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Study of astringency and pungency with multichannel taste sensor made of lipid membranes

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Abstract

Pungent and astringent substances have been studied using a multichannel taste sensor. The electric-potential pattern made up of eight outputs from the membranes of the sensor has information about taste quality and intensity. Pungent substances including capsaicin, piperine and allyl isothiocyanate have no effect on the electric potentials of the lipid membranes. On the other hand, astringent substances such as catechin, tannic acid, chlorogenic acid and gallic acid change the potentials remarkably. A principal-component analysis of the patterns in electric-potential change caused by the taste substances reveals that the taste quality of astringency is located between bitterness and sourness. A model for astringency is presented.

Keywords: Taste sensor; Lipid membrane; Electric potential; Pungency; Astringency; Principal-component analysis; Model for astringency sensation

1. Introduction

Lipid membranes are useful materials for transforming chemical information into electric signals [1–4]. Taste substances are especially effective on membrane potentials because of their electric charges. For these reasons, a multichannel taste sensor with a lipid membrane has been constructed. Using eight lipid membranes as the transducer of taste information and a computer as a data analyser, the sensor responds to different taste qualities by a unique pattern of output signals. As a result, the sensor can clearly distinguish between different brands of beverage including beer, coffee and an aqueous drink [5–10]. It has sensitivity, durability and reproducibility superior to those of humans.

Most studies on taste sensation have been focused on five basic tastes, salty, sour, bitter, sweet and umami. Accordingly the knowledge of taste sensation in terms of astringency and pungency is insufficient. However, these tastes are also indispensable in various foods. It is widely recognized that astringency and pungency are not chemical tastes. Although pungent substances such as capsaicin cause a strong sensation of irritation when applied to the skin [11,12], no systematic studies of this sensation in relation to the action on a biomembrane have been carried out.

Many investigators [13–17] consider that oral astringency, which is often described as drying or puckering, is related to the ability of some chemicals to precipitate or crosslink salivary proteins. Nevertheless, others [18,19] believe astringency to be a gustatory sensation that affects the biomembranes in taste buds. If the pungent or astringent substances affect the membrane potential of the taste sensor, they will produce a chemical taste, because the transducer of the sensor is made of lipids, the constituents of biomembranes.

This paper describes the responses of lipid membranes of the taste sensor to pungent and astringent substances. Astringent substances markedly affected the membrane potentials of the sensor, while pungent substances had no effect.

2. Experimental

2.1. Taste substances

Commercial products were used without further purification. Pungent substances including capsaicin, pi-

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perine and allyl isothiocyanate were dissolved in ethanol. Astringent substances, catechin, tannic acid, chlorogenic acid and gallic acid, were dissolved in 1 mM KCl solution. The pH of the solution was not corrected. Taste substances used for comparison were sodium picrate, quinine hydrochloride and HCl.

2.2. Lipid membranes

The taste sensor with a multichannel electrode was similar to that previously reported [5–10]. Eight kinds of lipid analogues were used for membranes; each lipid was mixed with 400 mg polyvinyl chloride and 0.5 ml dioctylphenyl phosphonate (plasticizer), which were dissolved in 10 ml tetrahydrofuran and dried on a glass plate. The lipid membrane thus prepared was a transparent colourless soft film about 0.2 mm thick. The lipids were abbreviated as follows: dioctyl phosphate, C; trioctylmethylammonium chloride, T; oleyl amine, N; decyl alcohol, DA; oleic acid, OA. Lipid membranes such as C:T=9:1, C:T=3:7 and C:T=5:5 indicate the mixture of two lipids with the ratio of molar concentrations. The membranes C:T=3:7, C:T=5:5, T and N are positively charged, whereas C:T=9:1, C, OA and DA are negatively charged.

2.3. Taste sensor measurements

The experimental set-up is shown in Fig. 1. The construction of this measuring system is as follows: Ag/AgCl electrode in 100 mM KCl solution | membrane | reference electrode in taste solution. Taste substances may change the membrane potential; then the electric signal from each membrane is converted to a digital code by a digital voltmeter (Advantest, R6551) through a high-impedance amplifier and eight-channel scanner, and recorded in a computer (NEC, PC-9801). The sensor output is composed of eight electric potentials from the lipid membranes.

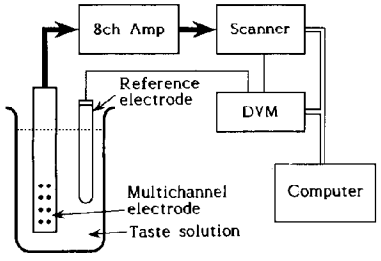

Fig. 1. Experimental set-up. The electric potential across the membrane was detected by the Ag/AgCl electrode in the hole filled with 100 mM KCl and a reference electrode. Eight kinds of lipid membranes were stuck on the multichannel electrode. The constitution of this measuring system is as follows; Ag/AgCl electrode in 100 mM KCl solution | membrane | reference electrode in taste solution. Taste substances may change the membrane potential, and then the electric signal from each membrane is converted to a digital code by a digital voltmeter through a high-input-impedance amplifier and an eight-channel scanner, and recorded in a computer. The sensor output is composed of eight electric potentials from the eight kinds of lipid membranes.

2.4. Principal-component analysis

The taste quality of astringent substances was investigated using principal-component analysis, which is very effective in reducing dimensional space without losing information [20]. Being expressed on an eight-dimensional space from eight kinds of lipid membranes, the original data were visualized on a two-dimensional space.

2.5. Effect of astringent substances on proteins

Albumin (egg white, Wako Jyunyaku), lysozyme (egg white, Wako Jyunyaku) and polypeptone (Nihon Seiyaku) were dissolved in water at a concentration of 0.5%. Astringent substances were added to these protein solutions, and the mixtures were allowed to stand for 30 min at 25 °C. The transmittance of the solutions was measured at 550 nm with a spectrometer (Shimadzu, Spectronic 20A).

3. Results

3.1. Responses of the taste sensor to pungent substances

Pungent substances were almost insoluble in water, so were dissolved in ethanol. When the responses of the taste sensor to pungent substances were measured, these substances were added to 1 mM KCl solution, and their concentration was increased stepwise. The effect of ethanol on the taste sensor was negligibly small.

Fig. 2 shows the response of the taste sensor to capsaicin. Capsaicin, which causes a violent stimulation in the oral cavity, is usually used at low concentrations (3–6 μM) in experiments on taste sensation [21,22]. However, at these concentrations, it was substantially inactive on the electric potentials of lipid membranes. Piperine and allyl isothiocyanate also had no effect on the membrane potentials, similar to capsaicin. Experiments with higher concentrations of pungent substances were difficult because of their precipitation.

3.2. Responses of the taste sensor to astringent substances

The effects of catechin, tannic acid, chlorogenic acid and gallic acid on the taste sensor were investigated. The pH values of these solutions at a concentration

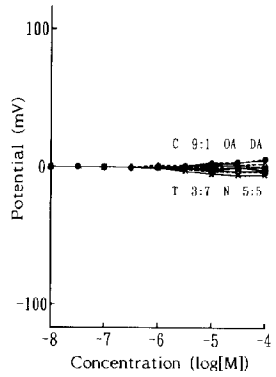

Fig. 2. Response of lipid membrane potential of taste sensor to capsaicin. Each data point was obtained from eight kinds of lipid membranes: O–O, C:T=3:7; ×–×, C:T=5:5; ●–●, T; △–△, N; ● - - ●, C:T=9:1; O - - O, C; △ - - △, OA; × - - - ×, DA.

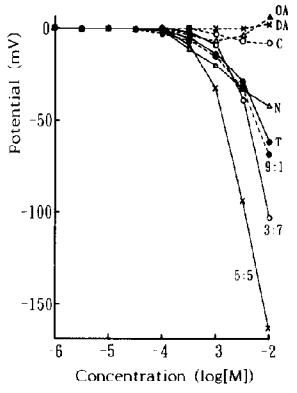

Fig. 3. Response of lipid membrane potential of taste sensor to catechin.

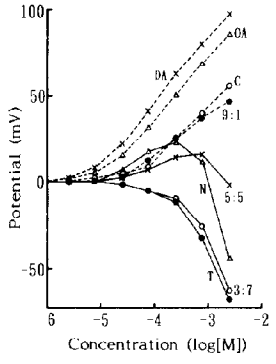

Fig. 4. Response of lipid membrane potential of taste sensor to chlorogenic acid.

of 1 mM were 5.68, 3.93, 3.61 and 3.27, respectively. The response potentials of lipid membranes of the taste sensor to catechin and chlorogenic acid are shown in Figs. 3 and 4, respectively. The astringent substances caused marked changes in the membrane potentials of the sensor. The standard deviations with five measurements on each taste substance were 1–2% in Figs. 3 and 4.

The responses in Figs. 3 and 4 do not resemble each other, although catechin and chlorogenic acid belong to the same group of astringent substances. Catechin changed remarkably the potentials of positively charged lipids such as C:T=5:5 and C:T=3:7, while it had no effect on the potential of negatively charged lipids such as OA and DA. On the other hand, chlorogenic acid effectively changed the potentials of both positively and negatively charged lipids. Tannic acid resembled catechin, while gallic acid resembled chlorogenic acid in the response pattern.

3.3. Principal-component analysis

The taste sensor with lipid membranes responded well to astringent substances. Therefore we searched for qualities of taste similar to astringency. A response pattern like that of chlorogenic acid was easily found in the data obtained in this study. It is the response pattern to HCl (sourness). However, it differs from Fig. 4 in the lipid membranes such as C:T=5:5 and C:T=3:7, indicating the subtle difference between astringency and sourness.

Lea and Arnold [18] suggested that astringency is closely linked to bitterness. In fact, a response pattern similar to that of catechin was obtained with a bitter substance, sodium picrate. However, there are some exceptions in the response of the membranes composed of lipids N and C:T=5:5. Quinine, which is also a bitter substance, did not show a response pattern similar to that of catechin.

These results indicate that among the astringent substances, some of them are close to acidic substances, and others to bitter substances. Therefore, a principal-component analysis of the patterns obtained with the sensor was carried out to confirm the similarity to a bitter or a sour taste. The responses of the lipid membranes at the following concentrations (mM) were used for the analysis: catechin, 0.1, 0.3, 1, 3, 10; tannic acid, 0.1, 0.3, 1, 3, 10; chlorogenic acid, 0.025, 0.075, 0.25, 0.75, 2.5; gallic acid, 0.05, 0.15, 0.5, 1.5, 5; sodium picrate, 0.1, 0.3, 1; HCl, 0.1, 0.3, 1, 3, 10; quinine hydrochloride, 0.1, 0.3, 1, 3, 10. As shown in Fig. 5, the qualities of catechin and tannic acid are close to those of sodium picrate (bitter), whereas those of chlorogenic acid and gallic acid are located between the qualities of sodium picrate (bitter) and HCl (sour).

3.4. Effect of astringent substances on proteins

Astringent substances markedly affected the lipids in membranes. On the other hand, it is well known that these substances precipitate or crosslink proteins

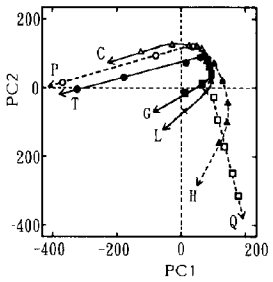

Fig. 5. Principal-component analysis. The first and second principal components are denoted by PC1 and PC2, respectively. The unit of PC1 and PC2 is mV, but these axes do not always indicate absolute values because of a linear transformation, which visualizes the original data from eight kinds of membranes on the two-dimensional plane: C, catechin; T, tannic acid; L, chlorogenic acid; G, gallic acid; P, sodium picrate; H, HCl; Q, quinine hydrochloride. Concentration of each taste substance increases in the direction of the arrow.

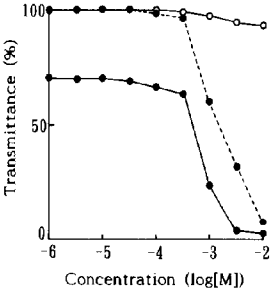

Fig. 6. Effect of tannic acid on transmittance of protein solutions: O–O, lysozyme; ● - - ●, polypeptone; ●–●, albumin.

[14,15]. Therefore the interaction of astringent substances with proteins was investigated. Fig. 6 shows the effect of tannic acid on the transmittance of a protein solution. Albumin was somewhat turbid originally. Tannic acid decreased the transmittance of polypeptone and albumin solutions, while the effect on lysozyme was quite small. A conformational change of proteins caused by astringent substances may have induced the decrease of solubility and the decrease of transmittance. The effect of catechin was smaller than that of tannic acid.

4. Discussion and conclusions

Although pungent substances such as capsaicin, piperine and allyl isothiocyanate offer a violent stimulation in the oral cavity, they did not affect the membrane potential of the taste sensor. Some reports suggest that the capsaicin-sensitive neurons, which are associated with taste buds, are separated from primary taste neurons [11,23]. These substance may not produce a chemical taste as widely recognized in the field of taste sensation. The results from the taste sensor agree with this recognition.

The astringent substances, which are well known to generate a tactile sensation [13–17], altered the membrane potentials of the taste sensor markedly. The naturally occurring phenolic compounds, which dissociate into ions in solution, are correlated with astringency. Fig. 4 shows that the negatively charged portion of chlorogenic acid would have affected the positively charged lipids such as C:T = 3:7 and T to lower the membrane potentials. On the other hand, dissociated protons would have affected the negatively charged lipids such as DA, OA and C to increase the membrane potentials.

It is natural to deduce from these results that the astringent substances produce a chemical taste in the oral cavity, because the transducer of the sensor is made of lipids, which are the constituents of biomembranes. The important role of lipids on taste and odour reception is suggested by Kurihara et al. [24]. The principal-component analysis in Fig. 5 indicates that the quality of astringency is located between those of bitter and sour tastes. In contrast, as is shown in Fig. 6, astringent substances precipitate some proteins; this may produce the widely recognized tactile sensation [13–17]. Therefore, they will have two kinds of sensation, namely a tactile sensation and a gustatory sensation. More detailed study of the effect of astringent substances on biological membranes may be necessary.

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