Bitterness Suppression of BCAA Solutions by L-Ornithine

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The purpose of the present study was to evaluate the bitterness-suppressing effect of L-ornithine (L-Orn) on single or mixed solutions of branched-chain amino acids (BCAAs) using human gustatory sensation tests and an artificial taste sensor. The BCAAs tested (L-isoleucine (L-Ile), L-leucine (L-Leu), and L-valine (L-Val)) are the main components of various enteral nutrients or supplements. The bitterness-suppression effect of L-Orn was also compared with the effect of L-Arg. L-Orn was effective in suppressing the bitterness of single or mixed solutions of BCAAs in human gustatory sensation tests, the effect being similar to or greater than that of L-Arg. The artificial taste sensor was able to predict the bitterness-suppressing effects of L-Orn and L-Arg. The response electric potential patterns of L-Val, L-Leu and L-Ile solutions to which 100 mM L-Arg had been added were quite similar to the sensor response patterns of the 100 mM L-Arg solutions alone. The relative response electric potential patterns of L-Val, L-Leu or L-Ile solutions containing 100 mM L-Orn in channels 5—8 (positively charged) are similar to that of single solution of 100 mM L-Orn.

Key words L-ornithine; L-arginine; branched-chain amino acid; bitterness; taste sensor; sensor response potential pattern

Amino acids are found throughout the body, both as constituents of proteins and as free amino acids. L-Ornithine (L-Orn) is found only as a free amino acid. It is found in various foods, such as Corbicula (an Asian clam), and is common in the natural world. The primary physiological function of L-Orn is in the liver, where it is an intermediate in the urea cycle.[1—3] It is also involved in muscle building, through actions on the pituitary, and facilitates the secretion of growth hormone.[4] L-Orn is an essential part of a healthy diet and is important in the build-up of muscle protein. It has also been reported that L-Orn increases immunopotency by macrophage activation.[5]

The taste and flavour of L-Orn are reported to differ considerably from those of most other amino acids.[6] In the present study, we clarified the bitterness-suppressing effect of L-Orn on single or mixed solutions of branched-chain amino acids (BCAAs), using human gustatory sensation tests and the artificial taste sensor. BCAAs are common components of enteral nutrient solutions, such as those used in severe hepatic disease (*e.g.*, Aminoleban®EN, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan).

The bitterness-suppression effect of L-Orn on BCAAs was examined and its effect was compared with the effect of L-Arg on BCAAs, since we demonstrated L-Arg being effective in suppression of bitterness of BCAAs in our previous paper.[7]

Secondly, we examined the effects of adding L-Orn and L-Arg to solutions of L-valine, L-leucine, and L-isoleucine on the sensor response output pattern of the taste sensor.

Experimental

Materials The following five amino acids were used in the present study: L-leucine (L-Leu), L-isoleucine (L-Ile), L-valine (L-Val), L-arginine (L-Arg) and L-ornithine monohydrochloride (L-Orn). They were all gifts from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). All amino acids were diluted with 10 mM KCl in this study. In the bitterness suppression experiments, using either gustatory sensation tests or the taste sensor, single 100 mM BCAA solutions containing L-Ile, L-Leu, or L-Val plus 1, 10 or 100 mM of L-Orn or L-Arg, were used as test solutions. The mixed BCAA solution contained 28.1 mM L-Ile, 12.1 mM L-Leu and 60.7 mM L-Val, to which 1, 10 or 100 mM of L-Orn or L-Arg were added. In the study performed to clarify sensor profile changes, 100 mM L-Orn or L-Arg was added to single BCAA solutions.

Quinine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), used as a bitterness standard, was dissolved to produce 0.01, 0.03, 0.10, 0.30 and 1.00 mM solutions with 10 mM KCl. All other reagents were of special reagent grade.

Gustatory Sensation Tests The gustatory sensation tests were performed with six to eight well-trained volunteers according to a previously described method.[8] 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 volunteers were asked to keep the abovementioned standard quinine solutions in their mouths, and were told the concentrations and bitterness scores of each solution. They were then asked to give the samples an equivalent bitterness score. The sample size was 2 ml, and all samples were kept in the mouth for 5 s. After tasting each sample, subjects gargled well and waited for at least 20 min before tasting the next sample. The differences between the bitterness scores of the various samples were analyzed using the Mann Whitney *U*-test, non-parametric method.

Sensor Measurement and Data Analysis The taste-sensing system SA402 (Intelligent Sensor Technology Co., Ltd., Atsugi, Japan) was used to measure the electric potential of the amino acid solutions. The electrode set is attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes composed of lipid/polymer membranes. The lipid components of the sensor used in the present study are the same as those described in a previous paper.[9] Each lipid was mixed in a test tube containing poly(vinylchloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran, and dried on a glass plate at 30 °C to form a transparent thin film, almost 200 μm thick. The electrodes consisted of a silver wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3 M KCl solution. The potential difference between the working electrode and the reference electrode is known as the sensor output, and is sent to the computer through the robot arm.

Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference sample (Vr) and also to rinse the electrodes after every measurement. The method used for the measurements is shown in Chart 1. The electrode is first dipped into the reference solution (Vr) and then into the sample solution (Vs). The relative sensor output is represented as the difference (Vs−Vr) between the potentials of the sample and the reference solution. 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) and corresponds to aftertaste. The measuring intervals were set at 30 s, and the electrodes were rinsed after each measurement. In the present study, we used only the relative sensor output for data analysis, since the CPA values of the amino

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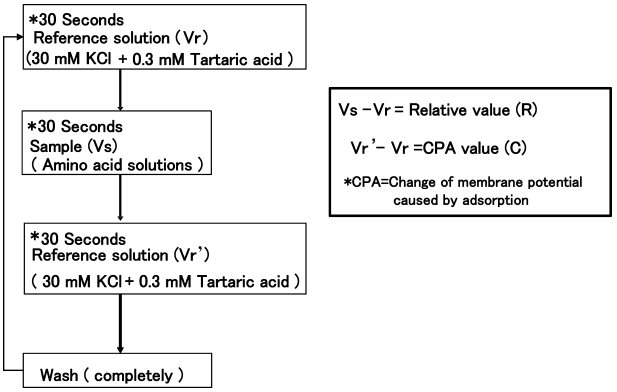

Chart 1. Measuring Procedure in This Study

acids were very small.

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

Results and Discussion

Bitterness-Suppressing Effect of L-Orn and L-Arg on Single or Mixed Solutions of BCAAs As reported in previous articles,[10,11] the bitterness of BCAAs is greater than that of other amino acids. Figure 1 shows the bitterness-suppressing effects of L-Orn and L-Arg on single BCAA solutions as evaluated in gustatory sensation tests. The bitterness-suppressing effect of L-Orn was almost the same as or greater than that of L-Arg. The bitterness of the L-Ile and L-Leu solutions could be reduced significantly by the addition of L-Orn. For example, the bitterness score of 100 mM L-Ile was 1.76, while the bitterness scores of L-Ile solutions containing 1, 10, or 100 mM L-Orn were 1.64, 1.51, and 1.01, respectively.

The bitterness score of 100 mM L-Val was 1.20 and the bitterness scores of L-Val solutions containing 100 mM L-Orn was 0.58. But statistical significance could not be obtained between the bitterness of 100 mM L-Val solution alone and that of 100 mM L-Val solution containing 100 mM L-Orn. This phenomenon might be due to low bitterness score of 100 mM L-Val (1.20), as an initial point, since we sometimes observed variations in bitterness score of drug solutions which corresponding to 0 or 1 of bitterness score in gustatory sensation test. In our pilot study, the bitterness score of 300 mM L-Val (1.81) could be significantly decreased by the addition of 100 mM L-Arg (1.38) or 100 mM L-Orn (0.88) (detail data not shown). These results suggest that L-Orn is effective in suppressing the bitterness of L-Val.

L-Orn and L-Arg were also effective in suppressing the bitterness of mixed BCAA solutions containing L-Ile, L-Leu, and L-Val at concentrations similar to those in commercially available enteral nutrition solutions (see Fig. 2). The bitterness scores of mixed BCAA solutions (containing 28.1 mM L-Ile, 12.1 mM L-Leu and 60.7 mM L-Val) to which 100 mM L-Orn or 100 mM L-Arg were added, were 0.17 and 0.50, respectively. These values are significantly smaller than that of the mixed BCAA solution alone (1.33). Thus, L-Orn and L-Arg were effective in suppressing the bitterness score of a mixed BCAA solution.

Figures 3 and 4 show the relationship between the bitterness score predicted by regression analysis of taste sensor data and that obtained in gustatory sensation tests for single BCAA solutions and mixed BCAA solutions containing

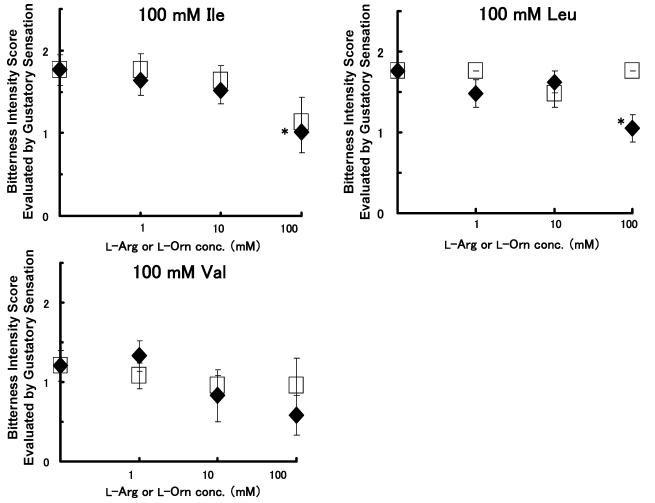

Fig. 1. The Relationship between Human Gustatory Bitterness Scores of Single BCAA Solutions and Increasing Concentrations of L-Arg (□) or L-Orn (◆)

Error bars represent the mean plus or minus standard error ($n=8$). $*p<0.050$ compared with control (in the absence of bitterness suppressant).

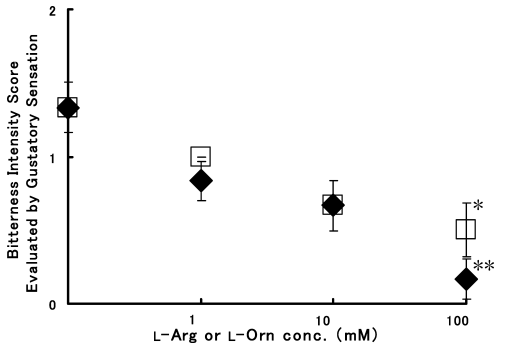

Fig. 2. The Relationship between Human Gustatory Bitterness Scores of Mixed BCAA Solutions and Increasing Concentrations of L-Arg (□) or L-Orn (◆)

Error bars represent the mean plus or minus standard error ($n=6$). $*p<0.050$, $**p<0.010$ compared with control (in the absence of bitterness suppressant).

L-Orn or L-Arg, respectively. In these figures, data from taste sensor channels 8, 1, and 4 were used for the analysis of L-Ile, L-Leu and L-Val, respectively. In the case of Ile+Orn, Val+Orn and Ile+Arg, a comparatively good correlation was found between the bitterness scores predicted by the sensor and the bitterness obtained in the human gustatory tests.

In gustatory sensation tests, bitterness score of L-Leu and L-Val were rather enhanced by addition of 100 mM L-Arg as shown in Fig. 1. While their taste sensor output were decreased by addition of L-Arg. Therefore, in case of Leu+Arg and Val+Arg solutions, good correlation was not obtained due to discrepancy between bitterness score predicted by taste sensor and that obtained in gustatory sensation test.

In the case of Leu+Orn solution, good correlation between obtained and predicted bitterness score, was not also obtained due to the variance of bitterness score of 100 mM L-Leu containing L-Orn in gustatory sensation test. The sensor output value shows that the bitterness-suppressing effect of L-Orn was similar or superior to that of L-Arg.

Figure 5 shows two bitterness-perception pathways, using

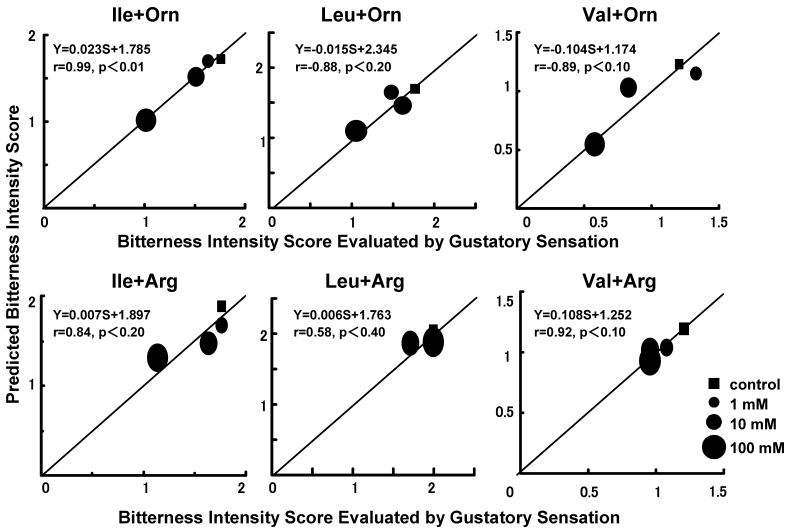

Fig. 3. Comparison between Bitterness Scores Predicted by the Taste Sensor and Those Obtained in Human Gustatory Sensation Tests of BCAA Single Solutions Containing L-Orn or L-Arg

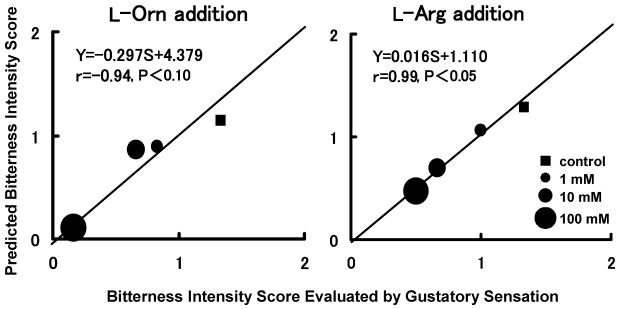

Fig. 4. Comparison between Bitterness Scores Predicted by the Taste Sensor and Those Obtained in Human Gustatory Sensation Tests of Mixed BCAA Solutions Containing L-Orn or L-Arg

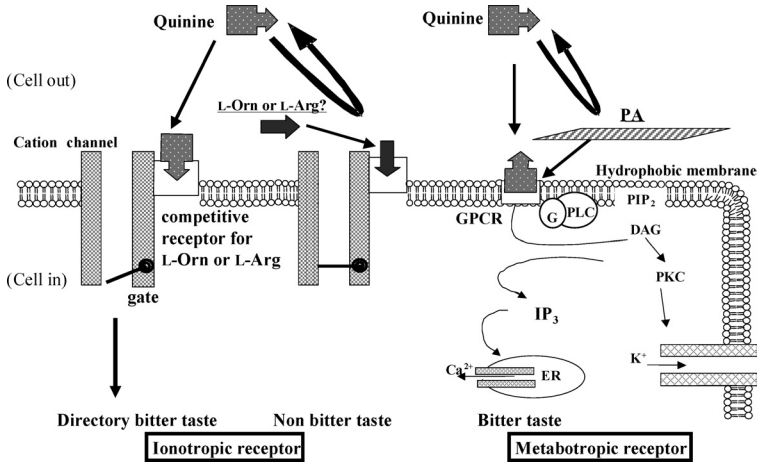

Fig. 5. Schematic Representation of Possible Bitterness Perception Pathways for Quinine

The right side of the figure illustrates bitterness perception *via* a metabotropic receptor. Phosphatidic acid (PA) prevents the quinine molecule from binding to the hydrophobic region of the receptor membranes uniformly, and then blocks the ensuing bitterness perception *via* the inositol triphosphate (IP_3) system. G: G-protein, which binds with the cell membrane and mediates activation of phospholipase C (PLC); PIP_2: phosphatidylinositol 4,5-bisphosphate; DAG: diacylglycerol; PKC: protein kinase C; ER: endoplasmic reticulum. The left side of the figure illustrates bitterness perception *via* the ionotropic receptor. Binding of quinine to receptors located near the cation channel usually opens the gate of the channel so that the bitterness signal is given. We suggest that L-Orn competes with quinine for the receptor, thus closing the gate of the cation channel, and inhibiting the perception of bitterness.

quinine as a model bitter substance. The metabotropic receptor pathway, which utilizes the phospholipase C/inositol 1,4,5-triphosphate (PLC/IP_3) system, is shown on the right side of the figure. The mechanism of the bitterness sensation produced is reportedly due to stimulation of IP_3 production *via* the PLC/IP_3 system, which leads to a decrease in intracellular cyclic adenine monophosphate (cAMP), and to direct blockage of K^+ channels.[12,13] Phosphatidic acid (PA) has hydrophobic characteristics and is known to be a bitterness-suppressing agent.[14]

Another pathway, utilizing the ionotropic receptor, is shown in the left side of the figure. Quinine has been reported to induce an inward current in bullfrog taste receptor cells by opening non-selective cation channels under conditions in which none of the second messenger candidates or their precursors (*e.g.* IP_3, ATP, GTP, *etc.*), were present on either side of the membrane.[15,16] Thus, two pathways seem to be involved in bitterness perception for substances such as quinine.

We recently demonstrated that L-arginine (L-Arg) was capable of significantly suppressing the bitterness of quinine and BCAAs.[7] In that study, L-Arg was as effective as PA in suppressing the bitterness of quinine. However, the bitterness-suppressant mechanism of L-Arg seems to be quite different to that of PA. L-Orn whose structure resembles that of L-Arg, was effective in restricting the bitterness of BCAAs with an efficiency equal or superior to that of L-Arg. On the other hand, L-Lys, whose structure and polarity also closely resemble those of L-Org or L-Arg, was not effective in bitterness suppression in gustatory sensation tests.

In a previous study on catfish,[17] L-Orn, L-Arg, and related compounds were shown to bind to sodium-channel sites. However, our understanding of the detailed mechanism of bitterness perception by this ionotropic receptor remains incomplete.

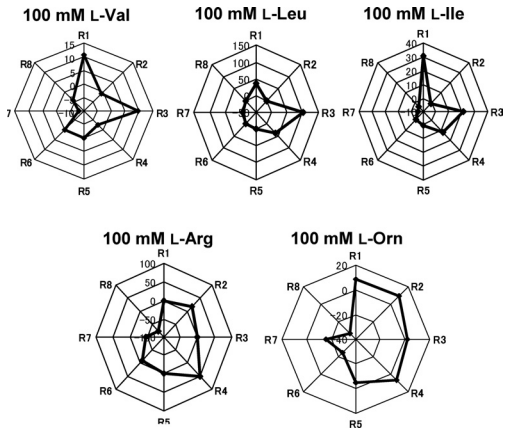

Fig. 6. Sensor Response Output Profiles for 100 mM L-Ile, L-Leu, L-Val, L-Arg, and L-Orn alone

Change of Sensor Response Output Profile by Addition of L-Orn and L-Arg Figure 6 shows the sensor response output electric potential profiles of L-Val, L-Leu, L-Ile, L-Orn and L-Arg. The relative response electric potentials of L-Val, L-Leu and L-Ile were comparatively large in channels 1 and 3; the electric potential patterns of these BCAAs were similar. L-Orn showed a comparatively strong response in channels 5—8 (which have a positive charge). L-Arg also showed a strong response in channels 5—8, but the sensor output values of L-Arg were larger than L-Orn. Unlike L-Orn, L-Arg also showed a response in channel 4.

Figure 7A shows the response electric potential patterns of 100 mM solutions of L-Val, L-Leu and L-Ile containing 100 mM L-Arg, while Fig. 7B shows the sensor output profile of L-Val, L-Leu and L-Ile solutions containing 100 mM L-Orn.

The relative response electric potential patterns of L-Val, L-Leu and L-Ile solutions containing 100 mM L-Arg in channels 1—8 were almost the same as that of L-Arg alone, while those of L-Val, L-Leu or L-Ile solutions containing 100 mM L-Orn in channels 5—8 (positively charged) are similar to that of L-Orn alone, even though simultaneous change was not obtained in channels 1—4 (negatively charged).

It was concluded that the response electric potential patterns of L-Val, L-Leu and L-Ile solutions with added 100 mM L-Orn or 100 mM L-Arg do not show markedly different sensor response patterns to single solutions of L-Arg and L-Orn.

Conclusions

In gustatory sensation tests, the bitterness-suppressing effect of L-Orn was similar to or greater than that of L-Arg. The bitterness-suppressing effects of L-Orn and L-Arg on mixed solutions of BCAAs could be predicted using a taste sensor.

The response electric potential patterns of L-Val, L-Leu and L-Ile solutions to which 100 mM L-Arg had been added were quite similar to the sensor response patterns of the 100 mM L-Arg solutions alone. The response electric poten-

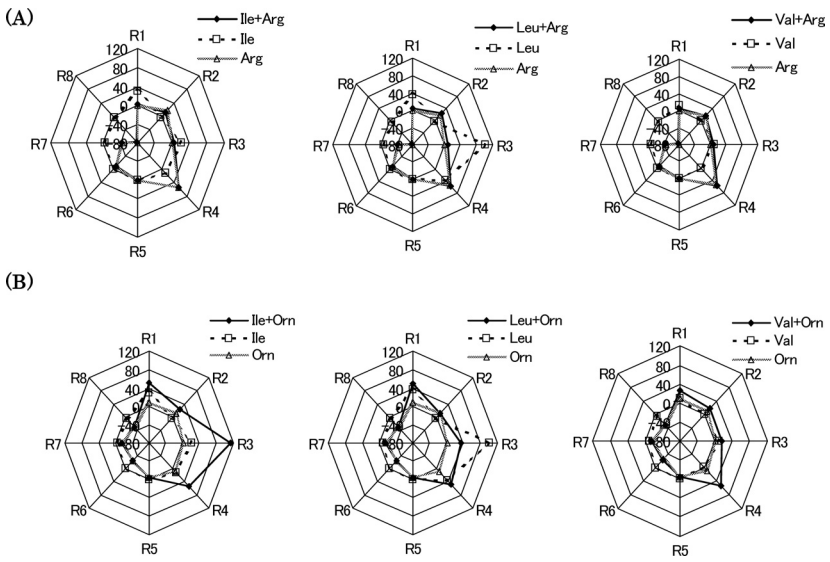

Fig. 7. The Change of Sensor Response Output Profile Caused by the Addition of L-Arg (A) or L-Orn (B)

For example, R1 means the relative response electric potential in channel 1.

tial patterns of L-Val, L-Leu or L-Ile solutions containing 100 mM L-Orn in channels 5—8 (positively charged) are similar to that of single solution of 100 mM L-Orn.

L-Orn was shown to be effective in suppressing the bitterness of BCAAs. We are planning to also investigate whether it is effective in the bitterness suppression of other medicines, and to elucidate the mechanism behind this bitterness suppression.

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