Effect of Bitter Substances on a Model Membrane System of Taste Reception

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Received March 12, 1986

The effect of bitter substances on the self-sustained electric oscillation of a DOPH (dioleoyl phosphate)-Millipore membrane was investigated as a model system of taste reception. Caffeine and theobromine, having higher taste thresholds, had slight effect, but quinine, strychnine and nicotine, exhibiting strong bitterness, increased the frequency of the oscillation. The mode of action of bitter substances on the DOPH-Millipore membrane in the non-oscillatory state suggested that they cause the receptor potential by a change in the phase boundary potential. The intensity of action on the electric potential was associated with the threshold values of bitterness.

It is generally accepted that taste reception occurs on the gustatory receptor membrane,^{1,2)} mainly composed of lipid and protein. Hiji³⁾ found that the taste responses to sugar and amino acids were selectively eliminated by the application of proteolytic enzyme to a rat tongue. This suggests that other taste sensations such as salty, sour and bitter tastes arise by the interaction with lipid.

The mechanism of the generation of receptor potential is one of the main problems to be solved, but it is not known at present. This partially stems from the smallness of the receptor cell, which prevents investigation of the molecular process of taste reception at the membrane level. This situation compels us to undertake experiments using a model membrane for clarifying the physicochemical mechanism of taste sensation. Furthermore, this kind of study gives some possibility of constructing a taste sensing system with an artificial membrane.

The self-sustained oscillation of electric potential in a synthetic lipid dioleoyl phosphate (DOPH)-Millipore membrane which was found by Kobatake *et al.*⁴⁾ has been investigated experimentally and theoretically by

Toko and Yamafuji.^{5~7)} Recently, it was found that the electric oscillation responds to several taste substances.⁸⁾ For example, the frequency of the oscillation changed proportionately with the concentration of potassium chloride as a salt, and with the logarithm of the concentration of hydrogen ions as a sour substance. In the present study, the effect of bitter substances on the model membrane was investigated.

MATERIALS AND METHODS

DOPH-Millipore membrane. A DOPH-Millipore membrane was prepared by the same method as reported previously.^{5~8)} DOPH was synthesized by hydrolyzing the reactant of oleyl alcohol with phosphorus oxychloride. A Millipore filter of cellulose ester with a normal pore size of 8 μ m was immersed for a few minutes in a solution of DOPH in benzene, and was dried in air. The quantity of DOPH adsorbed within the filter was adjusted to about 3 mg/cm² by controlling the concentration of the DOPH-benzene solution. The DOPH-Millipore membrane was conditioned in 100 mM potassium chloride solution over 12 hours, and later was immersed in 5 mM potassium chloride solution for a few hours.

Measurement of membrane potential and membrane resistance. Figure 1 shows the experimental arrangement

FIG. 1. Schematic Illustration of Experimental Apparatus.

A DOPH-Millipore membrane was placed between two cells containing 5 mM and 100 mM potassium chloride solution, respectively. An area of about 1 cm^2 of the membrane was exposed to the solution. Membrane potential was detected with Ag-AgCl electrodes and recorded on a pen-type X-Y recorder (X-Y). When direct current and/or pressure were necessary, they were applied using a current sweeper (CS) and manometer, respectively. Bitter substances were added to the 5 mM potassium chloride solution.

used for measuring the membrane potential and the membrane resistance. Each membrane was placed between two cells with two pairs of Ag-AgCl electrodes, one of which was used for the measurement of membrane potential, and the other for the constant current supply. The potential change accompanying the application of an electric current makes it possible to evaluate the membrane resistance. The cells were also made so that the pressure difference could be imposed on the membrane.

Treatment with bitter substances. One cell is occupied by 100 mM potassium chloride solution and the other by 5 mM solution. Bitter substances were added to the 5 mM potassium chloride solution, which was stirred throughout the experiment. The reason why chemicals were added to the 5 mM solution is that the self-oscillation occurs within a pore near the side of this solution.⁸⁾ The temperature was kept at 25°C except during the examination of the effect of temperature.

RESULTS

Effect of bitter substances on the self-oscillation of the DOPH-Millipore membrane

The DOPH-Millipore membrane exhibits self-oscillation of electric potential, with a frequency of $0.1 \sim 1\text{ Hz}$ and an amplitude of about 100 mV, when a pressure difference of about 30 cm H_2O and an electric current of about $0.3\text{ }\mu\text{A}$ are imposed on the membrane from the 100 mM to the 5 mM side. Under

FIG. 2. Induction of a Burst of Oscillation by Quinine.

The influence of quinine on the self-sustained oscillation of the DOPH-Millipore membrane is displayed. Time passes from left to right. In the lowest chart, the time scale of the burst caused by $840\text{ }\mu\text{M}$ quinine has been extended.

constant conditions, these oscillations continue stably for a few hours.

As is well known,²⁾ the threshold values of bitter substances are very much lower than other taste stimulants in a real system. The values for humans are as follows: strychnine $1.6\text{ }\mu\text{M}$, picric acid $3.7\text{ }\mu\text{M}$, quinine $8\text{ }\mu\text{M}$, nicotine $19\text{ }\mu\text{M}$, caffeine $700\text{ }\mu\text{M}$, theobromine $750\text{ }\mu\text{M}$.

In Fig. 2, the effect of quinine on the self-oscillating DOPH is shown in part. The membrane potential was oscillating at a frequency of about 1 Hz before the treatment. At a concentration of $10\text{ }\mu\text{M}$ to $160\text{ }\mu\text{M}$, quinine suppressed the oscillation a little, resulting in a decrease in the frequency. It stopped the oscillation at $320\text{ }\mu\text{M}$, and the same situation continued even at $640\text{ }\mu\text{M}$. However, quinine at a concentration of $840\text{ }\mu\text{M}$ decreased the base line of electric potential to some extent, and later it caused a sudden and fast oscillation, namely a burst. The oscillation is expressed as a burst, but it is a very rapid and regular one. The frequency reaches approximately 10 Hz, as shown in the lowest part of the same figure. A burst usually continued for almost one hour. This type of response was not observed in treatment with sour, salty and sweet substances.⁸⁾

Six kinds of bitter substances were used, and tests were conducted four times on each. Strychnine, quinine, and nicotine always brought about a burst at a concentration within a narrow range. The average concen-

TABLE I. THE CONCENTRATIONS OF BITTER SUBSTANCES CAUSING A BURST OF OSCILLATION

Experimental No.	Concentration (mM)		
	Strychnine	Quinine	Nicotine
1	1.20	0.84	0.80
2	0.80	1.60	1.60
3	0.64	1.28	1.20
4	1.00	0.84	0.64

FIG. 3. Effect of Caffeine on the Oscillation of the DOPH-Millipore Membrane.

Time passes from left to right.

tration which caused a burst of oscillation was about 1 mM, as shown in Table I.

The three remaining bitter substances, caffeine, picric acid and theobromine did not exhibit a pronounced influence on the oscillation. As is shown in Fig. 3, these substances caused disturbances, such as an increase of amplitude and an abnormality of oscillation. They did not induce the typical burst, even at 10 mM or above. But the oscillation which was not appreciably altered with 10 mM caffeine was changed to a burst by the treatment of 1 mM quinine.

Effect of bitter substances on the potential and resistance of the DOPH-Millipore membrane

Bitter substances like quinine caused a marked change in the oscillation of DOPH. Next, their influence on the membrane potential and resistance in the non-oscillatory state of the DOPH-Millipore was investigated. The oscillation of the DOPH can easily be stopped by removing the current application.

The mode of change of membrane potential and resistance is shown in Fig. 4, when the

FIG. 4. Effect of Quinine on Electric Potential and Membrane Resistance.

During the measurement of electric potential, quinine was added to 5 mM potassium chloride and its effect on electric potential and membrane resistance was investigated. One division of the time scale corresponds to 1 min. The ordinate E means electric potential. The time of quinine application is shown by an arrow. The vertical and momental change of potential was derived from the application of a 0.01 μ A current.

DOPH, not oscillating, was treated with quinine. The measured electric potential was about -200 mV, when the DOPH was interposed between 5 mM and 100 mM potassium chloride. It consists of electrode, diffusion and phase boundary potentials. The sum of the last two potentials usually corresponds to the membrane potential. The potential decreased with an increase of the concentration of quinine, and finally attained the electrode potential (-77 mV).

The effect of each bitter substance on the electric potential of the DOPH-Millipore is summarized in Fig. 5. The three bitter substances, quinine, strychnine and nicotine lowered the potential at about 100μ M. The possibility that a constituent ion participated in the change of the potential was not ignored, because the bitter substances used were in the form of salt which is composed of two ion species with opposite charges. As a control experiment, therefore, to study the influence of ionic strength, the effect of potassium chloride was investigated. In order to change the potential simply with an ion, ten to hundred times the concentration was needed, as shown in the same figure. Caffeine, picric acid and theobromine had a slight effect.

FIG. 5. Changes of Electric Potential of the DOPH-Millipore Membrane Caused by Bitter Substances.

Only a pressure of 30 cm H_2O was imposed on the membrane from the 100 mm to 5 mm side. The ordinate E means the electric potential. S, strychnine; Q, quinine; N, nicotine; K, potassium chloride; P, picric acid; C, caffeine; T, theobromine.

FIG. 6. Changes of Resistance of the DOPH-Millipore Membrane Caused by Bitter Substances.

The ordinate R means the membrane resistance. All others are the same as in Fig. 5 except S' , which indicates only this strychnine treatment was done without pressure application.

The effect of bitter substances on the membrane resistance is shown in Fig. 6, where the effects of strychnine, quinine and nicotine were also large. Above a concentration of 1 mM, the fall of the resistance was remarkable. When the pressure application was removed, strychnine changed the electric potential in a similar manner, as shown in Fig. 5, but its influence on the resistance was smaller (refer to S' in Fig. 6).

Influence of salt and temperature on the potential change caused by bitter substances

Taste mixture interactions are especially important because most taste stimuli outside of

the laboratory are complex. In mixtures of substances with different taste qualities, the components can still be recognized but are usually perceived as less intense than when unmixed.^{9,10)}

Figure 7 exhibits the effect of potassium chloride on the potential change of the DOPH membrane caused by quinine. The threshold value which caused the change of electric potential increased with increasing concentrations of salt. The increase of the threshold value of quinine implies the weakening of its effect.

Temperature also affected the change of the electric potential caused by quinine as shown

FIG. 7. Decrease of the Effect of Quinine on Electric Potential Caused by Salt.

Current and pressure were not applied. Quinine was added to potassium chloride solution whose concentration differed from 0.1 to 10 mM, and the electric potential was measured. When the concentration of one cell was 100 mM consecutively and that of the other was 0.1, 0.3, 1, 3, 10 mM, the potentials were -358 , -312 , -253 , -190 , and -136 mV, respectively. These values were normalized as relative to the value 100. When an initial potential was -358 mV and it was decreased to -179 ($= -358 \times 0.5$) mV, it is displayed as 50. The concentrations (mM) of potassium chloride are as follows: $\bigcirc$ — $\bigcirc$, 0.1; $\bullet$ — $\bullet$, 0.3; $\triangle$ — $\triangle$, 1; $\square$ — $\square$, 3; $\bullet$ — $\bullet$, 10.

FIG. 8. Decrease of the Effect of Quinine on Electric Potential Caused by Temperature.

The concentrations of potassium chloride on both sides of the membrane were kept at 1 mM and 100 mM, and the effect of quinine was investigated at each temperature. Current and pressure were not applied. Initial potentials differed a little with temperature, so they were normalized in the same manner as in Fig. 7. Temperatures ($^{\circ}\text{C}$) were as follows: $\bigcirc$ — $\bigcirc$, 10; $\bullet$ — $\bullet$, 20; $\triangle$ — $\triangle$, 30; $\square$ — $\square$, 40; $\bullet$ — $\bullet$, 50.

in Fig. 8. It increased the threshold value of quinine, acting to decrease the potential.

DISCUSSION

The strongly bitter substances such as quinine, strychnine and nicotine caused a burst of electric oscillation of the DOPH–Millipore membrane at a concentration within a narrow range. It is considered that the burst is a characteristic effect of these bitter substances. There is a possibility that this type of model membrane could be devised into a kind of taste sensor.

Between the concentrations of 0.1 mM and 1 mM, quinine altered the electric potential markedly, but only slightly affected the membrane resistance. It coincides with the *in vivo* observation that quinine produced a large depolarization, but did not reduce the membrane resistance, in the taste cell of the frog tongue.¹¹⁾ Still more, in this concentration range, a phase boundary potential rather than a diffusion potential can be considered as responsible, because the change of the resistance is small.

The reason why bitter substances induce the burst may be explained simply as follows. The oscillation of electric potential is attributable to the phase transition of DOPH molecules between hydrophobic oil droplets and hydrophilic multilayers.^{5–7)} On the other hand, bitter substances decrease the phase boundary potential as shown in Fig. 5. The decrease of the potential is based on the reduction of the charge of DOPH molecules. The reduction of the charge may increase the rate constant of the phase transition as described in a previous paper,⁸⁾ and may result in the increase of the frequency of oscillation.

The penetration of bitter substances into a lipid membrane has already been shown by Kurihara *et al.*^{12,13)} Recently, they found that bitter substances change the phase boundary potential of the neuroblastoma cell.¹⁴⁾ On the potential change, they assumed that the adsorption of a bitter substance will induce a conformational change of membrane lipid, and decrease its charge density.

Another consideration may be possible. As mentioned by Kurihara *et al.*, hydrophobicity

of a bitter substance accounts for the penetration into a membrane made of DOPH. But strychnine, quinine and nicotine are charged positively at neutral pH. On the other side, the phosphate group of DOPH is charged negatively. The penetration of bitter substances into the membrane may neutralize the charge of the lipid. It results in a reduction of the effective charge and is attributable to a decrease of the electric potential. Taking into account the charge of bitter substances, the reason why strongly bitter picric acid is inactive on DOPH may be explicable. It is charged negatively. A mutual repulsion between negatively charged picric acid and DOPH will make physical contact impossible. Another type of lipid which has a plus charge locally may be influenced by picric acid.

Repression of the effect of quinine by a salt, potassium chloride, agrees well with our familiar experience. As discussed above, bitter substances neutralize the charge of the membrane lipid by penetration into it. Salt may mask the ability of charge neutralization of bitter substances. With the elevation of temperature, the effect of quinine decreased linearly. This result is partly incompatible with our taste sensation, because the greatest sensitivity to quinine is found at 30~35°C.¹⁵⁾ However, the linearity of temperature depen-

dence will be utilized in a taste sensor.

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