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Bitterness Prediction or Bitterness Suppression in Human Medicines Using a Taste Sensor

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The purpose of the present study was to develop a method for the quantitative evaluation and bitterness prediction or bitterness suppression in commercial medicines using a taste sensor. The bitterness of 16 commercial medicines was evaluated in human gustatory sensation tests with 11 volunteers and using a multichannel taste sensor. For sensor measurement, three variables were used to predict estimated bitterness in multiple regression analysis or principal component analysis: sensor output (S), the change of membrane potential caused by adsorption (C), and C/S. For the 10 drugs with a positive sensor output, multiple regression analysis was applied. A particularly good correlation (r=0.822) was obtained between bitterness scores predicted using C/S values for channels 2 and 4 (which have high sensitivity for drugs with positive charge; see text for detail). Six drugs with no positive charge inside the molecule did not show any sensor output, although they had a low bitterness score in gustatory tests. Finally, an artificial taste sensor was used to evaluate or predict the bitterness suppression in quinine by sucrose, aspartame, NaCl, phosphatidic acid and tannic acid. The sensor output profile was shown to reflect the suppressant effect (human gustatory sensation result) of phophatidic and tannic acids at the receptor site well, whereas no sensor output changes by sucrose and aspartame were observed.

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1. Introduction

Humans can perceive and distinguish between five taste components, namely, sourness, saltiness, sweetness, bitterness, and umami (in Japanese). In general, bitter-tasting medicines are difficult for patients to swallow, which leads to noncompliance and thus decreased therapeutic efficacy. Therefore, quantitative evaluation of the bitterness of human medicines is an important factor in drug design. The taste sensor, an electric "tongue" with global selectivity, comprises several kinds of lipid/polymer membranes which transform information about substances producing taste into electrical signals.[1–3] These signals are analyzed by a computer and the sensor output has been shown to produce different patterns for groups of chemical substances with similar tastes. The tastes of various foodstuffs such as beer,[4] coffee,[5] sake,[6] rice, soybean paste and vegetables can be expressed quantitatively using the sensor. Nevertheless, there have been no systemic studies of evaluation of the bitterness of medicines by a taste sensor.

Recently, we evaluated the bitterness of 11 commercial medicines (basic or acidic drugs) using multichannel sensor output as an explanatory variable.[7] In that study, however, we only demonstrated the possibility of the system to evaluate bitterness quantitatively or to predict the bitterness of a substance to human gustatory senses. In the present study, therefore, we developed a more quantitative prediction method for estimating bitterness using sensor output (S), change of membrane potential caused by adsorption (C), and C/S as three explanatory variables. These variables can then be analyzed using multiple regression analysis. In addition, the taste sensor was used to evaluate or predict the bitterness suppression in quinine by sucrose, aspartame, NaCl, phosphatidic acid and tannic acid, and its possibility for predicting the bitterness-suppressant of human medicines at receptor sites in human taste cells was demonstrated.

2. Materials and Methods

2.1 *Chemicals*

Sixteen commercial drugs (shown in Table 1) were purchased by Sigma Chemical Co., St Louis, MO, USA, dissolved, and diluted to 0.3 mM solutions with 10 mM KCl. Sucrose, aspartame, NaCl, 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.

2.2 *Sensor measurement and data analysis*

The taste-sensing system SA402 of Intelligent Sensor Technology, Ltd,. Japan, as shown in Fig. 1 was used to measure the electric potential of the 16 drugs. The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes made of lipid/polymer membranes. The lipids used in the present study are listed in Table 2. Each lipid was mixed in a test tube containing poly(vinyl chloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran in a test tube, and dried on a glass plate at a temperature of 30°C to form a

Table 1
Commercial drugs.

Hydrochloride	Quinine
	Amitriptyline
	Dibucaine
	Diltiazem
	Imipramine
	Promethazine
	d-Chlorpheniramine Maleate
	Calcium Pantothenate
	Dextromethorphan Hydrobromide
	Trimebutine Maleate
	Acetaminophen
	Anhydrous caffeine
	Benzoic acid
	Metronidazale
	Salicylic acid
	Theophylline

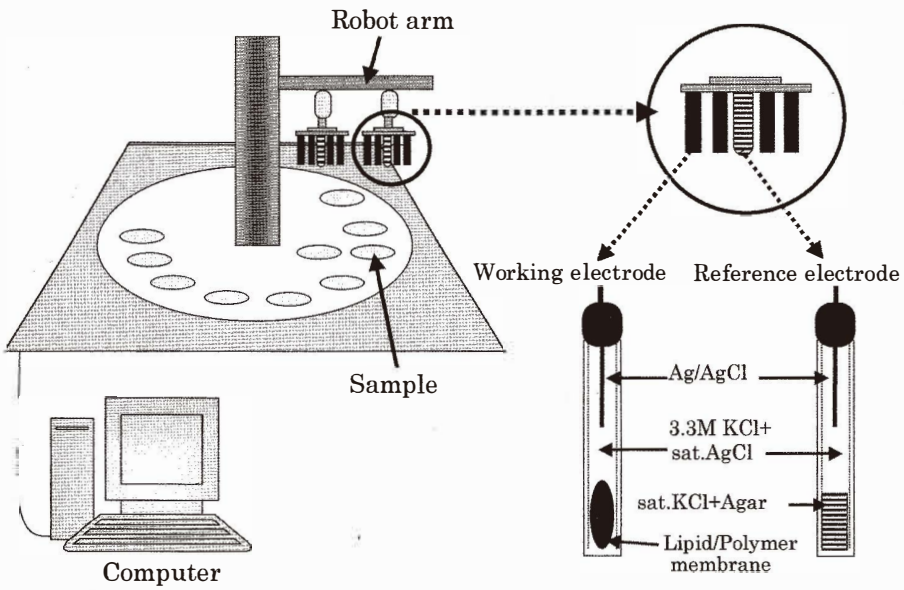

Fig. 1. The multichannel taste-sensing system (SA402) used in the present study.

Table 2
Lipid components for the sensor membranes.

Channel	Lipid component	Plasticizer
1	Phophoric acid di-n-decyl ester	Dioctyl phenyl-phosphonate
2	Phophoric acid di-n-decyl ester	2-Nitrophenyl octyl ether
3	Hexadecanoic acid	Dioctyl phenyl-phosphonate
4		Dioctyl phenyl-phosphonate
5,6	Tetradodecyl ammonium bromide	Dioctyl phenyl-phosphonate
7,8	Tetradodecyl ammonium bromide	2-Nitrophenyl octyl ether

transparent thin film, almost 200 μm thick. Each electrode was made of a silver wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3M KCl solution. The difference between the electric potential of the working electrode and the reference electrode was measured by means of a high input impedance amplifier connected to a computer.

Samples consisting of 0.3 mM solutions of the 16 drugs diluted with 10 mM KCl 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 method used to maximize the sensitivity and the selectivity of adsorption of the test substances is summarized in Fig. 2. The relative sensor output was represented as the difference (Vs–Vr) between the potential of the sample (Vs) and that of the reference solution (Vr). After sample evaluation, the electrode was dipped into the reference solution again, and the potential then obtained was defined as Vr'. The difference between the potentials of the reference solution before and after sample measurement (Vr'–Vr), was defined as C (change of membrane potential caused by adsorption) and represents the value corresponding to aftertaste. Each measurement interval was set at 30 s, and electrodes were thoroughly rinsed after each measurement.

S-PLUS 2000J (Mathematical Systems, Inc., Tokyo, Japan) was used as calculation software for multiple regression analysis.

2.3 *Gustatory sensation tests*

The standard quinine hydrochloride concentrations used in this study 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. The concentration of quinine and the bitterness score were defined according to the previous articles.[8,9] Before testing, the volunteer subjects (n=11) kept the above standard samples in the mouth, and were told their concentrations and their bitterness scores. After tasting 5 ml of a 0.3 mM sample test drug solution, the volunteers 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.

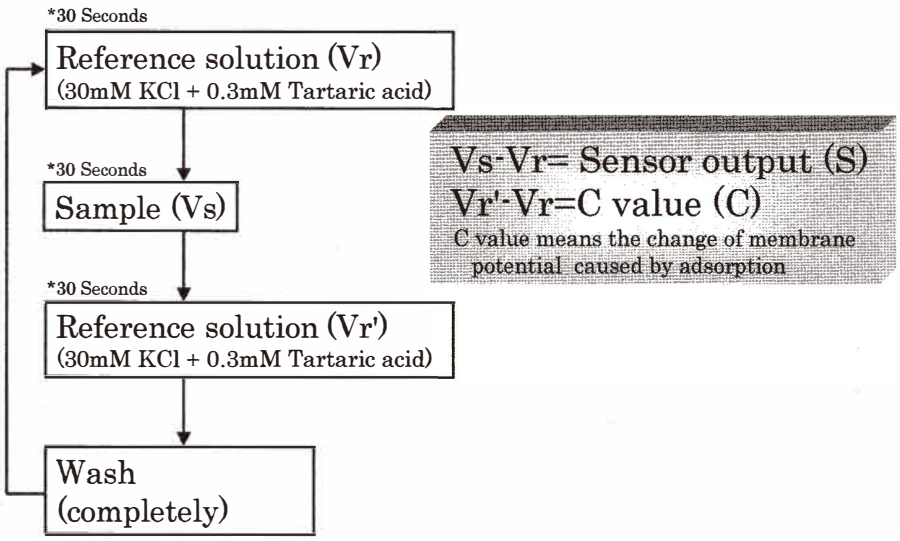

Fig. 2. Measurement procedure in this study.

3. Results and Discussion

3.1 *Evaluation of bitterness of medicines by the taste sensor*

Figure 3 shows the principal component analysis data for 16 drugs using *S*, *C*, and *C/S* as three explanatory variables in relation to channels 2 and 4. We could not define the true meaning of the components of PC1 and PC2. But at least, in the figure, 7 symbols corresponding to 6 drugs and the control were concentrated at one point. Four of these drugs (benzoic acid, salicylic acid, acetaminophen, and theophylline) and calcium pantothenate, are acidic drugs and did not show any sensor output in channels 2 and 4. This is presumably due to the fact that the carboxylic acid (anionic) group in the molecules did not interact with the membrane surfaces of channel 2 or 4, which are also negatively charged. However, benzoic acid and salicylic acid showed comparatively large sensor output in channels 5 and 7, the membranes of which were positively charged (data not shown). Metronidazol is also a weak basic compound and anhydrous caffeine seems to have no charge at neutral pH. These drugs did not show large sensor output values, and their bitterness scores in the gustatory sensation test were lower than those of drugs with large sensor output values.

Kurihara *et al.* have reviewed the receptor mechanisms of bitter substances.[10] They note that, while bitter alkaloids such as quinine or strychnine carry a comparatively large positive charge inside the molecule, they also carry a hydrophobic residue which contributes to their binding to the receptor site. The positive charge seems to be particularly important in giving rise to bitterness, since electrical interaction between the positive charge of bitter substances and the negative charge at the receptor sites or their surrounding

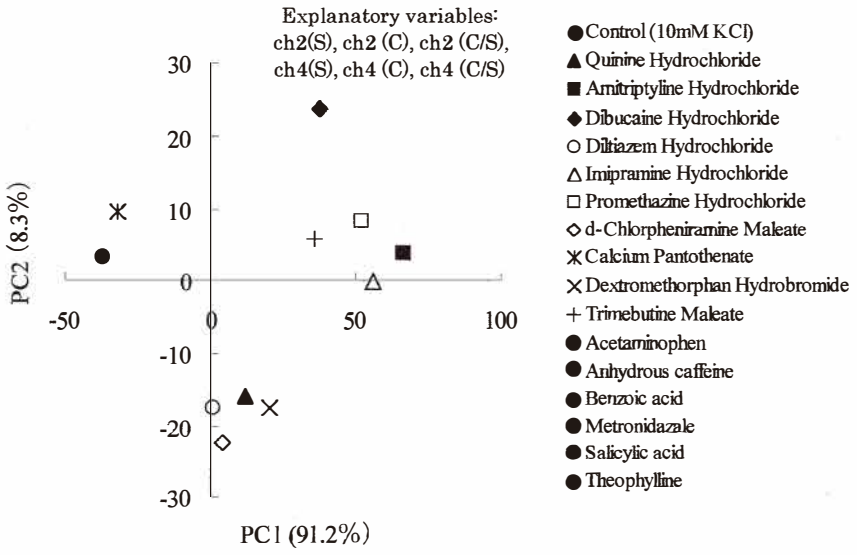

Fig. 3. Principal component analysis of 16 drugs using sensor output (*S*), change ofmembrane potential caused by adsorption (*C*), and *C/S* as three explanatory variables. Seven symbols corresponding to seven drugs and the control (30 mM KCl solution) were concentrated at one place.

region seems to be the trigger for sensing bitterness. Caffeine and theophylline are bitter drugs, although they have no positive charge inside the molecule at neutral pH. They nevertheless induced summated responses of the frog glossopharyngeal nerve to drugs after the tongue had been adapted with 50 mM $CaCl_2$ solution for 2 min. Caffeine did not show any sensor output in the present study. The above phenomena suggest that the present sensor system could not evaluate the bitterness of drugs which have no charge inside the drug molecule itself.

Takahashi and Kaneko reported that extracellular Ca^{2+} plays a critical role in sensing bitterness.[11] Thus, although the mechanism giving rise to bitterness seems to be rather complicated as reported,[12,13] at least drugs with a comparatively large positive charge, such as quinine, probably induce bitterness according to the above scheme of electrical interaction. Certainly, the multichannel sensor with negatively charged membranes used in the present study seems to be useful for evaluating the bitterness score of positively charged drugs.

Multiple regression analysis was applied to the data for the 10 drugs with a positive sensor output in channels 2 and 4. The model equation for bitterness can be represented as follows.

$$Y = aX1 + bX2 + cX3 + \cdots zXn \cdots, \quad (1)$$

where Y: Estimated bitterness score, Xn: Explanatory variables.

The results are shown in Fig. 3. Sensor output (S) and the change of membrane potential caused by adsorption (C) were measured, and their ratio (C/S) for channels 2 or 4 was calculated. A comparatively good correlation (r=0.822) was found between the estimated bitterness scores and the scores derived from the human gustatory sensation tests. The surfaces of all the membranes in channels 1–4 were charged negatively, due to proton dissociation. The results from channels 2 and 4 were used in the multiple regression analysis because of their greater sensitivity. An electric double layer is formed near the surface of the membrane in aqueous solution; cations such as amino groups accumulate near the surface of the negatively charged membrane. The electric potential then changes gradually from a negative value to zero. Therefore, basic drugs with amino groups are likely to show an increased relative response in electric potential (mV). In particular, drugs with quaternary amino groups in the molecule, such as quinine, trimebutine and dibucaine, showed a comparatively strong electric response in the sensor.

3.2 *Evaluation of bitterness suppression of quinine by the taste sensor*

The artificial taste sensor[1,3,4] 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

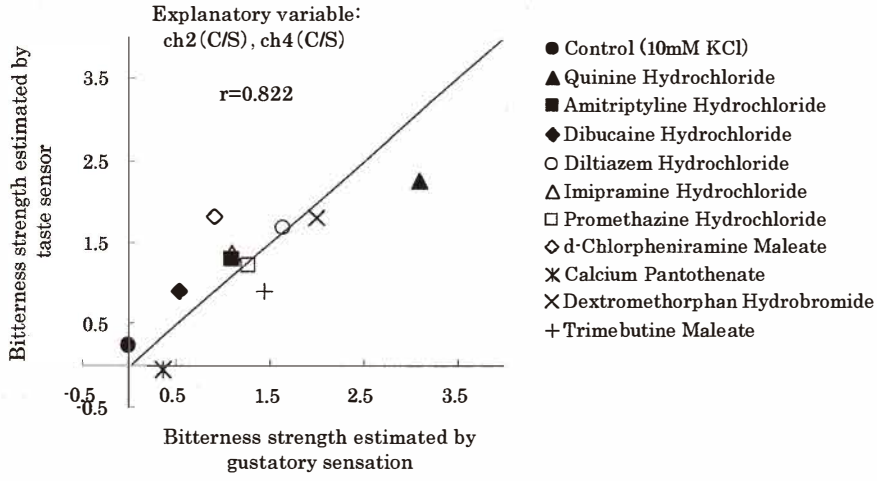

Fig. 4. Multiple regression analysis of 10 drugs using C/S data as explanatory variables for channels 2 and 4. Vertical axis shows the bitterness score predicted by principal component analysis; horizontal axis shows the bitterness score based on human gustatory sensation test results.

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.[6,14] 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 the most useful for quantitative evaluation of bitterness. In the present study, we investigated the *C* (change of membrane potential caused by adsorption) of the candidate bitterness suppressants, as this value has been shown to correspond to a bitter aftertaste.

Figure 5 shows the relationship between gustatory sensation and sensory data (*C* value) for five bitterness-suppressant substances (sucrose, aspartame, NaCl, phosphatidic acid and tannic acid) added to a 0.1 mM quinine solution. Sucrose and aspartame did not reduce the *C* 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.*[14] also reported that very high concentrations of sucrose slightly reduced sensor output using a membrane with a negative charge. Nevertheless, nobody has examined the bitterness-suppressant effect of various substances using the *C* value as a criterion. The results shown in Fig. 5 indicate that sucrose and aspartame do not compete with quinine binding in the sensory membrane. The *C* values were not changed, although the bitterness strength

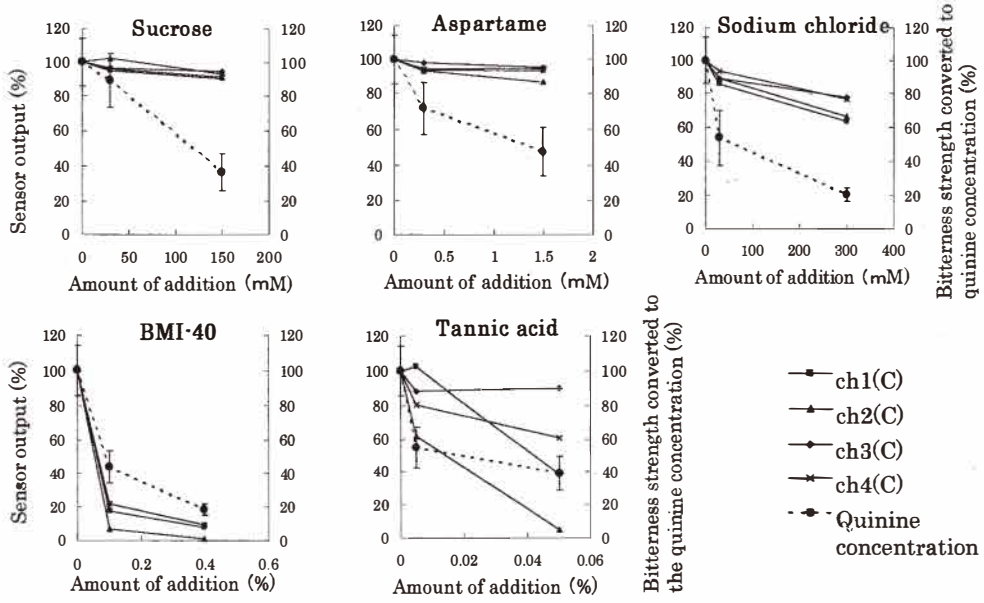

Fig. 5. Relationship between sensory *C* value profile (continuous lines) and bitterness strength (dotted lines) expressed as the equivalent quinine concentration (relative value %). The gustatory sensation data was obtained in human volunteers (n=11). The sensor data was the mean value obtained in three experiments

markedly decreased, with increasing sucrose and aspartame concentrations. This phenomenon suggests that the bitterness suppression in mixtures with sucrose and aspartame occurs centrally. As shown in Fig. 5, in the case of NaCl, the C value was decreased to almost 80%, while a marked 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. 5, the sensor *C* value 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. 5), 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.

A candidate taste-masking substance should therefore compete with bitter substances such as quinine at the level of the bitterness receptor, in other words, by exerting a peripheral rather than central effect. Sucrose and aspartame had a central effect since there is no inhibition of the output value in sensor. 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.

Finally, a brief taste perception model is proposed in Fig. 6. Quinine stimulates the bitterness perception route well and it could be inhibited by phosphatic acid effectively. The sensor data well reflect this suppression profile of the bitterness of quinine, as shown in Fig. 5. In the case of sucrose or aspartame, their bitterness-suppression effects were very large even though the effect of aspartame as an artificial sweetener has been reported to be 200-fold greater than the effect of sucrose.[16] The sucrose molecule is not charged while aspartame is. With addition of these substances, the sensor output did not change, which means their effects were felt mainly centrally, not peripherally. Thus, the competitive effect of aspartame and sucrose against the bitterness of quinine in the receptor of the human taste cell seems to be very small. Sodium chloride acts both peripherally and centrally in bitterness suppression. This peripheral effect could not be neglected and we could evaluate its participation by sensor measurement for many drugs with bitterness. In the case of tannic acid, a low concentration of tannic acid with moderate astringency could suppress the bitterness of quinine to some extent as shown in Fig. 5, but more concentrated (over 0.15%) tannic acid enhanced the bitterness of quinine (detailed gustatory sensation data not shown). The mechanism of this interaction (enhancement of bitterness) has not been clarified yet, but the interaction seems to occur centrally.

In conclusion, a taste sensor has potential to evaluate bitterness suppression in the receptor sites through bitterness perception. We are going to fabricate a sensor membrane which mimics the molecular structure of the receptor of bitterness in the future.

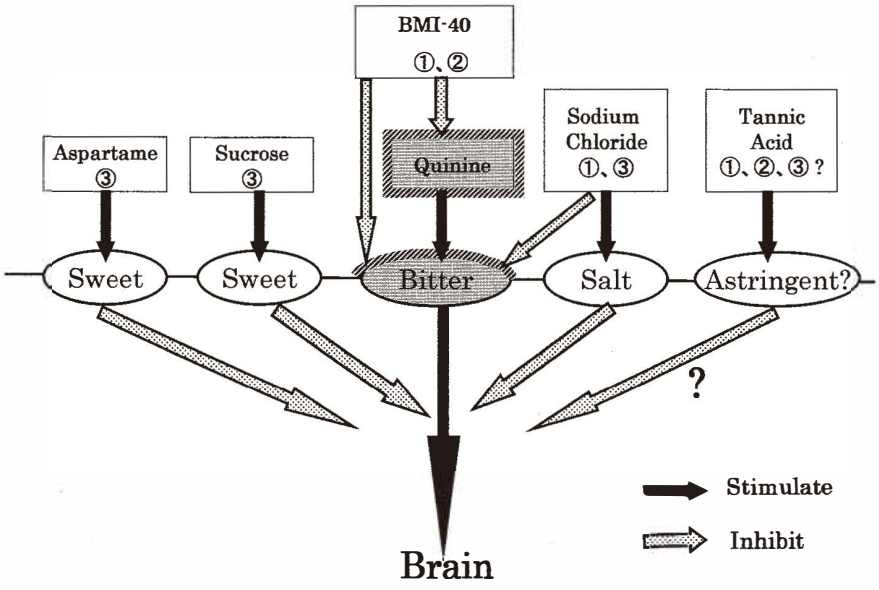

Fig. 6. Proposed taste perception model in the present study. In the figure, ① means the competition at receptor site (peripheral effect; for instance, ion channels, G proteins pathway route, etc.); ② means the chemical interaction in solution (for instance, adsorption of phsphatidic acid to quinine); ③ means the competition in the brain (central effect; neural inhibition).

4. Conclusions

In the present study, the bitterness of 16 commercial medicines was evaluated in human gustatory sensation and a multichannel taste sensor. For the 10 drugs with a positive sensor output, multiple regression analysis was applied, and good correlation (r=0.822) was obtained between human bitterness scores in gustatory sensation and scores predicted by the taste sensor. An artificial taste sensor was also found to be useful in evaluating or predicting the bitterness suppression of quinine by phosphatidic acid and tannic acid.

Thus, the taste sensor is potentially useful for predicting the effectiveness of bitterness or bitterness suppressants in human medicines at the receptor site in human taste cells.

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