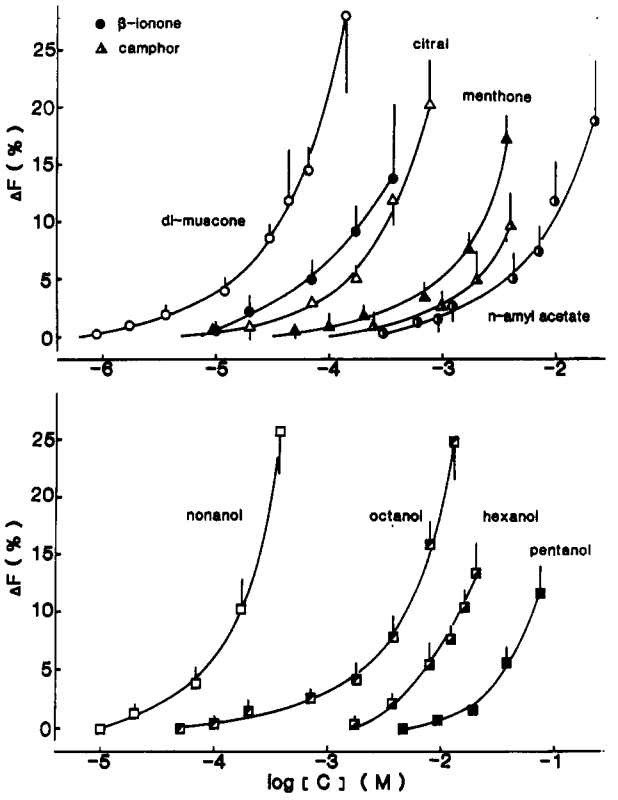

FIGURE 3: Changes in the fluorescence intensity (ΔF) of diS-$C_3(5)$ added to an azolectin liposome suspension as a function of odorant concentrations. Each point is the mean value of data obtained from at least four preparations.

diS-$C_3(5)$ in response to odorants closely correspond to the membrane potential changes.

Figure 2 shows the time course of the fluorescence intensity changes of diS-$C_3(5)$ added to azolectin liposomes in response to nonanol. The fluorescence intensity increases rapidly after addition of nonanol and reaches the maximal level, which is constant at least for 30 min. The maximal level of the fluorescence intensity changes depends on the amount of nonanol added.

Figure 3 shows changes in the fluorescence intensity of diS-$C_3(5)$ added to the azolectin liposome suspension as a function of odorant concentrations. Various odorants increase the fluorescence intensity in a dose-dependent manner, which indicates that odorants change the potential at the inside of the liposomes to a positive direction (we refer a potential change of this direction as depolarization in this paper). In olfactory systems, only depolarizing responses of olfactory cells are effective to generate impulses in the olfactory nerves (Anderson & Ache, 1985; Suzuki, 1977; Trotier & MacLeod, 1983). Concentrations of odorants that depolarize the lipo-

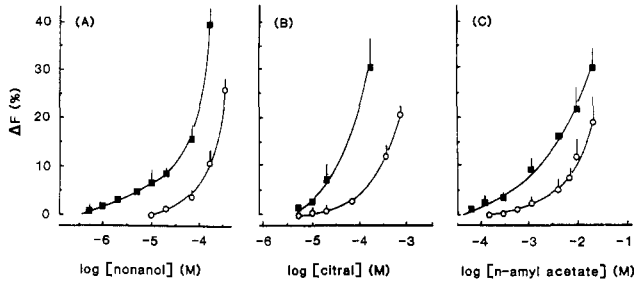

FIGURE 4: Effects of addition of SM to azolectin on the responses to nonanol (A), citral (B), and *n*-amyl acetate (C). Open circles represent data in azolectin liposomes that were taken from those in Figure 3. Closed squares represent data in the liposomes made of an azolectin–SM (2:1 w/w) mixture. Each point is the mean value of data obtained from at least four preparations.

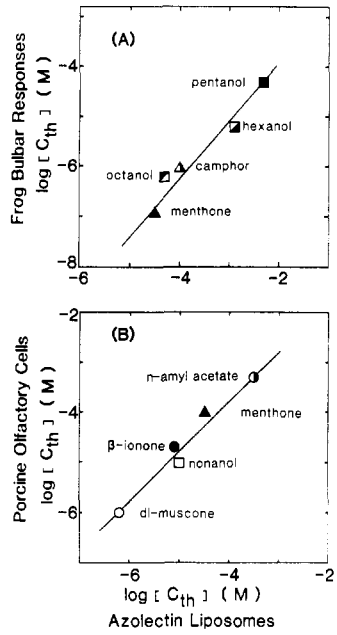

FIGURE 5: Comparisons of the minimum concentrations (C_{th}) of odorants to depolarize azolectin liposomes with those to induce the frog olfactory responses (A) and those to depolarize the porcine olfactory cell suspension (B). The minimum concentrations for the liposomes were taken from data in Figure 3. The data for the frog olfactory responses and the porcine olfactory cell suspension were taken from previous papers (Ohno et al., 1985; Kashiwayanagi et al., 1987).

somes greatly vary among odorants.

Figure 4 shows effects of addition of SM to azolectin on the responses to nonanol (A), citral (B), and *n*-amyl acetate (C) as a function of odorant concentrations. As seen from the figure, addition of SM leads to a large enhancement of the magnitude of depolarizations by the odorants and a lowering of the minimum concentrations of nonanol and *n*-amyl acetate to depolarize the liposomes. This indicates that changes in the lipid composition of liposomes lead to modification of the sensitivity to odorants.

Comparison of Responses to Odorants between Liposomes and Living Cell Systems. Figure 5A compares minimum concentrations of odorants to depolarize azolectin liposomes with those to induce the frog olfactory responses (bulbar responses) (Ohno et al., 1985). The minimum concentrations were obtained by extrapolating the curves in Figure 3 to zero level. There exists a good correlation between the two sets of the minimum concentrations. In Figure 5B, the minimum concentrations of odorants to depolarize azolectin liposomes are also compared with those to depolarize the porcine olfactory cell suspension (Kashiwayanagi et al., 1987). There also exists a good correlation between the two sets of the

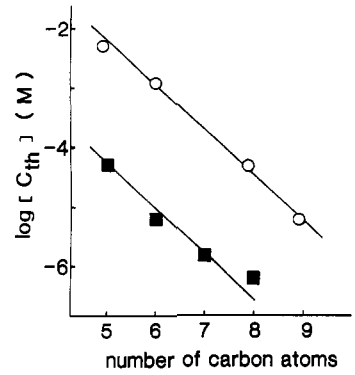

FIGURE 6: Dependence of the minimum concentrations (C_{th}) of alcohols to depolarize azolectin liposomes (○) and the threshold concentrations for the frog olfactor responses (■) on the carbon chain length of alcohols. The data for the liposomes were taken from Figure 3. The data for the frog olfactory responses were taken from a previous paper (Ohno et al., 1985).

minimum concentrations. The depolarization of the liposomes by odorants is induced by adsorption of odorants on the liposomes, which suggests that the order of the minimum concentrations of odorants to depolarize the liposomes is primarily determined by affinity of odorants to the lipid phase. The good correlation between data from the liposomes and those from the olfactory system suggests that the reception of odorants in olfactory cells is induced by adsorption of odorants on the hydrophobic region of the cell membranes.

Relationship between Thresholds and Carbon Chain Length. In the frog olfactory responses, an increase in carbon chain length of alcohols with long alkyl chains leads to a decrease of the threshold concentrations for the responses (Ohno et al., 1985). In Figure 6 (closed square mark), the threshold concentrations of the alcohols for the frog olfactory responses, which were taken from the previous paper (Ohno et al., 1985), are plotted against carbon chain length. There is a linear relationship between the thresholds and the chain length. In Figure 6 (open circle), minimum concentrations of alcohols to depolarize azolectin liposomes are plotted against carbon chain length of alchols, which also gives a straight line with the same slope as that for the olfactory responses. The straight line for the liposomes is shifted in parallel to the higher concentration region because of lower sensitivity of the liposomes to odorants.

The linear relationships shown in Figure 6 are explained in terms of solubility of the alcohols in the lipid phase. According to Tanford (1973), the following equation holds between standard free energies for transfer of hydrocarbons (alcohols in the present case) from aqueous solution to lipid phase ($\mu^\circ_{HC} - \mu^\circ_W$) and carbon chain length (n_C) when the term of activity coefficient of hydrocarbons is neglected:

$$\mu^\circ_{HC} - \mu^\circ_W = RT \ln (X_W/X_{HC}) = -\alpha - \beta n_C \quad (3)$$

Here, X_W and X_{HC} are the concentrations of hydrocarbon in mole fraction units in water and the lipid phase, respectively. Both α and β are proportionality constants. Equation 3 suggests that the linear relationship shown in Figure 6 can be explained by assuming that minimum concentrations of the alcohols to induce the depolarization in the frog olfactory cells and the liposomes mainly depend on solubility of the alcohols in the hydrophobic phase, In other words, the results in Figure 6 support the notion that the depolarizations in both the olfactory cells and the liposomes are induced by adsorption of the alcohols on the hydrophobic region of the membranes.

Changes in Membrane Fluidity by Odorants. Changes in the membrane fluidity of azolectin liposomes in response to

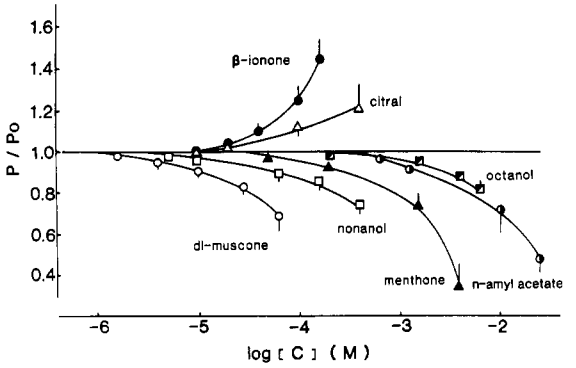

FIGURE 7: Changes in relative polarization value (P/P_0) of the DPH fluorescence in the presence of azolectin liposomes as a function of odorant concentrations. Each point is the mean value of data obtained from at least four preparations.

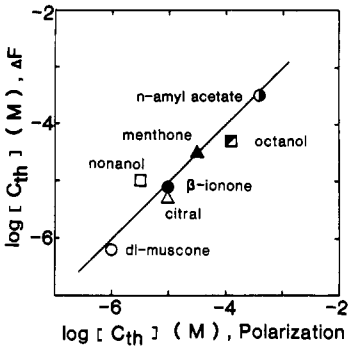

FIGURE 8: Relationships between the minimum concentrations (C_{th}) of odorants to induce the membrane potential changes and those to induce the membrane fluidity changes in azolectin liposomes. The data for the membrane potential changes and those for the membrane fluidity changes were taken from Figures 3 and 7, respectively.

various odorants were monitored by measuring the polarization of the DPH fluorescence. Figure 7 shows changes in the relative polarization value (P/P_0) of the DPH fluorescence in azolectin liposomes as a function of odorant concentrations. P and P_0 stand for the polarization values of the DPH fluorescence in the presence and the absence of odorants, respectively. The P_0 value for azolectin liposomes was 0.113 ± 0.003 (n = 27). As seen from the figure, β-ionone and citral increase the P/P_0 values, which indicates that these odorants decrease the membrane fluidity. Other odorants increase the membrane fluidity. These profiles of the membrane fluidity changes in response to various odorants closely resemble those observed with the porcine olfactory cell suspension (Kashiwayanagi et al., 1987) and the N-18 cell (Kashiwayanagi & Kurihara, 1985). In Figure 8, minimum concentrations of odorants to induce the membrane fluidity changes in azolectin liposomes are compared with those to induce the membrane potential changes. The minimum concentrations were obtained by extrapolating the curves in Figure 7 to the 1.0 level. As seen from the figure, there is a good correlation between the two sets of the minimum concentrations, which indicates that the membrane fluidity changes occur at concentrations similar to those where the membrane potential changes occur.

Membrane Potential Changes of Planar Lipid Bilayer. Figure 9 shows typical examples of the membrane potential changes in the planar bilayer made of a azolectin–SM (2:1 w/w) mixture in response to nonanol (A), citral (B), and *n*-amyl acetate (C). Ionic compositions of the water phases were similar to those for the liposomes: 7 mM KCl and 93 mM NaCl for the cis side and 93 mM KCl and 7 mM NaCl for the trans side. The odorants were added to the water phase at the cis side by bath application. The membrane potential

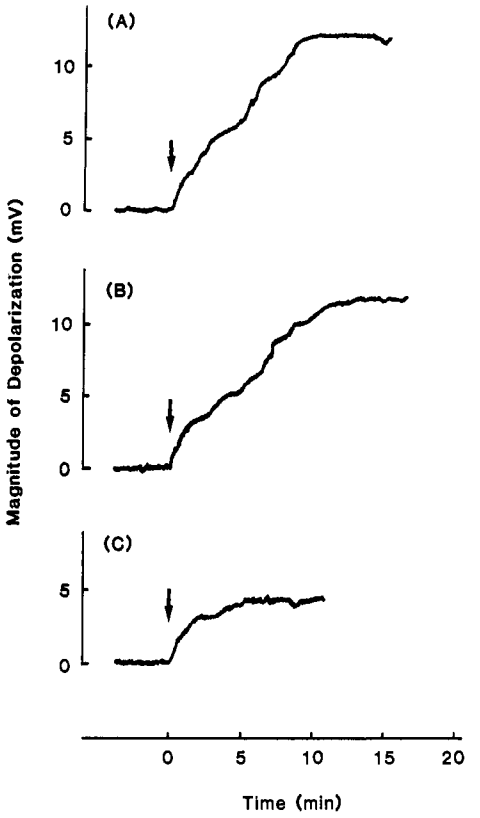

FIGURE 9: Time course of the membrane potential changes of the planar bilayers made of an azolectin–SM (2:1 w/w) mixture in response to 1 mM nonanol (A), 1 mM citral (B), and 22 mM *n*-amyl acetate (C). The arrow in the figure indicates the injection of an odorant solution to the water phase at the cis side.

across the bilayer is gradually depolarized by application of the odorants and reaches a steady level. The time course of the membrane potential changes greatly depends on the stirring speed of the water phase with a magnetic bar. A faster stirring led to a more rapid change in the membrane potential but also to an increase in the noise level of the potential record.

In the above experiments, odorants were added to one side of the chamber under the conditions that there was an ion gradient across the planar lipid layer. Recent preliminary data indicated that the membrane potential changes similar to those shown in Figure 9 were induced by addition of odorants even when 0.1 M NaCl solution was placed in both chambers across the bilayer (data not shown). In addition, depolarization of liposomes in response to odorants also occurred when 0.1 M NaCl solution was used as external and internal solutions for liposomes. In general, the membrane potential is composed of two phase boundary potentials at both sides 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 lipid membrane. Hence, the membrane potential changes in response to odorants seem to be brought about by changes in the phase boundary potential at the cis side of the membrane. In the membrane system having high permeability for ions, where ions easily reach an equilibrium state, changes in the phase boundary potential are canceled out by changes in the diffusion potential within the membrane (Kurihara et al., 1986), and hence, the changes in the phase boundary potential do not lead to the membrane potential changes under the equilibrium conditions. In liposomes, the diffusion potential does not easily change in response to changes in the phase boundary potential since ionic permeability of liposomes is very low. This type of the membrane potential is called

"asymmetric membrane potential" (Kamo & Kobatake, 1974), which is produced only in the nonequilibrium state.

The changes in the phase boundary potential in response to odorants seem to be induced as follows. Odorants 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 a change in the phase boundary potential. The above assumption that odorants induce a conformational change of the membrane is supported by the results that the membrane fluidity is changed at concentration ranges similar to those where the depolarization occurs (Figure 8). The direction of the membrane fluidity change is not identical for all odorants tested: β-ionone and citral decrease the membrane fluidity of azolection liposomes, and other odorants increase the fluidity, although all odorants tested depolarize the liposomes. In general, the membrane fluidity change induced by adsorption of chemicals does not occur homogeneously at all parts of the membrane (Kashiwayanagi & Kurihara, 1985). Probably the position of the membrane monitored with DPH is not identical with the position where the phase-boundary potential change occurs. In addition, the phase boundary potential change that leads to depolarization must be related to a certain direction of changes in orientation of the fixed charges and dipoles in the membrane. For these reasons, it seems that the direction of the membrane fluidity change monitored with DPH is not directly related to the direction of the membrane potential change.

The above mechanism of depolarization is essentially similar to that of depolarization of the N-18 cell in response to odorants that was discussed in the previous paper (Kashiwayanagi & Kurihara, 1985). A more detailed analysis of the membrane potential changes in liposomes will be made in a separate paper.

Conclusions. The present results demonstrate that the lipid membranes are depolarized by various odorants. There exists a good correlation between the minimum concentrations of odorants to depolarize azolectin liposomes and those to induce the olfactory responses in the frog and pig. These results suggest that odorant reception in the olfactory system is induced by adsorption of odorants onto the hydrophobic region of the lipid layer of the receptor membranes. This does not, however, exclude the possibility that odorants interact with the hydrophobic region of protein as well as lipids. The present model for the olfactory reception also well explains the fact that nonolfactory systems respond to various odorants.

Registry No. Nonanol, 143-08-8; β-ionone, 79-77-6; camphor, 76-22-2; *dl*-muscone, 956-82-1; citral, 5392-40-5; menthone, 89-80-5; *n*-amyl acetate, 628-63-7; octanol, 111-87-5; hexanol, 111-27-3; pentanol, 71-41-0.

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