Electric Responses of Bilayer-immobilized Films as Models of a Chemoreceptive Membrane

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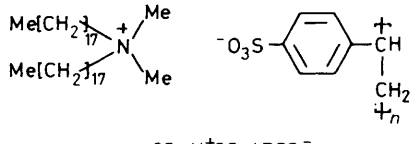
The membrane potential and resistance of a bilayer-immobilized film changed on addition of bitter substances or odorants in aqueous solution; there was a good correlation between the minimum concentration of these additives for inducing the membrane potential and the bitter taste or olfactory threshold in man.

The gustatory and olfactory cells in our bodies can detect multifarious substances in external environments. The transduction mechanism in these chemoreceptors is generally understood as follows:[1] when chemical substances such as sugars, amino acids, or odorants interact with receptor membranes, the membrane potential is changed (depolarized), and the depolarization is propagated electrically to the synaptic region or impulse-generating area of the taste or olfactory cells. The molecular mechanism of the reception of chemical substances and the transduction process are not well understood at present. It has been suggested[2] that although there are specific receptor proteins for sweet substances and amino acids in a taste cell, specific receptors are not involved in the reception of acids, salts, bitter substances, and odorants. The relatively hydrophobic bitter and odour substances are considered to interact directly with lipid bilayer matrices.[2–4]

In this communication, we report that a simple synthetic lipid bilayer cast film can detect some bitter substances and odorants which change the phase-boundary potential or the diffusion potential of the membrane, respectively. We have observed[5] that bitter substances are selectively adsorbed on lipid bilayer matrices, and can be detected by the frequency change of the piezoelectric microbalance crystal coated with a synthetic multibilayer-immobilized film.

The synthetic bilayer film was prepared from polyion complexes of dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1Br^-$) and sodium poly(styrenesulphonate) (PSS^-)[5] and cast on a polyester minigrid (270 mesh, 38 mm^2) attached to the bottom of a plastic tube (7 mm diameter), as previously reported.[6] The cast film ($2C_{18}N^+2C_1/PSS^-$, 100 μm thick) was confirmed to have a sharp phase-transition temperature (T_c) at 45 °C from solid to liquid crystal, in which the ($2C_{18}N^+2C_1$) amphiphiles form extended multibilayer structures parallel to the film plane in the polyion complex with PSS^-. The polyion complex, multibilayer-film was used because it was physically stable and water-insoluble. The



$2C_{18}N^+2C_1 / PSS^-$

electrical cell for membrane potential measurements consisted of Ag/AgCl|sat. KCl|solution I| membrane|solution II|sat. KCl|Ag/AgCl. The upper aqueous solution I in a plastic tube (1.5 ml) and the lower aqueous solution II (50 ml) contained 5mM and 0.5mM NaCl as electrolytes, respectively. The membrane resistance was measured with two platinum electrodes across the membrane, on both sides of which the NaCl concentration was 0.1 M. The membrane potentials and resistances were measured three times in each experiment (experimental error ±5%).

Figure 1 shows the responses of the membrane potential of the multibilayer film when an ethanolic solution (100 μl) of strychnine (a typical bitter substance) or octan-1-ol (a typical odorant) was injected into the lower aqueous solution II (50 ml) at 40 °C. In the case of the addition of strychnine (5×10^{-5} M), the membrane potential was immediately polarized positively within 30 s and a stepwise response was obtained on further additions. When octan-1-ol (2×10^{-4} M) was added, the membrane potential was polarized negatively and the response was very slow (5—10 min). The slow decay of the electrical response with time which was observed on addition of the odorant is not easy to explain.

These responses to strychnine and to octan-1-ol occurred only in the temperature range 35—40 °C, near T_c (45 °C) of the bilayer film; the membrane potential scarcely changed below 30 °C and above 45 °C. The membrane potential of the bilayer film was little changed on addition of a sweet substance (sugar) or L-glutamic acid. There were no significant changes of membrane potential when polystyrene, poly(amino acid), or acetyl cellulose was used as the cast film. Thus the bitter substance strychnine and the odorant octan-1-ol interact specifically with the fluid, disordered state of the multibilayer film changing the membrane potential.

Various bitter substances (caffeine, octa-acetylsucrose, quinine, and strychnine) and odorants (pentyl acetate, β-ionone, and octan-1-ol) were added in the concentration range 10^{-8} to 10^{-1} M, and the changes in membrane potential were plotted against concentrations of additives. The minimum concentration (threshold concentration, C_{th}) for inducing the potential change was observed, and it was found that the magnitude of the potential change increased linearly with the logarithm of the concentration of additive over the threshold concentration. The threshold concentration (C_{th}) varied widely according to the kind of bitter or odorant species applied. The C_{th} values obtained for the bitter substances were plotted against the threshold concentrations for bitter taste for the same additives in man,[7] both on logarithmic scales (Figure 2, closed symbols), giving a straight line. There was also a good correlation between log C_{th} values of odorants for inducing the membrane potential and the logarithm of the olfactory threshold in man[4] for the same odorants (Figure 2, open symbols). Thus, a bitter material or odorant having a lower bitter taste or olfactory threshold in man showed a lower threshold concentration for changing the potential of the synthetic bilayer film. We have observed that bitter substances can be adsorbed on the lipid bilayer matrix, and there is a good correlation between partition coefficients of bitter substances on a lipid bilayer and bitter taste threshold concentration in a biological cell.[5] These results agree with the proposal that the primary process of bitter taste or olfactory reception is adsorption on the lipid bilayer matrix, followed by a change in the membrane potential of the gustatory or olfactory cells.[2—4] Thus, the synthetic multibilayer film can act as a simple model of a chemoreceptive membrane for these additives.

The mechanism for change of the membrane potential was found to be different for bitter substances as compared with odorants. Whenever bitter substances were added over the threshold concentration, the following responses were observed: (i) the membrane potential changed within 30 s (Figure 1), (ii) the potential shifted only positively when the NaCl concentration gradient between the upper solution I and the lower solution II was changed in the opposite direction (C_I/C_{II} = 10 or 0.1) (thus the phase-boundary potential, not the diffusion potential, is predominant in this potential change), and (iii) the membrane resistance was scarcely affected by the addition of 10^{-3} to 10^{-7} M of bitter substance.

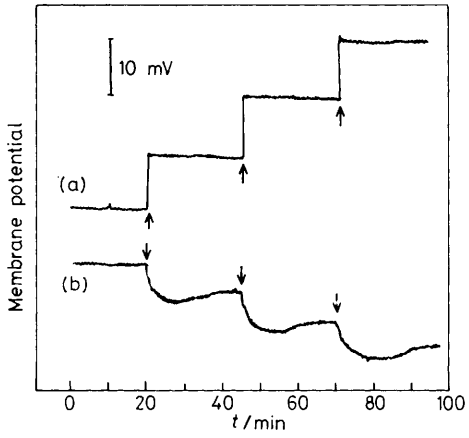


Figure 1. Changes of membrane potentials of the bilayer-immobilized film responding to three consecutive additions of (a) 5×10^{-5} M strychnine and (b) 2×10^{-4} M octan-1-ol at 40 °C. Additives were injected at arrows as ethanolic solutions into aqueous solution (50 ml).

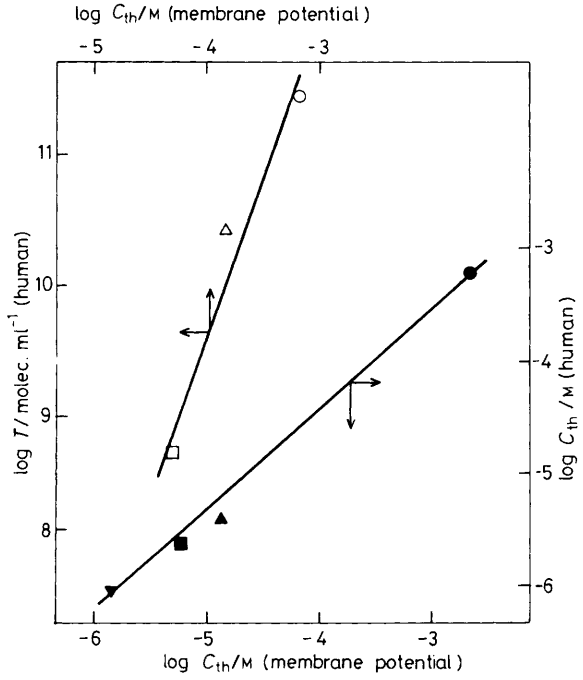


Figure 2. Relation between threshold concentration (C_{th}) of bitter substances (closed symbols) or odorants (open symbols) for inducing changes in the membrane potential and the bitter taste threshold (C_{th}/M in aqueous phase) in man[7] or the olfactory threshold (T/molecules ml^{-1} in air):[4] ▼, strychnine; ■, quinine; ▲, octa-acetylsucrose; ●, caffeine; □, β-ionone; △, octan-1-ol; ○, pentyl acetate.

In contrast, when odorants were added, (i) the membrane potential showed a very slow response (Figure 1), (ii) the negative shift of the membrane potential changed positively when the NaCl concentration gradient was reversed from $C_I/C_{II} = 10$ to 0.1 (thus the diffusion potential is predominant in potential changes), and (iii) the membrane resistance decreased markedly with increase in membrane potential. These results suggest that bitter substances are adsorbed on the surface of the bilayer film and the phase-boundary potential of the membrane is thereby changed; thus the membrane potential can respond quickly without change in membrane resistance. However the relatively hydrophobic odorants can penetrate into the middle of the bilayer film; thus the membrane potential (the diffusion potential) responds slowly to reduction of the membrane resistance (increase of ion permeability).

In conclusion, we have shown that a simple synthetic bilayer film responds specifically to both bitter substances and odorants by changes in the membrane potential, well correlated with the behaviour of gustatory or olfactory cells in man. Such simple membrane systems may provide useful models of chemoreceptors in biological membranes.

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