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Umami evaluation in taste epithelium on microelectrode array by extracellular electrophysiological recording

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ABSTRACT

Umami is one of the basic tastes along with sweet, bitter, sour and salty. It is often elicited by amino acids and can provide a palatable flavor for food. With taste epithelium as the sensing element, microelectrodes can be used to evaluate umami taste by biological responses of the tissue. The electrophysiological activities to umami stimuli are measured with a 60-channel microelectrode array (MEA). Local field potential (LFP) recorded by a MEA system showed different temporal characteristics respectively with L-glutamic acid (L-Glu), L-aspartic acid (L-Asp), L-monosodium glutamate (L-MSG) and L-monosodium aspartate (L-MSA), while remarkable differences were observed between amino acids and their sodium salts. Wealso found that a dose-dependent behavior in the increasing concentrations of umami stimulations and a synergistic enhancement between amino acids and purine nucleotides can be detected. The investigation of this evaluation for umami represents a promising approach for distinguishing and evaluating umami tastants.

1. Introduction

Taste sensory system of human beings is in charge of evaluating nutrient substances of food and preventing the ingestion of poisons. As one of the basic tastes along with sweet, bitter, sour and salty, umami plays a crucial role in improving the flavor of foods and the recognition of amino acids, which are important nutrients in foods to organisms [1–3]. Umami is elicited by many small chemical molecules, including amino acids, sodium salt of amino acids and some nucleotides. Notably, umami taste sensing of human and animals can be enhanced by some purine nucleotides which were widely applied in food industry [1,4]. Thus, measurement technology for umami chemicals, mainly containing glutamate and nucleotides, has attracted more attention of investigators in the world [5–8].

Cell and tissue based biosensors are special systems that utilize immobilized living cells and tissues as sensitive components, combined with sensors to produce easily detected responses, such as electrical and optical signals, through interactions between stimulus and cells or tissues [9–11]. In recent years, a number of studies have reported on cell and tissue based biosensors fabricated to be applied in various fields, containing pharmacological analyze, toxicological monitoring, developmental biology and basic research [12–14]. For taste compound detections, cells expressing special receptors were usually monitored by electrochemistry, Ca^{2+} imaging and electrophysiological techniques to develop bioelectrical tongues with high specificity and selectivity to taste compounds [15–17].

In our previous investigations, we employed a sensor chip with microelectrode array (MEA) to record electrophysiological activities of taste epithelium, building a platform to investigate detections and recognitions of basic tastes [17,18]. Unlike traditional methods applied for the electrophysiological investigations such as patch clamp and optical imaging technologies, MEA is non-invasive and allows monitoring electrophysiological activities of cells and tissues over a long period [19–22]. Moreover, the advantages, including less demanding in respect to the experimental abilities of operators and no restricted to ion flows of Ca^{2+} imaging, make MEA become a promising approach with increasing interest for electrophysiological studies and biorecognition detections.

Sensitivity and specificity of taste epithelium for umami is derived from receptors expressed in a population of taste receptor cells (TRCs) of taste buds, including mGluR1, mGluR4 and a heterodimer of the taste-specific T1R1 and T1R3 [23–25]. When these specific receptors contract umami substances through taste pores, interactions between them will elicit second messenger cascades, activate a member of the transient receptor potential (TRP) chan-

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nel family named TRPM5 and depolarize TRCs. During this process, chemical signals are converted into action potentials of TRCs. Then electrical signals are delivered to afferent nerves to encode and decode, ultimately the taste information about umami forms in the brain [26,27].

In this further study, we discussed detections for umami compounds at different concentrations and recognition of different kinds of umami tastants with MEA. In experiment, taste epithelium was isolated from the rat tongue with a mixture of collagenase and proteolytic enzymes, and fixed on the surface of MEA as the sensing element for umami taste detection. Owning to the particular mechanism of umami sensing in biology, special electrophysiological signals elicited by umami chemicals could be measured through the multichannel recording system with MEA and analyzed with mathematical statistics. Notably, the characteristics which were extracted from recording signals revealed some intrinsic features of umami sensing such as synergistic enhancement and interaction between Na^+ and amino acids. Hence, we fabricated the method for umami detections successfully, which can provide a functional detection and evaluation for umami tastants by mimicking umami sensing of human and animals.

2. Materials and methods

2.1. Isolation and fixation of taste epithelium

Sprague–Dawley rats weighting about 250 g were purchased from the Animal Research Center of Zhejiang Province, China. After anesthetized by intraperitoneal injection of 20% urethane, the tongue was dissected free and transferred to Phosphate Buffer Solution (PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4 and 2 mM KH_2PO_4). Epithelium with taste buds was then stripped from base of the tongue by injection of the mixture of 1 mg/ml collagenase and 3 mg/ml protease. The isolated epithelium (about 5 mm × 5 mm), from the tongue root on which many circumvallate papillae scatter, was rinsed with PBS, placed with taste pores side up on the surface of MEA and fixed by a plastic ring-shaped frame covered with a tightly stretched piece of mesh. The natural structures of taste buds were preserved with basic receptor cells population maintained integrated.

2.2. Preparation of MEA and recording system

Electrical signals of taste epithelium were recorded by MEA. Just as shown in Fig. 1A, taste epithelium with taste buds was stripped from the rat tongue with enzyme treatments, and attached on the surface of MEA, fabricating the umami sensory system to taste stimuli. The fabrication, preparation and exact measurement methodology of MEA were described concretely in our previous olfactory sensing work [28]. MEA that we applied was composed of an array of 60 electrodes where intact taste epithelium was fixed for detecting electrical signals produced by TRCs. The values of electrodes and inter-electrode spacing were 10 μm and 30 μm, which can avoid the electric interference between the neighboring electrodes effectively and help record more signals from a fairly small tissue.

Electrophysiological measurement was performed by applying the MEA1060-Inv system from Multichannel Systems (MCS, Reutlingen, Germany) for synchronously recording signals from 60 channels. A MC-RACK software (MCS, Reutlingen, Germany) was used to display and store up signals in real time with sampling rate of 10 kHz.

2.3. Taste experimental protocol

The concentrations of L-glutamic acid (L-Glu), L-aspartic acid (L-Asp), L-monosodium glutamate (L-MSG) and L-monosodium aspartate (L-MSA) were chosen from 0.1 mM to 10 mM to observe responses of the epithelium to umami compounds with increasing concentrations. Experiments were separated into two parts. First, umami compounds of L-Glu, L-Asp, L-MSG and L-MSA were applied to stimulate taste epithelium for detecting responses to different umami. Second, the umami compounds at increasing concentrations were delivered into the MEA chamber to explore the dose-dependent characteristic of the measurement.

Before stimulations, intrinsic excitability of the epithelium in PBS was recorded for 150 s and employed as control condition. Then, umami stimuli were added into the bath solution in the MEA chamber automatically and electrophysiological signals of the epithelium were also recorded for 150 s. After an administration of umami component, PBS was injected into MEA chamber again and repeated twice to wash out stimuli totally. In order to rule out the influence of residual tastants and make electrodes and the tissue return to a stable state, the minimum interval between the taste injections was 300 s. The temperature of recording chamber was maintained at 37 ± 0.5 °C, which was controlled by a temperature module in the recording system.

2.4. Mathematical statistics

Characteristics such as amplitude and duration were extracted from signals of different experiments respectively. In the discrimination of different umami tastants, the aspect ratios of V_p/V_{pp} and V_{pp}/duration were calculated to represent characteristics of responses while these values showed more differences in stimulations of four umami tastants than direct extracting features such as V_{pp} and duration. Indeed, the values of ratios were believed to show more accurate discriminations of single units from extracellular recording in electrophysiological measurement [29]. For each series with increasing concentrations, data were normalized to the mean value at the highest concentration or the largest response signal. All data were given as mean ± standard deviation of samples. Mean values were also statistically compared using the Student's *t*-test. Responses have significant differences from each other if $p < 0.05$, and have remarkable differences if $p < 0.01$.

3. Results

3.1. Extracellular recording from multichannel MEA

In Fig. 1, extracellular potentials of epithelium with taste buds were recorded by MEA. While stripped from the rat tongue, taste epithelium with papillae was coupled to the surface of MEA. In Fig. 1B, taste papillae, containing basic taste sensing units of taste buds, were observed obviously to scatter on taste epithelium with a microscope and could respond to umami stimuli. Based on the multichannel property of MEA, electrophysiological signals can be detected in parallel in conditions of spontaneity and umami stimulations (Fig. 1C). Typically, signals were revealed as a representative multi-recording. It can be seen that every channel from channel 01 to channel 60 recorded similar negative peaks with amplitudes and durations about 100 μV and 1 s. For each event, the initial stage was a rapid downstroke to the minimum field potential amplitude, then a repolarization to positive peak followed with a return to baseline. The values of amplitudes were maintained at the range from 50 μV to 200 μV with variations between administrations of different umami stimuli, while durations were at the magnitude order of hundreds milliseconds. Indeed, these

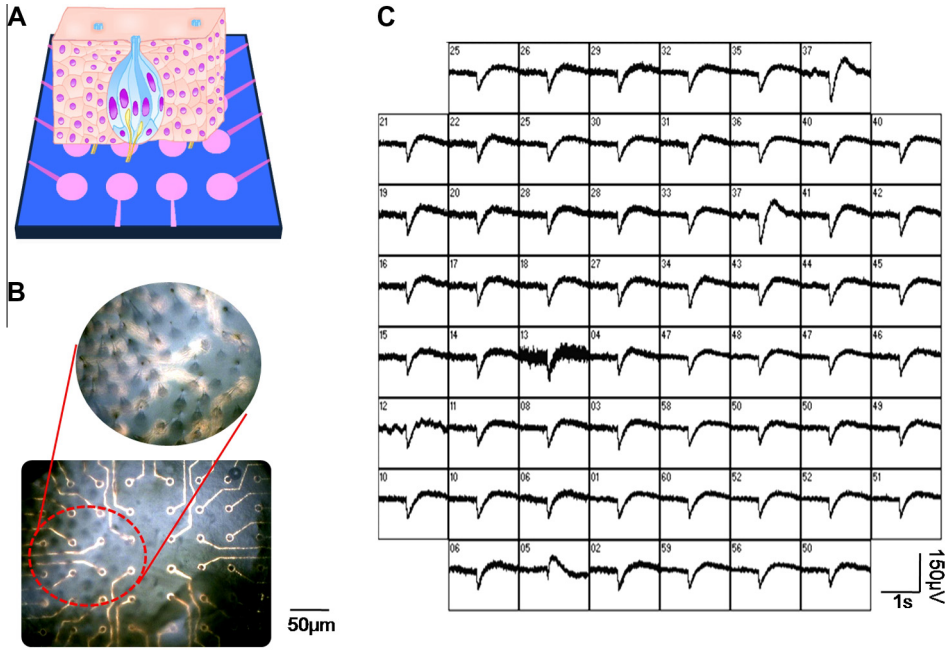

Fig. 1. Extracellular recording with MEA. (A) Schematic of taste epithelium fixed on a MEA surface (illustration not to scale). (B) Observation of taste epithelium coupled to microelectrodes with a light microscope. (C) Electrophysiological signals of epithelial tissue recorded with MEA, in stimuli of MSG at 1 mM as an example.

extracellular potentials recorded from taste epithelium showed high similarity in amplitudes and durations with local field potential (LFP) which were often detected in thin slices of tissue.

3.2. Detections for umami tastants

L-Glu, L-Asp, L-MSG and L-MSA at 1 mM were chosen as stimuli to taste epithelium in experiment trials. Fig. 2A showed the potential recordings with 100 s in the control and umami stimulations, while a single waveform of LFP was displayed in Fig. 2B. Compared to the control group, umami tastants of L-Glu, L-Asp, L-MSG and L-MSA can provoke electrophysiological activities of taste epithelium respectively and these signals can be recorded in LFP. The durations which were defined as time consumption between the depolarization and the return to baseline are 827.5 ± 30.48 ms, 930.6 ± 29.70 ms, 972.1 ± 21.83 ms and 855.3 ± 17.15 ms for

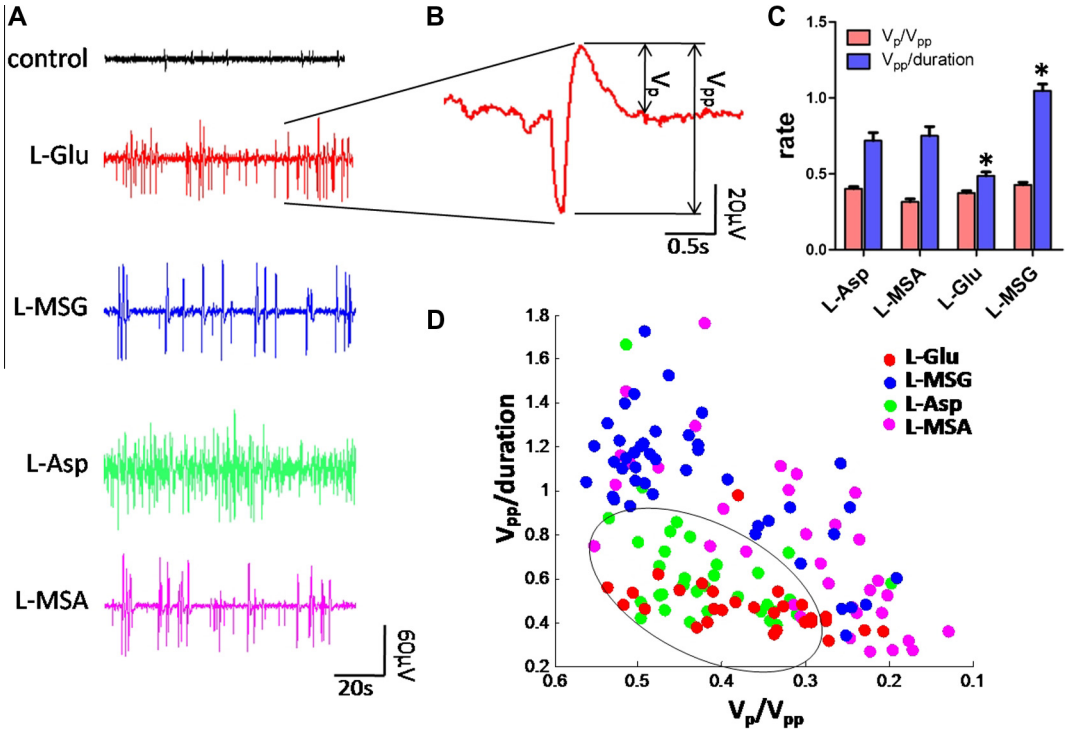

Fig. 2. Responses of the biosensor to different umami stimulations. (A) Field potentials of extracellular potential recording in stimuli of L-MSG, L-MSA, L-Glu and L-Asp at 1 mM. (B) A single waveform of field potential with feature of V_p/V_{pp} and V_{pp}/duration, which were extracted from signal trains. (C) Quantification of electrophysiological responses to umami tastants with values of V_p/V_{pp} and V_{pp}/duration ($n \geqslant 11$, mean ± s.e.m.). ∗ indicates statistically significant differences from other three groups ($P < 0.05$). (D) Classify of signals elicited by diverse compounds in a two domain space.

L-Glu, L-Asp, L-MSG and L-MSA, and varied slightly with umami stimuli. In contrast, the amplitudes of field potentials for L-Glu, L-Asp, L-MSG and L-MSA, equaling to 38.96 ± 1.37 μV, 42.98 ± 1.76 μV, 100.9 ± 4.32 μV and 65.26 ± 4.85 μV, were obviously different between two groups of amino acids and their sodium salts. To distinguish different umami, the aspect ratios of V_p/V_{pp} and V_{pp}/duration were calculated based on LFP extracted from response traces with mathematical statistics (Fig. 2C). As shown in Fig. 2D, responses to L-Glu, L-Asp, L-MSG and L-MSA were scattered into two clusters in a two-dimensional spacewith the axis of V_p/V_{pp} and V_{pp}/duration. Responses to L-Glu and L-Asp shared similar characteristics while L-MSG and L-MSA got together with larger values of two ratios.

The administrations of four umami tastants at 0.1 mM, 0.5 mM, 1 mM, 5 mM and 10 mM were also applied respectively to measure responses of the epithelium to the same umami compounds at increasing concentrations. Individual waveforms evoked by different concentrations and extracted from recording trains were plotted in Fig. 3A to reveal the relation between increasingconcentrations of umami and shapes of potentials. Similar to above responses to different umami, amplitudes of displayed waveforms ranged from 20 μV to 300 μV with variation of concentrations separately. The durations were about 900 ms, which had no noticeable changes of each other. All amplitudes of LFP in stimuli of L-Glu, L-Asp, L-MSG and L-MSA revealed a visible increase with concentrations in a dose-dependent manner. To L-Glu and L-Asp, the curve of amplitudes slightly increased after low-dose treatment (0.1 mM and 0.5 mM), but rose drastically after application of umami with concentration of 1 mM. On the contrary, responses to L-MSG andL-MSA started a rapid growth from low concentration of 0.1 mM and tended to saturations at high concentrations.

3.3. Synergistic enhancement with IMP

A hallmark of umami taste is the synergistic enhancement of potency when umami compounds such as L-MSG and L-MSA is combined with monophosphate esters of inosine or guanosine nucleosides [inosine-5′- monophate (IMP) and guanosine-5′-monophate (GMP)]. This feature has been cleverly commandeered by the food industry as a mean of enhancing the flavor of a wide range of products. The synergistic enhancements were suggested to attribute to interactions between amino acids and nucleoside molecules [4,30]. In our experiment, taste interactionsbetween sodium salts of amino acid (L-MSG and L-MSA) and IMP were measured repeatedly at various concentrations to investigate whether responses of epithelium was evoked specifically by the quality of umami. The waveforms recorded in stimulations of L-MSG and L-MSA with IMP at 0.1 mM showed larger responses in amplitudes than signals without IMP, but durations shared similar values (Fig. 4A). Importantly, Fig. 4B showed the quantitative analysis with mathematical methods for response with and without IMP, which indicated that existences of IMP resulted in significant changes of signal elicited by umami stimulations.

4. Discussion

In our study, LFP of taste epithelium elicited by umami stimuli can be measured and recorded with the 60-channel system. In fact, LFP is a particular class of electrophysiological signals which are predominated by the electrical current flowing from all nearby dendritic synaptic activities within a volume of tissue. In recent years, several reports have suggested that changes in LFP are linked to many important processes, such as memory, movement and sensory function [31–33]. Thus, LFP, low-frequency components of the extracellular voltage, can be regarded as the characteristic responses to discriminate tastes, and extracted from original recording signals with a low-pass filter cutting off at about 100 Hz for analysis. Consequently, quantitative values of amplitudes and durations can be calculated with mathematical statistics to estimate taste stimuli because of the characteristic information that LFP own carried.

L-MSG, L-MSA, L-Glu and L-Asp were four well-known umami chemicals usually used in umami investigations [34,35]. Depending on a combination of genetic, behavioural and physiological studies, these umami components were demonstrated to elicit the taste sensing of humans and animals with thresholds ranging from 10 μM to 100 μM [23]. In our study, these four tastants at 1 mM all elicited specific umami responses of the epithelium with distinctions in analysis. The previous reported data have also suggested that presence of Na^+ will affect the umami sensory in the investigations of behavior and electrophysiology [36–38]. More specifically, sodium salt shows a great enhancement on electrophysiological activities of taste sensory system provoked by amino acids. In essence, multiple receptors are proposed as the potential receptors for umami sensing [23–25]. These receptors include two kinds of glutamate-selective G protein-coupled receptors, mGluR4 and mGluR1, and the taste bud-expressed heterodimer T1R1 + T1R3, and own dissimilar properties to umami tastants. The mGluRs are activated by glutamate and certain analogs how-

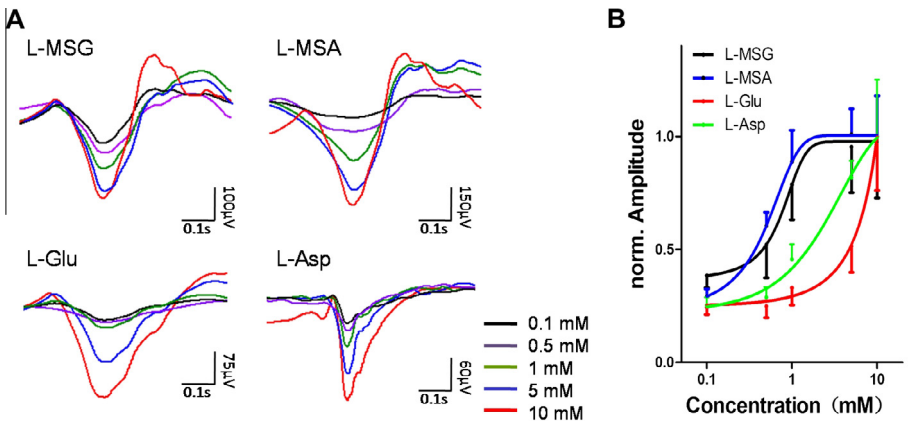

Fig. 3. Dose-dependent response of the biosensor to umami tastants at different concentrations. (A) Recordings of the biosensor during simulations of L-MSG, L-MSA, L-Glu and L-Asp at five increasing concentrations; shown are individual traces for different concentrations of 0.1 mM (black), 0.5 mM (purple), 1 mM (green), 5 mM (blue) and 10 mM (red). (B) Mathematical statistic curves on normalized amplitude for four umami tastants of L-MSG (black), L-MSA (blue), L-Glu (red) and L-Asp (green), which have a dose-dependent manner. Data are shown in mean ± s.e.m. ($n \geqslant 11$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

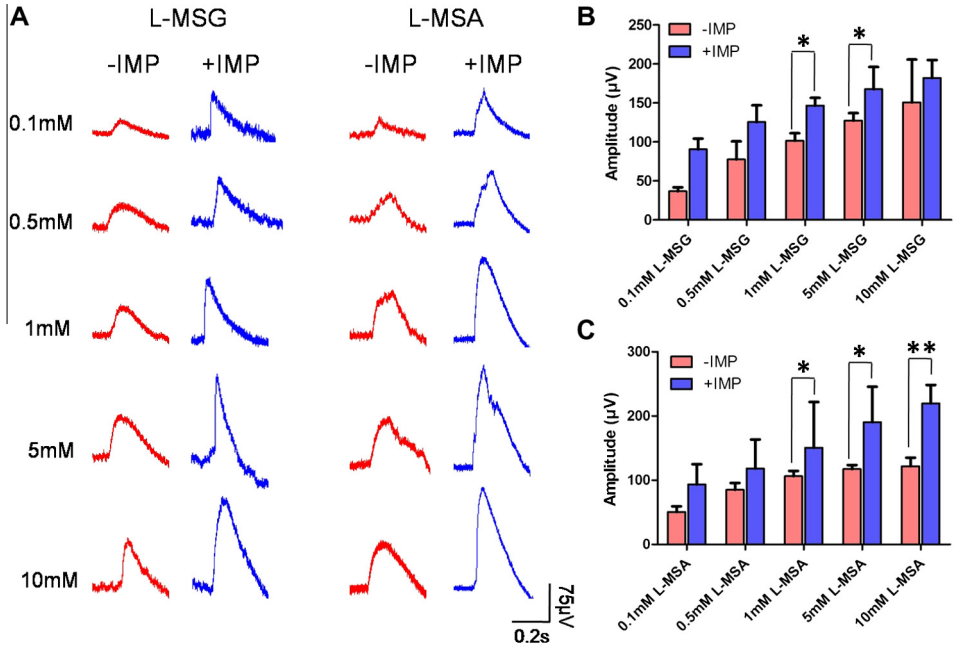

Fig. 4. Synergistic enhancement between umami tastants and IMP recorded by the biosensor. (A) Field potential recording of the biosensor to umami compounds (L-MSG and L-MSA) with and without IMP. (B) and (C) Comparison between response recorded in the presence and absence of IMP at 1 mM. Red bar, tastant alone; blue bar, tastant + 0.1 mM IMP. The values are mean ± s.e.m. ($n \geqslant 11$). * indicates statistically significant differences ($P < 0.05$) while ** indicates remarkable statistically significant differences ($P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to theweb version of this article.)

ever were not reported to be sensitive to nucleotides. In contrast, T1R1 + T1R3 can be activated by a broad range of amino acids and several nucleotides. In our study, with the quantitative analysis to waveforms of LFP, it showed different responses to amino acids and their sodium salts in shapes of LFP, which may resulted from the involvements of diverse receptors. Meanwhile, signals of amino acids and their sodium salts can be assembled respectively into two regions in a two-dimensional space with morphology parameters. It was demonstrated that the biosensor could respond specifically to amino acids (L-Glu and L-Asp) and sodium salt of amino acids (L-MSG and L-MSA), and response signals could be distinguished in a two-dimensional space.

A salient feature of umami taste in animals and human beings is their impressive potentiation by purine nucleotides such as IMP and GMP. Thus, the experiment with the administrations of mixture of amino acid salt (L-MSG and L-MSA) and IMP was also carried out to examine that recorded signals were elicited by umami quality rather than salt quality (Na^+). It was reasoned that if sodium salt modulated the potential of taste epithelium, presence of IMP should had no effect on the response signals recoded. Indeed, the responses measured by the MEA showed a significant enhancement with impact of IMP at several increasing concentrations of L-MSG and L-MSA, especially in the conditions of high concentrations. These results validated that the response signals recorded by our method was specifically by umami tastants rather than other four basic tastes. It also indicated that this approach can evaluate umami intensity of a mixture of amino acids and IMP with similar function to the biological system. Indeed, this characteristic showed a wide potential application of the measurement in umami taste evaluation of food flavorings containing several compounds.

We also found that applications of umami compounds at increasing concentrations would induce alterations in amplitudes of LFP. It has been reported that gustatory nerve recordings and behavior preference tests of organisms showed a visible variation with increasing concentrations of umami stimuli [23,34,39]. In our study, four umami compounds of L-Glu, L-Asp, L-MSG and L-MSA all elicited electrophysiological responses of taste epithelium, and increased the amplitudes of LFP in a dose-dependent manner. It was coincident with the consequences of genetic, behavioural and physiological experiments about umami taste in above studies. Notably, seen from the does-dependent curves, responses to L-MSG and L-MSA had larger values at range of low concentrations (0.1–1 mM) than L-Glu and L-Asp, and turned into saturated states after concentration of 5 mM. In contrast, the growth of responses to L-Glu and L-Asp was continuous until approximately maximum concentration limited by their own solubility at room temperature. These were also line with the results discussed above that sodium salt enhanced umami sensing in mammals. Consequently, the electrophysiological signals of epithelium recorded by MEA was demonstrated to reflect a dose-dependent behavior of taste cells in electrophysiological activities, when taste epithelium was stimulated with umami tastants at increasing concentrations.

5. Conclusions

MEA coupled with intact taste epithelium tissue *in vitro* constituted a platform for detecting umami compounds. By measuring LFP in taste epithelium through multichannel recording, the biosensor had specific responses to different umami tastants of L-Glu, L-Asp, L-MSG and L-MSA, especially can distinguish amino acids (L-Glu and L-Asp) and their sodium salts (L-MSG and L-MSA) in the shape of field potential. The responses of bioelectrical tongue reveal a dose-dependent behavior in amplitudes with stimulations of same umami compounds at different concentrations. Moreover, the synergistic enhancement between umami compounds and purine nucleotides measured with the MEA alsorepresent a potential application in umami evaluation of mixed flavorings. These consequences of analysis validate that the tissue-MEA biosensor is an attractive approach for umami detections by mimicking umami detection and evaluation of organisms.

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