A New Method for Evaluating the Bitterness of Medicines by Semi-continuous Measurement of Adsorption Using a Taste Sensor

Takahiro UCHIDA,*[,a] Yoshikazu KOBAYASHI,[b] Yohko MIYANAGA,[a] Rieko TOUKUBO,[b] Hidekazu IKEZAKI,[b] Akira TANIGUCHI,[b] Mayumi NISHIKATA,[a] and Kenji MATSUYAMA[a]

School of Pharmaceutical Sciences, Mukogawa Women's University,[a] 11–68, Koshien 9-Bancho, Nishinomiya 663–8179, Japan and Anritsu Corporation,[b] 1800 Onna, Atsugi, 243–8555, Japan. Received June 27, 2001; accepted July 18, 2001

We describe a new method for the evaluation of the bitterness of medicines by semi-continuous measurement of adsorption using a multichannel taste sensor or 'electric tongue'. The bitterness of 10 basic medicines was evaluated by both the taste sensor and in human gustatory sensation tests with 11 volunteers. The sensor part of the taste sensor consists of eight electrodes made of lipid/polymer membranes. Three variables were obtained from the taste sensor data: sensor output (*S*), the change of membrane potential caused by adsorption, corresponding to aftertaste (*C*), and the ratio *C*/*S*. These variables were used to predict an estimated bitterness score in multiple regression analysis. Semi-continuous measurement of *C* (every 30 s up to 150 s) was adopted as an additional explanatory variable, and the attenuation rate of *C* was defined as *C*′. These data were also subjected to multiple regression analysis. The correlation coefficient (*r*) estimated for the bitterness score predicted by the taste sensor, using *C*′ for channel 2 and *C*/*S* for channel 4, and the score obtained by human gustatory sensation, was 0.824. This value was greater than that obtained using *C*/*S* for both channels 2 and 4 (0.734). The method described in the present study seems to offer good predictability for the evaluation of bitterness.

Key words taste sensor; bitterness; human gustatory sensation

The five components of human taste, namely, sourness, saltiness, sweetness, bitterness, and umami, can be elicited by a wide variety of apparently unrelated molecules. Many medicinal drugs, including alkaloids such as quinine, which carry a positive charge, give rise to severe bitterness, even though their structures differ. In general, patients find bitter-tasting medicines difficult to swallow, causing noncompliance and thus decreasing therapeutic efficacy. Therefore, quantitative evaluation of the bitterness of medicines is an important factor in drug design.

A taste sensor, an electric 'tongue' with global selectivity, comprising several kinds of lipid/polymer membranes, has been applied to the evaluation of the taste of various foodstuffs.[1—3] The lipid/polymer membranes in the sensor transform information about substances producing taste into electrical signals.[4—6] These signals are analysed by the computer and the sensor output has been shown to produce different patterns for different groups of chemical substances with similar tastes.

We have previously investigated the bitterness of commercial medicines (basic or acidic drugs) using multichannel sensor output as a explanatory variable.[7] In that study, however, we did not clearly demonstrate the ability of the system to evaluate bitterness quantitatively or to predict the bitterness of a substance to human gustatory senses. In the present study, we report a new method for estimating the bitterness of medicines by semi-continuous measurement of *C*, the change of membrane potential caused by adsorption, and corresponding to aftertaste.

We evaluated the bitterness of 10 basic medicines, both by a multichannel taste sensor and in human gustatory sensation tests with 11 volunteers. As explanatory variables, sensor output (*S*), the change of membrane potential caused by adsorption (*C*), attenuation of *C* for up to 150 s after initial exposure (*C*′), and *C*/*S* were used to predict the bitterness score of the medicines in multiple regression analysis. The contribution of *C*′ (attenuation of *C*) to the bitterness score was also evaluated.

Experimental

Materials Ten commercial drugs (amitriptyline hydrochloride, D-chlorpheniramine maleate, dextromethorphan hydrobromide, dibucaine hydrochloride, diltiazem hydrochloride, imipramine hydrochloride, promethazine hydrochloride, propranolol hydrochloride, trimebutin maleate, quinine hydrochloride) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), dissolved, and diluted to 0.3 mM solutions with 30 mM KCl. Sucrose and aspartame were obtained from Nakai Tesque Co., Kyoto, Japan. All other reagents were of special reagent grade.

Sensor Measurement and Data Analysis The taste-sensing system SA402 of Anritsu Co., Ltd,. Japan, was used to measure the electric potential of 10 human pharmaceutical drugs. The electrode set is attached to a

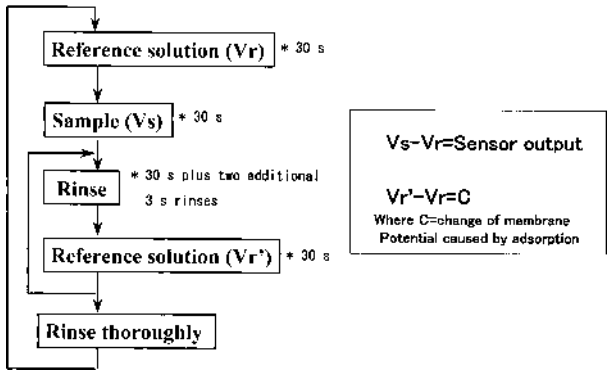

Chart 1. Measuring Procedure in this Study

Table 1. Lipid Components for the Sensor Membranes

Channel	Lipid component
1	Phosphoric acid di-*n*-decyl ester, dioctyl phenyl-phosphonate
2	Phosphoric 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

* To whom correspondence should be addressed. e-mail: takahiro@mwu.mukogawa-u.ac.jp

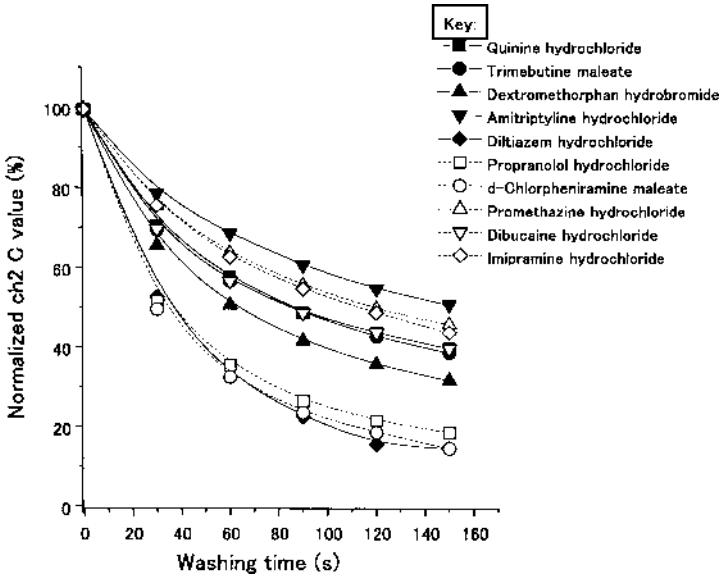

Fig. 1. Attenuation Profile of *C* from Channel 2 of the Taste Sensor for 10 Medicinal Drugs

The vertical axis shows the normalized *C* value for channel 2. The *C* value was measured every 30 s for 150 s. Each value is the average of five experiments (standard deviations not shown).

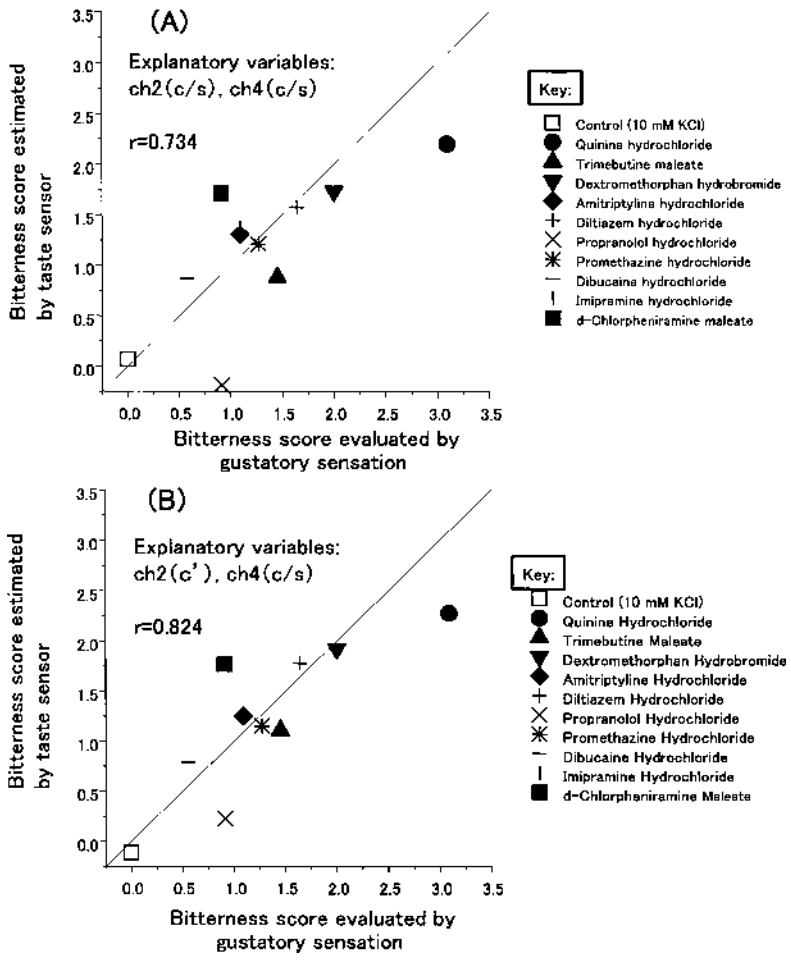

Fig. 2. Multiple Regression Analysis of the Data Obtained from the Taste Sensor for 10 Medicinal Drugs

The vertical axis shows the predicted bitterness score obtained from the taste sensor while the horizontal axis shows the bitterness score based on human gustatory sensation tests. (A) As explanatory variables, *C*/*S* values were used for channels 2 and 4. (B) As explanatory variables, *C*′ was used for channel 2, and *C*/*S* for channel 4.

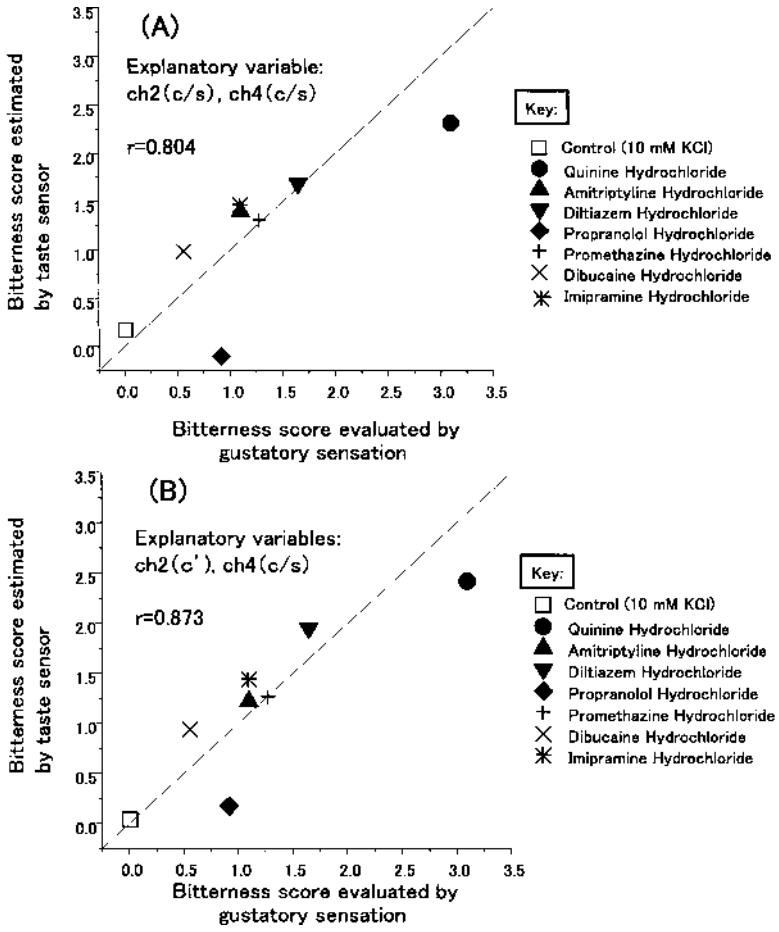

Fig. 3. Multiple Regression Analysis Data for Seven Medicinal Drugs Which Are Hydrochloride Salts

The vertical axis shows the predicted bitterness scores derived from the taste sensor data while the horizontal axis shows the bitterness scores based on human gustatory sensation tests. (A) As explanatory variables, *C*/*S* values were employed for channels 2 and 4. (B) As explanatory variables, *C*′ was used for channel 2, and *C*/*S* for channel 4.

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 1. Each lipid was mixed in a test tube containing poly(vinyl chloride) and dioctylphenylphosphonate as plasticizer, dissolved in tetrahydrofuran in a test tube, and dried on a glass plate at a temperature of 30 °C to form a 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 3 M 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.

The surfaces of the membranes in channels 1—4 were charged negatively, due to proton dissociation. Channels 2 and 4 were used in the multiple regression analysis because of their high sensitivities. 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 electric potential (mV).

Samples consisting of 0.3 mM solutions of 10 drugs 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. Firstly, the sensor was dipped into the reference solution (30 mM KCl solution plus 0.3 mM tartaric acid) for 30 s, and the potential of the reference solution, *Vr*, was recorded. The sensor was then dipped into the sample solution for 30 s, and the potential of the sample, *Vs*, was recorded. The difference between the potentials of the sample and the reference solution ($Vs-Vr$) represents the true sensor output (*S*).

The sensor was rinsed with the reference solution twice for 3 s, before being dipped into a fresh reference solution for 30 s. The detected potential was defined as Vr'. The difference ($Vr'-Vr$) between the potentials of the reference solution before and after sample measurement was defined as *C1* (where *C* represents the change of membrane potential caused by adsorption, and is a reflection of aftertaste). The sensor was then dipped in reference solution for another 30 s and a second value of *C* (*C2*) was obtained. The sensor was rinsed again (two 3-s rinses) and the process repeated a further four times to yield values for *C3*, *C4*, *C5*, and *C6*. The attenuation slope, *C*′, was calculated as the first-order slope.

Gustatory Sensation 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 ($n=11$) kept the above standard solutions in the mouth, and were told their concentration and bitterness scores. After tasting a 0.3 mM sample of test drug solution, 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.

Results and Discussion

Figure 1 shows the attenuation profile of *C* obtained from the data of channel 2 for the 10 drugs. The vertical axis represents the normalized *C* attenuation profile in relation to channel 2 following the washing process in reference buffer for up to 150 s, as described above. The *C* value decreased with time, the slope of attenuation reflecting decreasing drug affinity for the sensor membrane. We adopted this attenuation slope, *C*′, as an explanatory variable.

Secondly, multiple regression analysis was applied to the data. The general model equation for bitterness can be repre-

sented as follows:

$$Y=aX_1+bX_2+cX_3+\cdots+zX_n \quad (1)$$

where Y=estimated bitterness score, X_n=explanatory variables.

Figure 2 shows the results obtained by multiple regression analysis. The sensor output values (S) and the C values for channels 2 and 4 were measured, and their ratio, C/S, used as an explanatory variable (Fig. 2 A). For comparison, the attenuation of C (C') was employed instead of the C/S value for channel 2 (Fig. 2B). A comparatively good correlation (r=0.734) was obtained between the estimated bitterness scores obtained using the taste sensor and those derived from the gustatory sensation test, using the C/S ratio for both channels 2 and 4 (Fig. 2 A). However, a better correlation (r=0.824) between the bitterness scores obtained using the taste sensor and from the gustatory sensation test, was obtained when C' was used instead of C/S for channel 2 (Fig. 2B).

When the results for the seven hydrochloride salts were analysed separately (Fig. 3), the correlation coefficient for the bitterness score predicted using C' for channel 2 and C/S for channel 4 and the bitterness score determined by human gustatory sensation was 0.873, which was also greater than that obtained using C/S for both channels 2 and 4 (0.804).

The mechanism giving rise to a taste of bitterness in humans seems to be rather complicated, and has been the subject of several hypotheses. Kurihara[8] has proposed that bitter alkaloids such as quinine or strychnine have a large positively charged intramolecule. This positive charge seems to be important, as electrical interaction between the positive charge of bitter substances and the negative charge at the receptor sites or their surrounding region may be the trigger for sensing bitterness. However, drugs with no charge may also be bound or partitioned to the surface of the taste receptors. For example, humans can easily detect the bitterness of caffeine or theophylline, which do not have a positive charge inside the molecule. Some bitter drugs, such as quinine, which do have a positive charge, also have a hydrophobic residue, which may also contribute to receptor binding. A recent article has suggested that Ca^{2+} plays an important role in sensing bitterness.[9] Whatever the precise mechanism, it seems that the binding of bitter substances to receptors is essential for the perception of bitterness.

In conclusion, even for alkaloid drugs such as like quinine or caffeine, drug binding to the receptor in the electric tongue or taste sensor can provide a quantifiable measure of bitterness, and to offer good predictability for evaluation of the bitterness of human medicines. The adoption of C' as a explanatory variable seems to increase the accuracy of the method.

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