The Effect of Various Substances on the Suppression of the Bitterness of Quinine–Human Gustatory Sensation, Binding, and Taste Sensor Studies

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The purpose of this study was to quantify the degree of suppression of the perceived bitterness of quinine by various substances and to examine the mechanism of bitterness suppression. The following compounds were tested for their ability to suppress bitterness: sucrose, a natural sweetener; aspartame, a noncaloric sweetener; sodium chloride (NaCl) as the electrolyte; phosphatidic acid, a commercial bitterness suppression agent; and tannic acid, a component of green tea. These substances were examined in a gustatory sensation test in human volunteers, a binding study, and using an artificial taste sensor. Sucrose, aspartame, and NaCl were effective in suppressing bitterness, although at comparatively high concentrations. An almost 80% inhibition of bitterness (calculated as concentration %) of a 0.1 mM quinine hydrochloride solution required 800 mM of sucrose, 8 mM of aspartame, and 300 mM NaCl. Similar levels of bitterness inhibition by phosphatidic acid and tannic acid (81.7, 61.0%, respectively) were obtained at much lower concentrations (1.0 (w/v)% for phosphatidic acid and 0.05 (w/v)% for tannic acid). The mechanism of the bitterness-depressing effect of phosphatidic acid and tannic acid was investigated in terms of adsorption and masking at the receptor site. With phosphatidic acid, 36.1% of the bitterness-depressing effect was found to be due to adsorption, while 45.6% was due to suppression at the receptor site. In the case of 0.05 (w/v)% tannic acid, the total bitterness-masking effect was 61.0%. The contribution of the adsorption effect was about 27.5% while the residual masking effect at the receptor site was almost 33%. Further addition of tannic acid (0.15 (w/v)%), however, increased the bitterness score of quinine, which probably represents an effect of the astringency of tannic acid itself. Finally, an artificial taste sensor was used to evaluate or predict the bitterness-depressing effect. The sensor output profile was shown to reflect the depressant effect at the receptor site rather well. Therefore, the taste sensor is potentially useful for predicting the effectiveness of bitterness-depressant substances.

Key words bitterness depressant; quinine hydrochloride; phosphatidic acid; sucrose; sodium chloride; tannic acid

Humans can perceive and distinguish between five components of taste, namely, sourness, saltiness, sweetness, bitterness, and umami (in Japanese). In general, bitter-tasting medicines are difficult and unpleasant for patients to swallow, leading to noncompliance and thus decreased therapeutic efficacy. Although various physical methods, such as film coating, have been used in attempts to decrease the perception of bitterness of medicines, some drugs are still administered as a syrup or solution, using additives such as sucrose to reduce the bitterness of the formulation. Unfortunately, this method often fails to suppress the bitterness sufficiently.

In the present study, quinine was used as a bitterness standard, while the following substances were evaluated for their effectiveness as bitterness suppressants: sucrose, aspartame, electrolytic NaCl, phosphatidic acid, and tannic acid. Sucrose and aspartame are widely recognised as sweeteners, able to reduce the bitterness of bitter substances.[1,2] Sodium chloride as the electrolyte has also been reported to inhibit bitterness.[3,4] Phosphatidic acid has recently been developed as a bitterness depressant for use in the medical field,[5,6] while tannic-acid-related compounds are found in green tea, which has an astringent taste and is sometimes used to mask the bitter taste of medicine in Japan.

The bitterness-suppressing effects of these substances were examined in gustatory sensation tests using human volunteers. The binding ratios of quinine to each of these bitterness-depressant substances were determined using chromatographic analysis of various mixtures, and the relation between this binding ratio and the results of the gustatory sensation tests was investigated.

Finally, we also examined whether the artificial taste sensor could be used to predict the results of gustatory sensation tests, by examining the bitterness inhibitors in this system.

Experimental

Materials Quinine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), dissolved, and diluted to 0.1 mM with 10 mM KCl. Sucrose, aspartame, and tannic acid were obtained from Nacalai Tesque Co. (Kyoto, Japan). Phosphatidic acid (BMI-40®), as a commercial bitterness-suppression agent, was supplied by Kao Chemical Co., Ltd. (Tokyo, Japan). All other reagents were of special reagent grade.

Gustatory Sensation Study The standard quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30, and 1.00 mM and the corresponding bitterness scores were defined as 0, 1, 2, 3, and 4, respectively. Before testing, the volunteer subjects ($n=11$) were asked to keep the above standard samples in their mouths, and were told their concentrations and bitterness scores. After tasting a 5 ml of a test drug solution (5 ml), they were asked to give the sample a bitterness score. All samples were kept in the mouth for 15 s. After tasting the sample, subjects gargled well and waited for at least 20 min before tasting the next sample.

Bitterness Suppression of Quinine by Various Substances in Human Volunteers The 0.1 mM concentration of quinine hydrochloride was chosen as the control solution for the bitterness-suppression study. The concentrations of the test substances were: sucrose: 30, 150, and 750 mM; aspartame: 0.03, 0.15, and 7.5 mM; NaCl: 30, 150, and 300 mM; phosphatidic acid: 0.001, 0.01, 0.1, and 1.0% (w/v); and tannic acid: 0.005, 0.015, 0.05, and 0.15% (w/v).

Evaluation of Binding of Quinine to Phosphatidic Acid and Tannic Acid Quinine hydrochloride solutions (0.1 mM) containing various concentrations of phosphatidic acid (0.01, 0.1, 0.25, 0.5, 1.0 (w/v)%), tannic acid (0.005, 0.015, 0.05, 0.15 (w/v)%), sucrose (30, 150, 750 mM), aspartame (0.03, 0.15, 7.5 mM) or NaCl (30, 150, 300 mM) were prepared, mixed thoroughly, immediately centrifuged (3000 rev/min for 20 min, Hitachi CR5B2,

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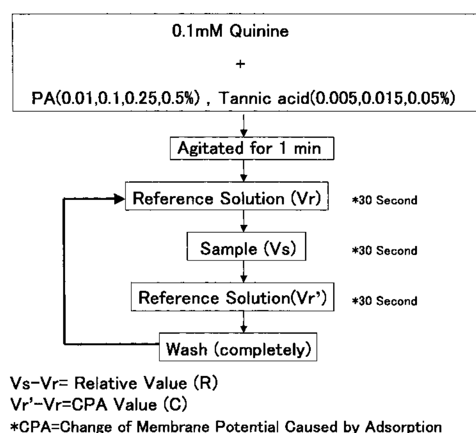

Chart 1. Measuring Procedure in This Study

Tokyo, Japan) and filtered by membrane filter with 0.45 μm-pore size (the recovery of quinine was over 98% and adsorption of quinine to filter was not observed). The concentration of the filtered solution was determined using HPLC: 100 μl was injected onto a chromatograph (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10AV), an integrator (Shimadzu C-R6A), and a reversed-phase column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai Tesque Co., Ltd., Kyoto). The following mobile phase system was used: A, water; B, acetonitirile; C, methanesulfonic acid; D, diethylamine (A : B : C : D=43 : 32 : 1 : 1). The flow rate was 1.0 ml/min and the wavelength was set at 235 nm. The procudure was repeated five times for each sample and the binding ratios of quinine to phosphatidic acid and tannic acid were calculated. Throughout above binding experiments, standard deviation of obtained values of binding faction were almost within 4% and reproducibility was confirmed (detail data not shown).

Sensor Measurement and Data Analysis The sensor measurement, data analysis, and lipid components used in the sensor are essentially the same as those described in a previous paper.[7] Samples of 0.1 mM quinine hydrochloride solution containing various concentrations of substances for evaluation of bitterness suppression were used in the study. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid was used as a reference sample, corresponding to saliva, and also to rinse the electrodes after every measurement. The measurement method used to maximise the sensitivity and the selectivity of adsorption of the test substances is summarized in Chart 1. The relative sensor output is represented as the difference (Vs−Vr) between the potentials of the sample (Vs) and of the 30 mM KCl solution containing 0.3 mM tartaric acid (reference solution, Vr). When the electrode was dipped into the reference solution again, the obtained potential is defined as Vr′. The difference (Vr′−Vr) between the potentials of the reference solution before and after sample measurement is defined as CPA (change of membrane potential caused by adsorption), which corresponds to a bitter aftertaste. Each measuring time was set at 30 s, and the electrodes were adequately rinsed after each measurement. SAS version 6.12 (SAS Ltd., Tokyo, Japan) was used for regression analysis. The CPA values for channel 4 were used in the present study, as this channel showed the largest absolute values of CPA.

Results and Discussion

Bitterness Suppression of Quinine by Various Substances in Human Volunteers Figure 1A shows the effects of sucrose, aspartame, and NaCl on the bitterness score of a 0.1 mM quinine hydrochloride solution in human volunteers. The bitterness score of the quinine solution decreased with increased amounts of all three sweeteners. The concentration of aspartame, however, was actually 1/100th of the concentration depicted. This means that aspartame was almost 100 times more efficient than sucrose with respect to its ability to decrease the bitterness score. This phenomena agrees with the findings of our previous study.[7] Bitterness was also dramatically decreased by 300 mM NaCl. Hellekant *et al.*[8] have reported that taste interactions between salts, such as NaCl and KCl, and bitter-tasting compounds, can lead to suppression of bitterness. They found NaCl to be more effective than KCl, and suggested that the sodium ion was the key for suppression of bitterness. Our gustatory sensation results agree with these findings.

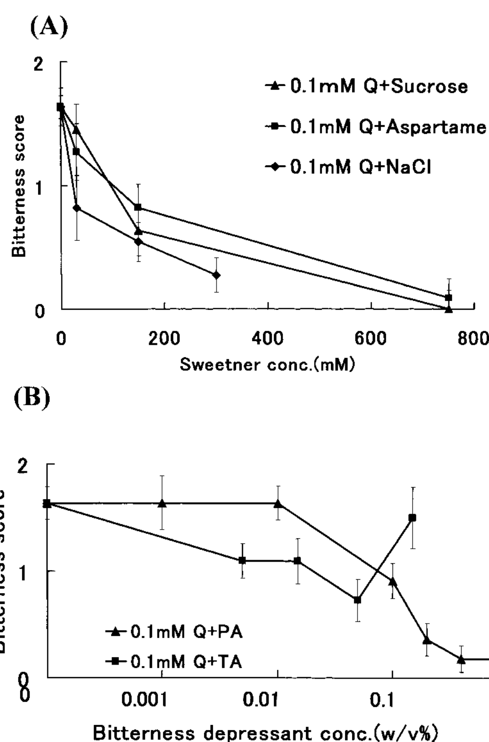

Fig. 1. Relationship between the Human Gustatory Bitterness Score and the Added Concentrations of (A) Sucrose, Aspartame, and NaCl, and (B) Phosphatidic Acid (PA) and Tannic Acid (TA)

The data represent the mean±S.E.M. (n=11). The concentration of aspartame is actually 1/100th of that stated.

Figure 1B shows the effects of phosphatidic acid and tannic acid on the bitterness score of a 0.1 mM quinine hydrochloride solution. Phosphatidic acid dramatically suppressed the bitterness of 0.1 mM quinine hydrochloride solution. On the addition of 1.0 (w/v)% of phosphatidic acid, the bitterness score was reduced to almost 10% of control values. In the case of tannic acid, the bitterness score was reduced with increasing concentrations up to 0.05 (w/v)%, but at a concentration of 0.15 (w/v)% no further bitterness inhibition was observed; in fact, bitterness suppression was reduced. Furthermore, when 0.15 (w/v)% tannic acid was added to a 0.1 mM quinine solution, the bitterness of the solution was enhanced, such that the bitterness score obtained was 1.5, significantly higher than control levels.

Evaluation of the Binding of Quinine to Phosphatidic and Tannic Acids Quinine did not bind to NaCl, sucrose, or aspartame (data not shown), the unbound fraction of quinine being almost 100% with these compounds. This result is as expected, as NaCl, aspartame, and sucrose are all hydrophilic and not likely to adsorb the quinine molecule. Figure 2 shows the relationship between the concentrations of (A) sucrose, aspartame and NaCl, and (B) phosphatidic acid and tannic acid and their bitterness-suppressing strength (left axis), or the unbound % of quinine (right axis). Bitterness strength (%) in left axis does not represent the bitterness score itself as shown in the case of Figs. 1A and B. It was ex-

pressed in terms of equivalent quinine concentrations as relative value % since right axis was simultaneously represented as unbounded ratio %. As shown in Fig. 2B, as the concentration of phosphatidic acid (PA) increased, the bitterness strength, calculated by converting the obtained bitterness score to its equivalent quinine concentration, decreased. The addition of 1.0 (w/v)% phosphatidic acid to 0.1 mM quinine solution gave almost 80% (precisely 81.7%±3.3%) bitterness depression. When expressed in terms of the binding of quinine to phosphatidic acid, the effect was not so great and was maintained in the range of 30—40%. In fact, the binding fraction of quinine in the presence of 1.0 (w/v)% of phosphatidic acid was 36.1±3.5%, and the unbound fraction of quinine was estimated to be almost 64%.

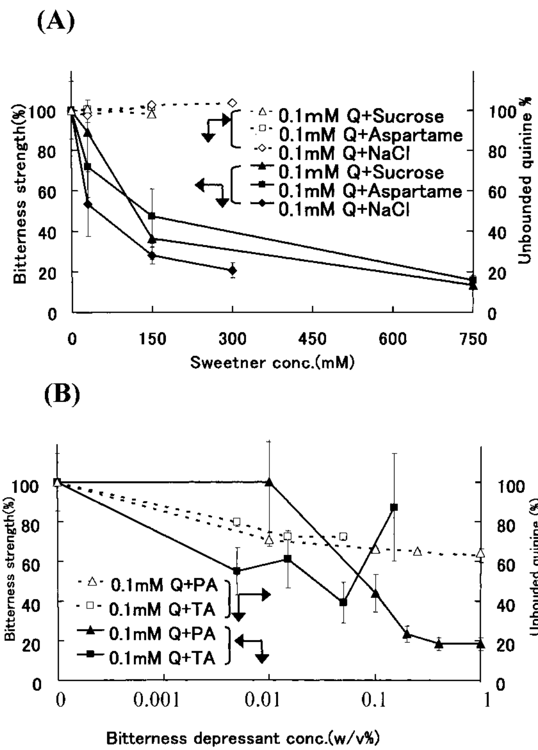

Fig. 2. The Relationship between the Added Concentrations of (A) Sucrose, Aspartame, and NaCl, and (B) Phosphatidic Acid and Tannic Acid, and the Bitterness Strength Expressed as Equivalent Quinine Concentrations (Left Axis, Continuous Lines), or Unbound Ratios (%) of Quinine (Right Axis, Dotted Lines)

The equivalent quinine concentrations are derived from human gustatory sensation data, while the unbound quinine ratios are derived from the binding study.

Finally, as summarized in Fig. 3, in the case of 1.0 (w/v)% PA, when the total bitterness-depressing effect is calculated in terms of equivalent quinine concentrations, the role of adsorption can be determined as 36.1%, while receptor blocking accounts for 45.6%, giving a total value of 81.7% bitterness depression. In the presence of over 0.1 (w/v)% phosphatidic acid, although the unbound quinine fraction was constant at about 60%, the calculated bitterness strength decreased considerably (from almost 44% to 18%), as the phosphatidic acid concentration increased from 0.1 (w/v)% to 0.5 (w/v)%. This suggests that phosphatidic acid competes with quinine to bind to the bitterness receptor site. Thus the role of phosphatidic acid at bitterness receptor site seems to be so important.

Figure 2B also shows that, when the concentration of tannic acid increased from 0.005 (w/v) up to 0.05 (w/v)%, the bitterness score as evaluated by the gustatory sensation test decreased to about 61% compared to control, even though comparatively large variances were observed. In the adsorption study, the unbound fraction was about 72%, and the variance was small. Nevertheless, when the concentration of tannic acid increased from 0.05 (w/v)% to 0.15 (w/v)%, the bitterness of quinine (closed square symbol in Fig. 2B) was enhanced. If tannic acid were to suppress the bitterness in the same way as phosphatidic acid, *i.e.*, *via* competition at the receptor site, the bitterness score would be expected to decrease with increasing concentrations of tannic acid, which is not the case. Tannic acid solution of 0.05 (w/v)% showed comparatively strong astringency. Increase of concentration up to 0.15 (w/v)% give rise to more significant increase of astringency (astringency strength data not shown). This severe astringency might affect bitterness strength for quinine.

The difference in effect between tannic acid and phosphatidic acid is thought to be due to their inherent tastes: phosphatidic acid has no taste while tannic acid is astringent. Thus, while the moderate astringency of low concentrations of tannic acid may reduce the bitterness of quinine, the astringency of more concentrated tannic acid solutions might enhance the bitterness. While we do not have direct evidence to support this hypothesis, this interaction, together with the astringency of tannic acid *per se*, seems to exert its main effect centrally. Phosphatidic acid, on the other hand, is not highly astringent and has its greatest effect peripherally, *i.e.*, mainly at the receptor site in taste cells. The proposed

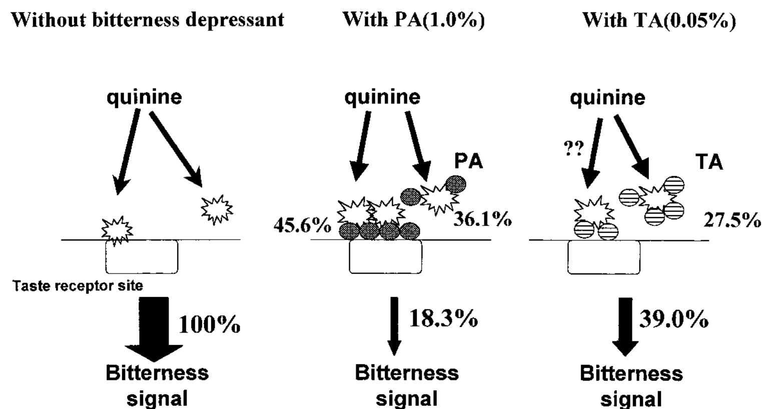

Fig. 3. Proposed Mechanism of Bitterness Suppression by Phosphatidic Acid (PA) and Tannic Acid (TA) in Human

scheme for tannic acid was also shown in Fig. 3.

Evaluation of Bitterness Suppression by the Taste Sensor As discussed above, there seem to be two mechanisms of bitterness suppression. The first mechanism, as exemplified by the adsorption effect of phosphatidic acid demonstrated in the present study, is the more complex. Evaluation of bitterness by measuring the % of unbound quinine is not very efficient, however, since in many cases equilibrium is reached, and free, dissolved, drug is immediately supplied from undissolved fractions, such that the free drug concentration remains almost constant. In this case, in order to achieve complete taste masking, a large quantity of additive would be required, which is a considerable disadvantage. A candidate taste-masking substance should therefore compete with bitter substances such as a quinine at the level of the bitterness receptor, in other words, exerting a peripheral rather that central effect.

The artificial taste sensor[9—12] provides a method in which sensor output value may be used to identify drugs which taste bitter when they are broken down peripherally at receptor sites in the tongue. When a bitter substance touches the human tongue, it is adsorbed by the microvilli of the taste cell. The surface of the taste cell is covered by a lipid bilayer membrane. When bitter substances are adsorbed by the lipid membrane on the taste cell, the electrical characteristics of the membrane change. Different output signals, or electric impulses, are obtained from the taste cells, with differential characteristics. It is thought that the neural network of the brain recognizes the different electrical patterns and is thus able to discriminate between various tastes.

We have been successful in evaluating the degree of bitterness of various drugs using the taste sensor.[13,14] In these studies, substances with a positive charge have been shown to exhibit the most bitterness, and a sensor membrane with a negative charge was therefore found to be most useful for quantitative evaluation of bitterness. In the present study, we investigated the CPA (change of membrane potential caused by adsorption) of the candidate bitterness-suppressants, as this value has been shown to correspond to a bitterness strength.

Figures 4A—E show the relationship between gustatory sensation and sensory data (CPA profile) for five bitterness-depressant substances (sucrose, aspartame, NaCl, phosphatidic acid and tannic acid) added to a 0.1 mM quinine solution. Sucrose and aspartame did not reduce the CPA value of quinine. We have previously reported that high concentrations of sucrose and aspartame themselves slightly reduce the sensor output value.[7] Takagi *et al.* also reported that very high concentrations of sucrose slightly reduced sensor output using a membrane with a negative charge.[13] Nevertheless, nobody has examined the bitterness-depressant effect of various substances using the CPA value as a criteria. The results shown in Figs. 4A and B indicate that sucrose and aspartame do not compete with quinine binding in the sensory membrane. The CPA values were not changed although the bitterness strength dramatically decreased with increasing sucrose and aspartame concentrations. This phenomenon suggests that the bitterness suppression in mixtures with sucrose and

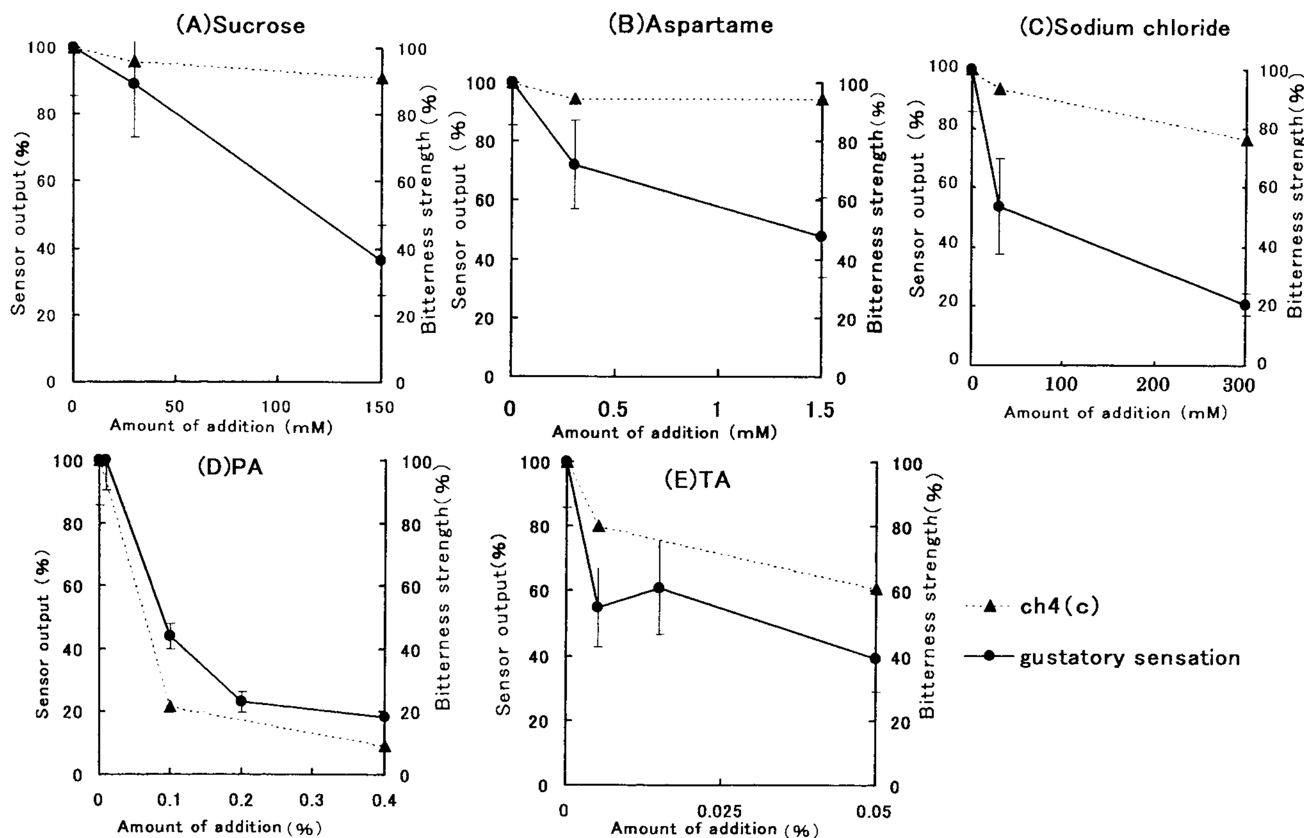

Fig. 4. Relationship between Sensory CPA Profile (Continuous Lines) and Bitterness Strength (Dotted Lines) Expressed as the Equivalent Quinine Concentration (Relative Value %)

The gustatory sensation data was obtained in human volunteers (n=11). The sensor data (channel 4) was the mean value obtained in three experiments.

aspartame occurs centrally. As shown in Fig. 4C, in the case of NaCl, the CPA value was decreased to almost 80%, while a dramatic reduction in bitterness strength was observed. It has been reported[15] that NaCl acts both peripherally and centrally in bitterness suppression, although the relative contributions of these mechanisms could not be determined precisely. If we assume that the sensor membranes resemble the bitterness receptor in the human tongue, the peripheral effect would be around 20% while the central effect is around 80%.

In the case of phosphatidic acid, as shown in Fig. 4D, the sensor (CPA) profile coincided well with the results of gustatory sensation tests. This result was not unexpected, as phosphatidic acid competes with quinine for binding to the human bitterness receptor, so that the sensor output should reflect the receptor membrane component. Finally, in the case of tannic acid, the sensor output also tended to decrease with increasing concentrations (Fig. 4E), although the decrease was less than that seen with phosphatidic acid. In this case, the decrease of sensor output reflects the decrease in the unbound fraction as well as the competitive effect of tannic acid at the surface sensor membrane.

Thus, using the taste sensor, it is possible to predict the ability of a substance to suppress bitterness as determined in human gustatory sensation tests. If the membrane components were to be modified to better reflect the actual components of the bitterness receptors on the human tongue, the sensor might give more predictable data.

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