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Award Review

Development of a Cultured Cell-Based Human-Taste Evaluation System

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Objective quantification of taste intensity would be extremely useful for product development and quality control in the food industry. Progress has been made toward using the responses of cultured cells expressing human taste receptors as an indicator in evaluating gustatory intensity objectively. Effective utilization of such evaluation systems should not only provide information regarding taste intensities as perceived by humans, but also make possible the characterization of taste modulators to be used for commercial food products. Moreover, such an evaluation system should be also useful in advancing our understanding of the ways tastants are recognized by taste receptors.

Key words: taste intensity; taste receptor; cultured cell; sensory evaluation

Taste is a critical determinant affecting the value of a food product, and elucidation of the mechanisms by which tastants are detected and recognized in the oral cavity is an important focus area for research in the food sciences. Taste cells, located in the epithelia of the mouth, acquire the ability to recognize tastants by expressing a set of proteins that are necessary for the detection of tastant stimulation.[1)] In recent years, taste receptors belonging to the G protein-coupled receptor (GPCR) family, which functions in the detection of sweet, umami, and bitter tastants, have been identified in mammals, including humans, and other vertebrate species.[2)] Candidate taste receptors that mediate the perception of sour and salty tastants have also been characterized.[3–5)] Thus, the long-standing hypothesis that the perception of the five basic tastes (sweet, sour, salty, bitter, and umami) is mediated by various kinds of receptors has been proven by progress in molecular and cellular biology.

Although the presence of taste receptors has been clearly established on the basis of the results of basic studies, the tastes of food products are still usually discriminated by a sensory evaluation test during industrial food product development. In this test, trained food panelists sense and score the tastes of foods by tasting the products themselves. Although the accuracy of the sensory evaluation test has improved through an accumulation of improved techniques over many years, it is not easy to quantify taste intensity by this method. A significant amount of training has to be provided to each panelist in order to obtain quantitative measurements that are free of individual subjectivity. Furthermore, since the products are tasted and confirmed by trained panelists, there is a limit the number of products that can be evaluated in a single sitting. This limitation poses a crucial disadvantage in the case of quality control in an industrial production area. Moreover, this method is highly inappropriate for the evaluation of products of which the safety has not yet been confirmed.

Given this background, the development of a simple, general-purpose, objective taste evaluation system is extremely useful in food science-related research. Recently, many efforts have been made to develop a highly sensitive method of measuring taste intensities of tastants using human taste receptors, and simultaneously to elucidate the mechanisms underlying various taste-related phenomena using this objective taste evaluation system.

I. Measurement of Taste Intensities Using Human Taste Receptors

Since mammalian taste receptor candidates of the GPCR family were first identified, progress has been made in developing effective methods of functional characterization, which should aid in determining the relationship between a receptor and its cognate ligands.[6–8)] Due to optimization of functional assays, an expression system using mammalian cultured cells is generally used (Fig. 1). Cultured cells are prepared to express each of the receptors transiently or stably along with a chimeric G protein that efficiently induces an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$) after receptor activation.[8)] Preloading with a fluorescent intracellular calcium indicator such as Fura-2 or Fluo-4 prior to the assay makes possible measurement of the increase in $[Ca^{2+}]_i$ resulting from receptor activation upon the application of its ligand. This measurement is obtained by evaluating the change in emission intensity of the fluorescent calcium indicator. That is, the activation level of taste receptors can be quantified objectively by measuring changes in the fluorescence of the indicator by calcium-imaging analysis performed using a fluorescence microscope or a high-

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Abbreviations: GPCR, G protein-coupled receptor; NHDC, neohesperidin dihydrochalcone

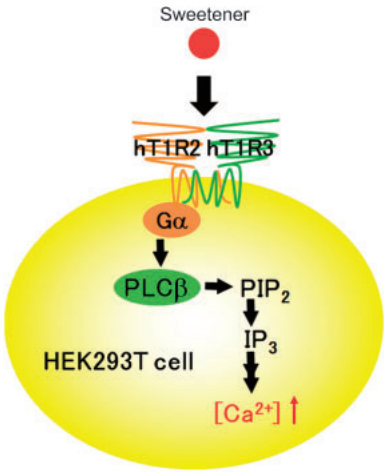

Fig. 1. Intracellular Signal Transduction in Cultured Cells Exogenously Expressing the Taste Receptor.

An example pertaining to the human sweet taste receptor. Cultured cells that express each of the taste receptors along with a chimeric G protein that efficiently induces an increase in intracellular calcium concentrations ($[Ca^{2+}]_i$) after receptor activation are prepared. An increase in $[Ca^{2+}]_i$ can be induced by receptor activation by application of its ligand.

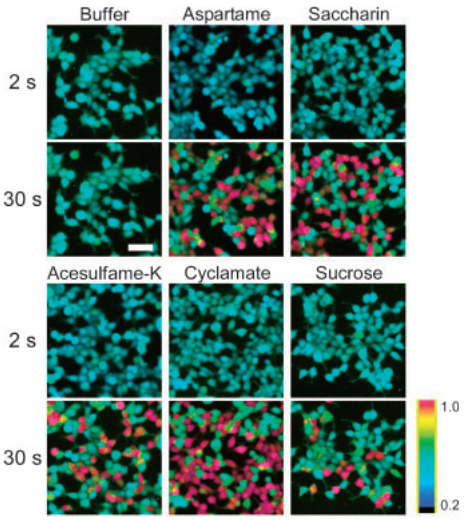

Fig. 2. Response of Human Sweet Taste Receptor Expressing Cells to Sweet Tastants.

Representative ratiometric images of fura-2-loaded cells expressing the human sweet taste receptor (hT1R2/hT1R3) and hG16gust44. Upper and lower columns show images obtained at 2 and 30 s after stimulation respectively. The sweet tastants used were aspartame (10 mM), saccharin (1 mM), acesulfame-K (3 mM), cyclamate (30 mM), and sucrose (200 mM).

throughput screening system involving a multi-well plate reader.

When novel taste receptors were identified in small fish such as the medaka fish and the zebrafish, which are used as model animals, the use of this cellular assay system showed experimentally that these fish-derived taste receptors exhibit high sensitivity to amino acids although their nucleotide sequences correspond to those of sweet taste receptors in mammals.[9] This finding also indicated that the fact that these small fish do not respond to sweet tastants such as sugars can be explained partly by the ligand specificity of the taste receptors in an animal. In contrast, in the case of a cellular assay system expressing each of human taste receptors, it is strongly expected that such a system would enable quantification of our perceived taste intensity by measurement of the responses of cells to an individual solution. In fact, many studies have reported that the concentration-dependent responses obtained in the case of human receptors are similar to the results obtained by sensory evaluation tests.[8,10,11]

The cellular assay system is also useful for determining which human taste receptors recognize a specific taste compound present in food products. There are 25 types of bitter taste receptors in humans, and each recognizes a wide variety of bitter tastants. Specific human bitter-taste receptors that recognize bitter compounds such as bitter peptides,[12] bitter saccharides,[13] and tea catechins[14] have been identified. Elucidation of the responses of receptors to specific compounds should provide useful information regarding the biochemical basis of taste modulation, as explained in detail below.

II. Development of a Sensitive Cellular Assay System for Sweet Tastants Capable of Providing a Long-Term Stable Response

Among the basic tastes, sweet, which is generally preferred by humans, is extremely important in the food industry. In humans, only one type of receptor, a heterodimeric GPCR (hT1R2/hT1R3), recognizes sweet tastants. It has also been demonstrated experimentally that many sweet tastants, including sugars, artificial sweeteners, and sweet proteins, are perceived by this receptor,[8] but since the frequency of the cellular response is not sufficiently high in cultured cell assay systems that transiently express the human sweet taste receptor, advanced techniques to detect and analyze the cellular responses upon the application of sweet tastants are required.

To solve these problems, our group spent several years determining the conditions necessary to generate cell lines that stably express both human taste receptor subunits (hT1R2 and hT1R3) and a chimeric G protein.[15] To obtain a cell line that exhibits long-term, stable responses to the application of sweet tastants, many expression constructs for the human sweet taste receptor have been developed, and several methods of generating stably expressing cell lines have also been applied. Our group succeeded in constructing a cell line that stably expresses the functional human sweet taste receptor.[15] This cell line was found to respond to all the sweeteners tested, *e.g.*, aspartame, saccharin, acesulfame K, and cyclamate, with extremely high responsiveness and frequency (Fig. 2).[15] Moreover, due to this increased sensitivity to sweet tastants, it should be very easy to detect and measure the cellular response to sucrose, which was previously extremely difficult to observe because of the weak responsiveness of the cells used (Fig. 2).

Moreover, by optimizing the conditions for the cellular assay system, *e.g.*, cell density, culture medium, and assay time, the response of the cell line stably expressing the human sweet taste receptor has become sensitive and reproducible. This system can also be used as a high-throughput assay system employing a multi-well plate reader.[15] Our high-throughput assay system enables the evaluation of sweet taste intensity in

multiple samples over a short period, 96 samples in approximately 30 min. Moreover, on evaluating the stability of the cell response over the long term, it was found that the sensitivity of the response of the cell lines to sweet tastants did not decline for more than 1 year. Hence we conclude that the assay system developed by our team using a cultured cell line stably expressing the human sweet taste receptor enables simple and stable evaluation of sweetness intensity as perceived by humans.[15)]

III. Application of the Cellular Assay System to Screening for Effective Taste Enhancers

The practical application of cellular assay systems using cells expressing the human taste receptor has shown that a variety of low-molecular-weight compounds can control taste receptor activation and thereby modulate the perceived intensity of taste. The most famous example of such taste synergism (increase in perceived taste intensity due to specific combinations of compounds) is umami synergism. An example of this is seen in the case of a combination of glutamate present in seaweed and inosine 5′-monophosphate (IMP) present in bonito soup stock,[16)] but the molecular mechanism underlying this synergism related to taste receptor function was long unclear. Experiments using cultured cells expressing the human umami taste receptor (hT1R1/hT1R3) have been conducted to elucidate such mechanisms.[17)] A study using a combination of molecular modeling and experiments using cultured cells expressing a variety of mutant receptors found that IMP binds to a site adjacent to the glutamate-binding site of the umami taste receptor and synergistically increases the activation of the receptor.[17)]

Sweet-taste enhancers are considered extremely important in the food industry in view of their potential effects with respect to producing the same level of perceived sweetness while simultaneously reducing calorie intake due to sugars. Their use also helps to decrease the use of artificial sweeteners that have unpleasant aftertastes. Previous studies involving sensory evaluations of sweeteners have indicated that some sweeteners alter sweet taste intensity when combined with other sweeteners.[18)] Based on these observations, experimental screening has been carried out on numerous compounds in order to determine whether the addition of a small quantity of the compound to sugar increases the response of cells expressing the human sweet taste receptor.[19)] Neohesperidin dihydrochalcone (NHDC) and cyclamate have been identified as sweet-taste enhancers that enhance the sweetness not only of sucrose but also of several other sweet tastants (Figs. 3, 4).[19)]

Recently, novel sweet-taste enhancers that increase the activation of the human sweet taste receptor for the artificial sweetener sucralose were identified in a chemical library of low-molecular-weight compounds with diverse chemical structures (Fig. 4).[20)] These newly identified sweet-taste enhancers do not exhibit sweetness themselves, but increase the perceived sweetness of sucralose.[20)] In view of the potential application of these sweet-taste enhancers in the food industry, researchers

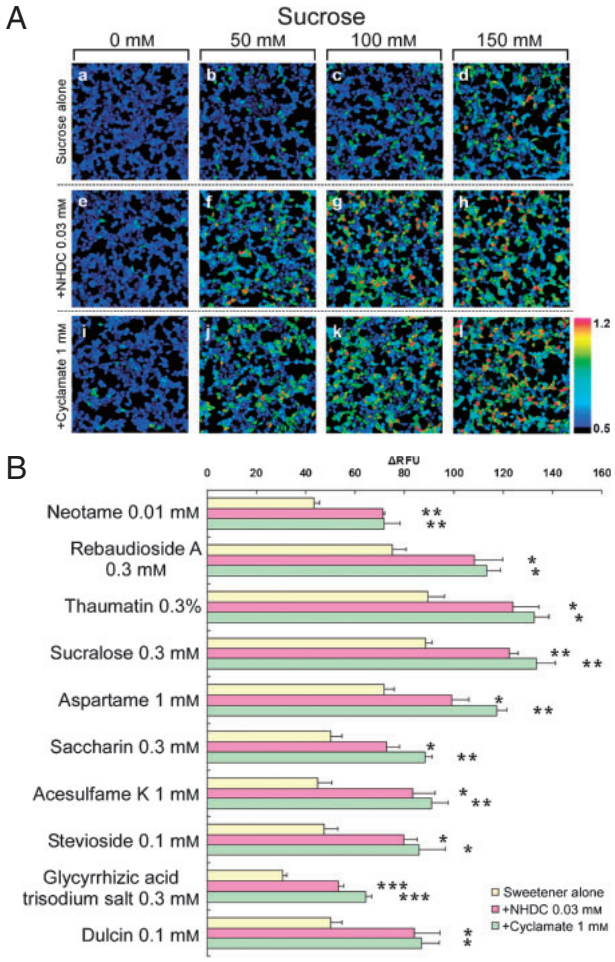

Fig. 3. Response of Human Sweet Taste Receptor Expressing Cells to the Application of Various Sweeteners with NHDC or Cyclamate.

A, Representative ratiometric images of fura-2-loaded human sweet taste receptor expressing cells are shown. Concentrations of 0–150 mM sucrose alone (a–d) and together with 0.03 mM NHDC (e–h) or 1 mM cyclamate (i–l) were applied to human sweet receptor expressing cells. Each image was obtained about 30 s after ligand application. Color scale indicates the fura-2 F_{340}/F_{380} ratio as a pseudocolor. B, Cellar responses of the human sweet taste receptor expressing cells were examined by application of various sweeteners in the absence and the presence of 0.03 mM NHDC or 1 mM cyclamate. Bars indicate mean ± SEM for three independent experiments. The significance of the differences between the control (sweetener alone) and the test values were determined by one-way ANOVA followed by Dunnett's test. $^{*}p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$ *vs.* a sweetener alone.

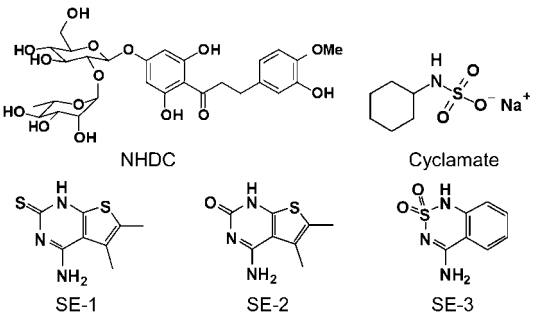

Fig. 4. Chemical Structures of the Sweet-Taste Enhancers.

Examples of sweet-taste enhancers that were identified by functional analysis using cells expressing the human sweet taste receptor are shown.[19,20)]

around the world are engaged in intense competition to identify sweetness enhancers that can be used for commercial products. It is expected that the identification of novel sweetness enhancers with the aid of human sweet taste sensor cells will soon be possible, and that these sweetness enhancers will actually be used in food products.

IV. Perspectives for the Future

Research in the field of taste science has advanced rapidly since the identification of the molecular entities of the human taste receptors.[1,2] The above-mentioned taste-sensing cultured cells with mechanisms similar to that in the human oral cavity might help indicate taste intensities for basic tastes by measurement of cellular responses to tastant solutions. Moreover, this evaluation system would also be useful in advancing understanding of the ways tastants are recognized by taste receptors.

Nevertheless, fluorescent indicator-based assays that use cultured cell pose many intrinsic fundamental problems. Some samples may contain biologically active compounds that induce a non taste-receptor mediated cellular response in the cultured cells. In such cases, accurate evaluation of taste intensity is not possible due to these responses. In addition, measurement of fluorescence intensity is impeded by the use of cloudy or colored samples, and interference is also observed in the case of samples containing intrinsically fluorescent compounds. Furthermore, highly acidic solutions and ones with high osmotic pressure can damage cultured cells and thereby hinder accurate measurement of the cellular response. The development of novel techniques to resolve these problems is essential for the creation of biosensors with an expanded range of applicability.

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