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Biosensor analysis of natural and artificial sweeteners in intact taste epithelium

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ABSTRACT

Sweeteners are commonly used as food additives in our daily life, which, however, have been causing a number of undesirable diseases since the last century. Therefore, the detection and quantification of sweeteners are of great value for food safety. In this study, we used a taste biosensor to measure and analyze different sweeteners, both natural and artificial sweeteners included. Electrophysiological activities from taste epithelium were detected by the multi-channel biosensors and analyzed with spatiotemporal methods. The longtime signal result showed different temporal-frequency properties with stimulations of individual sweeteners such as glucose, sucrose, saccharin, and cyclamate, while the multi-channel results in our study revealed the spatial expression of taste epithelium to sweet stimuli. Furthermore, in the analysis of sweetener with different concentrations, the result showed obvious dose-dependent increases in signal responses of the taste epithelium, which indicated promising applications in sweetness evaluation. Besides, the mixture experiment of two natural sweeteners with a similar functional unit (glucose and sucrose) presented two signal patterns, which turned out to be similar with responses of each individual stimulus involved. The biosensor analysis of common sweeteners provided new approaches for both natural and artificial sweeteners evaluation.

1. Introduction

Sweetness is generally regarded as a gratifying experience. Studies indicate that the perception of sugars and sweeteners has very ancient evolutionary beginnings, since sweet perception might have helped our early ancestors to seek for carbohydrate-rich food to ensure their intake of energy (Blass, 1987; Behrens et al., 2011). However, since the last century, the world has been facing a cascade of undesirable health effects of sweet intake, such as dental caries (Grenby, 1991), as well as cardiovascular diseases and its risk factors such as obesity and type-2 diabetes (Howard and Wylie-Rosett, 2002). These are all associated with sweeteners and have increased the demands for low calorie sweeteners worldwide. Meanwhile, the health concern of artificial sweeteners has also become a more serious problem in recent years (Kim and Kinghorn, 2002). Researchers suggested that artificial sweeteners may not only prevent us from associating sweetness with caloric intake, but also be addictive, leading us to eat more high-energy food unconsciously, and then gain weight (Whitehouse et al., 2008; Swithers and Davidson, 2008; Malik et al., 2010). Therefore, the detection and evaluation of sweetness, both natural sugars and artificial sweeteners, are gathering more and more attention in many fields, such as foods, beverages, and pharmaceuticals.

In a biological taste system, the signal responses to sweeteners are initially coded in taste buds of the epithelium by action potentials. It is known that all sweeteners are mediated by heterodimeric G-protein coupled receptors in Type II cells in the bud, which have a plurality of binding sites to correspondingly identify sweet stimuli of different structures (Bachmanov and Beauchamp, 2007; Nelson et al., 2001; Zhang et al., 2003; Zhao et al., 2003). However, studies have suggested that there are two kinds of sweetness signal transduction models which are different but interdependent to conduct different types of sweet stimuli. One is the cyclic adenosine monophosphate (cAMP) pathway utilized by natural sugars such as sucrose to elicit membrane depolarization, and the other is the inositol triphosphate (IP_3) and diacylglycerol (DAG) pathway used by artificial sweeteners for signal transduction (Doty, 2003; Bernhardt et al., 1996; Cummings et al., 1993; Striem et al., 1991; Behe et al., 1990). Especially, many research works have pointed out that receptor activities toward the artificial sweeteners greatly depend on residues in the amino terminal domains of the sweet taste receptors as ligand binding sites (Cui et al., 2006; Liu et al., 2011; Fernstrom et al., 2012). This means the signal responses of taste receptor cells to natural sugars and artificial sweeteners may be different and can be used to characterize different sweeteners.

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During the last decade, lots of efforts have been made to explore effective methods for sweetness detection and evaluation. Among all the approaches, high performance liquid chromatographic (HPLC) and ion chromatographic methods, which use a chromatographic technique to separate a mixture of compounds, have been extensively used (Casals et al., 1996; Biemer, 1989; Serdar and Kneževíc, 2011). However, these methods are often complicated and laborious, which always involve particular pretreatment and large scale equipment. To develop a more effective method, researchers combined lipid films with electrochemical techniques as biosensors for rapid and sensitive screening of sweeteners (Nikolelis et al., 1999). Many achievements have been attained with the techniques, which implied the efficient application of biosensors in sweeteners detection and evaluation (Nikolelis and Pantoulias, 2000; Tien and Salamon, 1989; Zviman and Ti Tien, 1991; Otto et al., 1992).

Cell- and tissue-based biosensors, which treat biometric units of cells and tissue as sensing elements, can collect the functional information of bioactive analytes with high sensitivity and specificity (Bousse, 1996; Rudolph and Reasor, 2001; Wang and Liu, 2009). In our previous work, we established a taste biosensor system for taste detection by electrophysiological sensing measurements of taste epithelium from rats (Liu et al., 2013a). Compared to the traditional electrochemical methods, our epithelium biosensor has realized the effective detection and classification of salt, bitter and even mixed tastants (Liu et al., 2013b). The spatial information of taste perception is well preserved in an intact tissue, and can be explored by our multi-channel recording biosensor. In this study, we applied the epithelium biosensor for the detection and evaluation of different sweeteners. Considering the differences between natural sugars and artificial sweeteners, four sweet stimuli (glucose, sucrose, saccharin, and cyclamate) were used to explore the spatiotemporal information of sweet taste transduction.

2. Methods

2.1. Tissue preparation on microelectrodes

In the biological taste system, taste buds can sense and recognize five basic tastes, while Type II taste cells in taste epithelium respond to sweeteners. In the preparation of taste epithelium, we chose a tissue slice with an intact taste bud to preserve the sensing unit of Type II taste cells for sweet perception. The isolation and cultivation process of taste epithelium was similar to our previous work (Liu et al., 2013a). The isolated epithelium (about 5 mm × 5 mm) was rinsed with Ringer's solution and placed with taste pores side up on the surface of microelectrode array (MEA) chip for signal recording, as shown in Fig. 1a. The functional unit of taste buds was well preserved with Type II taste receptor cells and microenvironment intact for sweeteners detection (Fig. 1b).

The MEA chip can realize the multi-channel recording of sweetener-induced signals for temporal–spatial analysis. The MEA chip was composed of an array of 60 electrodes, and the electrodes were 30 μm in diameter with 200 μm center to center spacing, which could avoid the electric interference between the neighboring electrodes effectively (Fig. 1c). After the fixation of taste epithelium on MEA, the micrograph of fabricated gold electrode array covered with an intact taste epithelium was shown in Fig. 1d. Observing through the scanning electron microscope (SEM, SU-70, Hitachi), filiform papillae and fungiform papillae formed a dense meshwork on the epithelium for sweet sensing, as shown in Fig. 1e. The native structures of the taste buds were well preserved in the isolated epithelium, with receptor cell populations intact. The epithelium was then fixed by a plastic ring-shaped frame covered with a tightly stretched piece of mesh on the chip.

2.2. Electrophysiological recording to sweet stimuli

During recording, the taste epithelium was perfused with oxygenated Ringer's solution at a flow rate of 1 ml/min. The sweet compounds were bath-applied through an active perfusion system. Responses of sweet compounds were recorded using the MEA1060-Inv system from Multichannel Systems (MCS, Reutlingen, Germany). The analog extracellular neuronal signals from 60 channels were AC amplified, sampled at 20 kHz, and stored in a compatible computer for subsequent off-line analysis. All recordings were subsequently subjected to spatial and temporal analysis using MATLAB (MathWorks, Natick, MA).

The responses were elicited by typical sweet stimulations. We used common sweeteners of glucose, sucrose, saccharin, and cyclamate as taste stimuli to taste epithelium, representing both natural sugars and artificial sweeteners. The sweetener chemicals were all obtained from Sigma (St. Louis, MO). The concentrations of natural sugars (glucose and sucrose) were fixed at 50 mM, 100 mM and 150 mM, while the concentrations of artificial sweeteners (saccharin and cyclamate) were 5 mM, 10 mM and 15 mM, under references and preliminary experiments. Then, in mixture sweeteners experiments we used 10 mM for each sweetener. And, in sweetened beverages experiments, we selected Coca-Cola and Coca-Cola Zero as examples due to their popularity. The carbonated beverage samples were pretreated by ultrasonication for 20 min to remove gas bubbles for the biosensor detection. Before stimulation, native electrophysiological activities of the epithelium were recorded for 150 s as a control trial. After taste substances being injected into the MEA chamber, the recording also lasted for about 150 s. Then the stimulus was thoroughly washed out from the chamber by standard perfusate to make the tissue back to native state. All solutions were added by a peristaltic pump and a selective valve controlled by a personal computer. All recordings were performed at 25 °C (room temperature).

2.3. Signal analysis of sweet signals

Signals were analyzed by MATLAB in both spatial and temporal domains to explore the feature information of different sweeteners. In spatial domain, we plotted the firing signals of 60 channels and calculated the corresponding magnitude of potentials to each sweetener. While in temporal domain, we analyzed the recorded long-time signals by their response patterns and basic characteristics. Furthermore, to explore the signal information of taste epithelium to sweet stimuli with different concentrations, we calculated and plotted the comparison diagram of basic characteristics after the stimulations of sweeteners with different concentrations.

To cluster and classify signals, the *K*-means cluster algorithm was used to calculate the spike sorting maps of different sweeteners induced signals, with the basic characteristics of amplitude (voltage difference between the maximal positive and negative peaks) and duration (interval between onset and end of one complete potential) shown in typical waveforms. *K*-means clustering is a heuristic algorithm which is easy to implement and apply even on large data sets. It has been widely used for image segmentation, signal classification and even for semi-supervised learning in Natural language processing. There are three steps in *K*-means clustering: first of all, cluster all the signals with randomly chosen centers by calculating the distances; then rematch the centers by average; finally plot all the sorting maps of different waveforms. With these typical waveforms, a pattern clustering was plotted based on the values of amplitude and duration from signals to sweeteners. At the

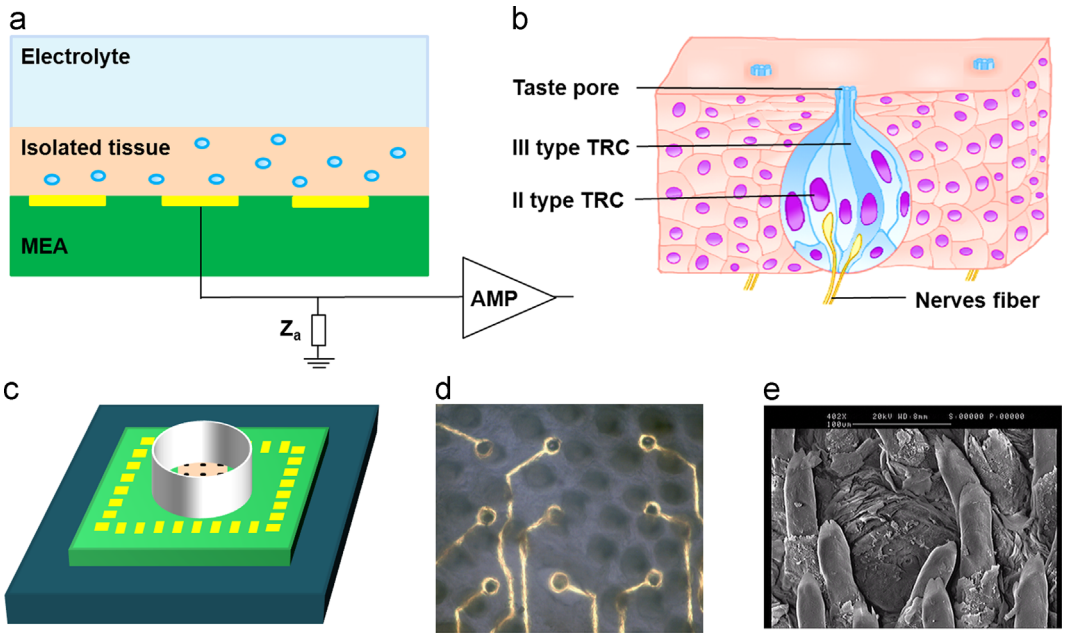

Fig. 1. Electrophysiological recording of taste epithelium by microelectrode arrays (MEA) for sweet analysis. (a) Schematic diagram of the hybrid biosensor. (b) Biological structure of taste epithelium with Type II taste cells in the tissue for sweet analysis. (c) Schematic diagram of 60-channel MEA device. (d) Micrograph of gold microelectrodes covered with a taste epithelium. (e) Fungiform papillae of the taste epithelium for sweet perception observed by SEM.

same time, a sonogram was used to implement the time–frequency analysis in a visualized way. It is a novel way of showing the frequency distribution of every short time point, and has been found useful in speech analysis and medical image processing. It is achieved through following steps: first, divide the signals into short time periods with a window function; then compute the energy density spectrum of every short time period; finally, display the energy density spectrum in pseudo-color. Horizontal axis represents time, vertical axis represents frequency, and the size of each pixel gray value reflects the energy density of the signal in corresponding time and frequency.

3. Results and discussion

3.1. Sweet induced signals recording

As mentioned, sweet taste has become the hot topic of taste research because of their influences on health and diseases. The detection and analysis of different sweeteners are thought to be the basis and main content of sweet taste research. Therefore, by combining the taste epithelium with MEA, we applied the epithelium biosensor as a new method for common sweeteners analysis, aiming at analyzing different sweeteners that are commonly used in food additions. Both natural and artificial sweeteners that can evoke the typical sweet sensing were discussed and evaluated by the electrophysiological activities from taste epithelium.

In our experiments, glucose and sucrose with concentration of 100 mM were chosen as the representative of natural sugars, while saccharin and cyclamate with concentration of 10 mM acted as artificial sweetener stimuli for taste analysis. Fig. 2 displays the original extracellular signals from taste epithelium for sweet taste analysis, with Fig. 2a representing a 60-channel signal as an example for multi-channel information under saccharin stimulation. The multi-channel results showed that only a specific region recorded dominated potentials with similar firing patterns, while other channels responded only with small peaks to sweet stimulus. As shown, channels 6, 7, and 8 recorded big sustained potentials with mean amplitude of about 110 μV, while channels 9 and 14 detected slightly small positive signals with mean amplitude of about 70 μV. The multi-channel results revealed that only taste receptor cells in a specific region of taste epithelium were activated in sweet taste sensing, showing the spatial expression for sweet taste analysis, and the result matched well with our previous study (Liu et al., 2013a). In the study of five basic tastes perception, we found that different subsets of taste receptor cells were activated by five tastes stimulations, which also meant that only one specific region of taste epithelium participated in sweet taste sensing with unique spatial expressions. That is to say, the biosensor analysis of sweet taste is able to code the spatial pattern of taste epithelium with multi-channel signals.

Fig. 2b shows the preprocessed long-time potential responses recorded by the epithelium biosensor in control and four sweet stimulations. In the stimulations of glucose, sucrose, saccharin, and cyclamate, channels 6, 7, 8, and 9, respectively, recorded the signals with highest firing rates of the multiple channels, so we chose signals in these channels as the typical long-time responses. It can be observed that the epithelium biosensor recorded obvious potential responses to both natural and artificial sweeteners compared with the control recording trial, providing the original long-time signals for sweet analysis. Furthermore, it is evident that the signals present distinct differences in response patterns to different sweeteners. When applied with glucose stimulus, taste epithelium delivered sparse signals with similar positive waveforms, while in the stimulation of sucrose, sustained signals with different negative spikes were recorded. When it came to the stimulation of artificial sweeteners, the taste epithelium responded with more intensive signals, even if the concentration was lower than that of natural sugars. It can be observed that big sustained signals with intensive firings were recorded in the stimulation of saccharin. The big signals continued for about 100 s, then rested for a while and went on being released with small burst spikes. While in the stimulation of cyclamate, the epithelium responded with an irregular positive signal firing pattern. The potential patterns of signals usually correspond to the unique responding process of taste epithelium, which meant that the taste-induced long time signals may be used well to characterize different sweeteners. Furthermore, it is obvious that

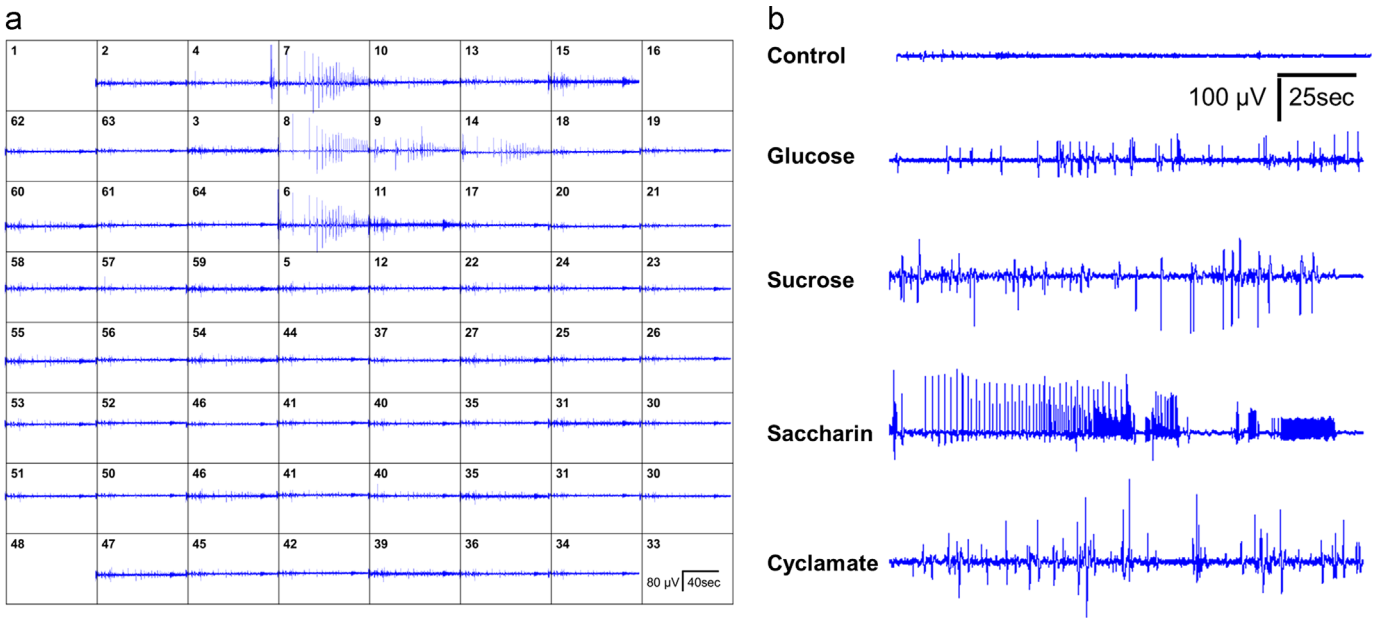

Fig. 2. Extracellular signals from taste epithelium to sweet taste stimuli. (a) Multi-channel recording of taste epithelium during saccharin stimulation. (b) Long time changes of one channel electrophysiological signals after the stimulations of glucose, sucrose, saccharin, and cyclamate.

the artificial sweeteners with low concentration can evoke more intensive signals than natural sugars in high concentration, especially for the saccharin, which is thought to be the sweetest substance (Cardello et al., 1999; Stone and Oliver, 1969; Giovanni and Pangborn, 1983; Moskowitz, 1970).

From the original extracellular signals in Fig. 2, we found that the biosensor can realize the long-time detecting and recording of extracellular signals simultaneously from multiple sites, which revealed the multiple-channel information as well as the long-time signals from specific channels for sweetener analysis. And, in the biosensor the taste epithelium was cultured *in vitro* with suitable environment after isolation and preserved the functional integrity of taste buds and their microenvironment for sweet taste, providing a good candidate for sweet taste sensing.

3.2. Temporal discrimination of sweet-induced signals

In signal analysis, the typical waveforms usually represent the temporal response properties. To derive more information for sweetener analysis, we analyzed the spike sorting map of all the sweetener-induced signals by the *K*-means algorithm, which clustered the corresponding typical waveforms of signals to each stimulus, as shown in Fig. 3a. It is evident that the spike-sorting map presented different shapes of waveform to different stimuli. The signals have obvious differences in basic temporal characteristics, such as amplitude and duration. To be more specific, we extracted and calculated all the values of amplitude and duration to characterize the features of sweeteners and plotted the signal-clustering pattern based on the values, which is shown in Fig. 3b. It can be seen that signals recorded were clustered into several regions, with the artificial sweeteners induced signals clustered with large amplitude and short duration, and the signals of natural sugars showing small amplitude and long duration. Actually, the mean amplitudes of signals after the stimulations of glucose, sucrose, saccharin, and cyclamate were 92.16 ± 2.863 μV, 98.77 ± 10.52 μV, 169.9 ± 2.796 μV, and 108.16 ± 5.405 μV. The corresponding mean durations equaled 550.1 ± 24.86 ms, 302.4 ± 12.09 ms, 142.4 ± 5.813 ms, and 267.0 ± 7.021 ms. The mean amplitudes, which were related to the signal intensity, showed obvious difference to different sweeteners, and the values of artificial sweeteners with low concentration were bigger than that of natural sugars with higher concentration. The difference in signal intensity likely resulted from the difference of sweetness in natural and artificial sweeteners. Studies showed that the relative sweetness of artificial sweeteners with concentration of about 10 mM was much larger than that of natural sugars with the concentration of about 100 mM (Cardello et al., 1999; Stone and Oliver, 1969). This meant that the relative sweetness of different sweeteners can be effectively evaluated by the amplitude of signals recorded with our epithelium biosensor.

From the results of Fig. 3, we found that all the sweeteners sensed by taste epithelium can be distinguished effectively by temporal properties clustering of characteristic waveforms. The maps of regions activated by one sweetener can be visualized with special pattern recognition. The results may reveal that both the natural and artificial sweeteners can be reflected by the epithelium biosensor in the form of different signal characteristics. Thus, the recognition of a particular sweetener can be converted into a specific pattern by deriving the characteristics.

3.3. Time–frequency analysis of sweet-induced signals

After the temporal analysis of long-time responses, we extracted the single waveform of the typical signals and calculated the time–frequency distribution by the sonogram, a color-coded method, to derive more information from the differences of the taste epithelium responses to different sweeteners. The visualized time–frequency distributions of our recorded typical waveforms to sweeteners are shown in Fig. 4. The response waveforms of taste epithelium have obvious characteristic time–frequency distribution under the stimulation of each sweet stimulus. Under the stimulation of artificial sweeteners, the frequency peak emerged with the spike peak, and showed a broad frequency band mainly ranging from 0 to about 40 Hz, especially for saccharin which reached the band of 60 Hz, while in the stimulations of natural sugars, the main frequency peak centralized to the low frequency band of 0–10 Hz. Furthermore, the time distributions of the spikes are also displayed in the color maps, which meant different time–frequency response patterns to different sweeteners.

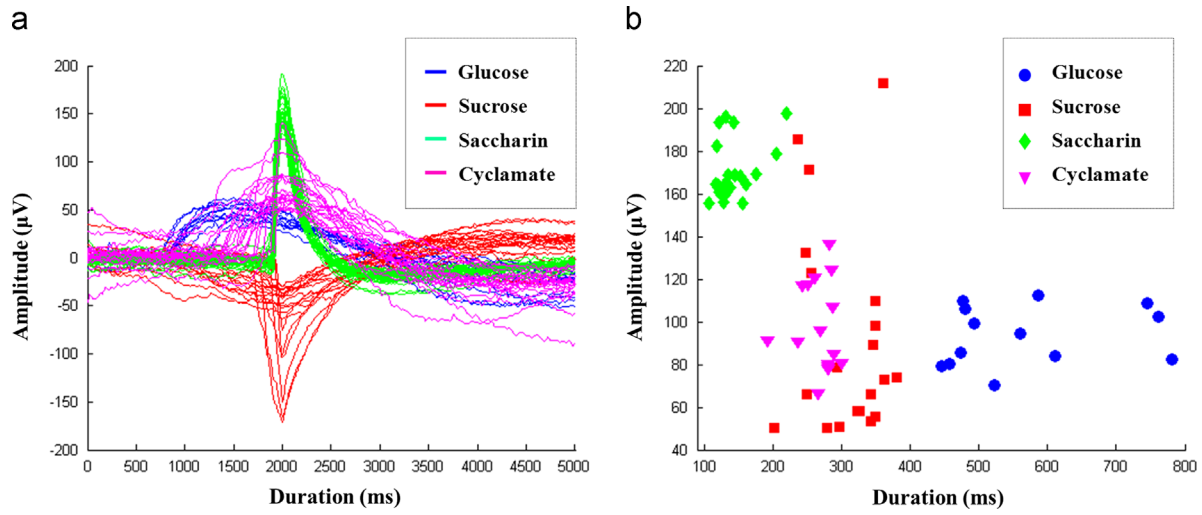

Fig. 3. Temporal discrimination of sweet-induced signals under stimulation of glucose, sucrose, saccharin, and cyclamate. (a) Spike sorting map of signals to different sweeteners. (b) Recognition pattern sensed by the biosensor. The amplitude and duration were derived from original signals.

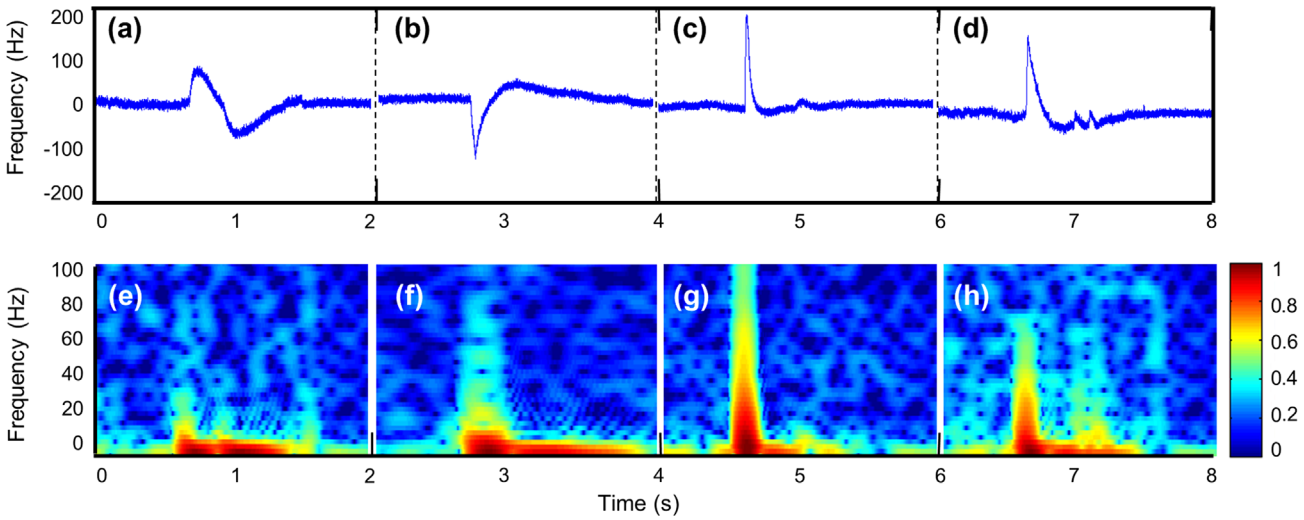

Fig. 4. Sonogram spectrum analysis for single waveform of signals under different sweet stimulations. Single waveform of glucose (a), sucrose (b), saccharin (c), and cyclamate (d), and sonogram spectrum of glucose (e), sucrose (f), saccharin (g), and cyclamate (h) respectively.

From the time–frequency result of sonogram, we can easily get the time–frequency information of typical waveform to sweeteners. Compared with the traditional frequency calculating method of power spectrum analysis, this image expression method merged the time and frequency information together, and presented in an accurate and visualized way. Furthermore, it has advantages in expressing high frequency information versus power spectrum, displaying distinct frequency bands in different signals. All these will benefit the practical application of the biosensor greatly.

3.4. *Evaluation of sweeteners with different concentrations*

After the analysis of different sweeteners, we used the biosensor for the evaluation of the same sweetener with different concentrations. Here, glucose and saccharin were used as examples for concentration analysis. The concentrations of glucose were 50 mM, 100 mM, and 150 mM, while the concentrations of saccharin were 5 mM, 10 mM, and 15 mM. Long-time potentials evoked by different concentrations and the normalized comparison diagram of basic characteristics were extracted and plotted in Fig. 5.

For glucose at increasing concentrations, the basic characteristics of both amplitude and frequency displayed obvious increasing trends. In the stimulations of saccharin, the amplitude of signals also demonstrated an evident increase in a dose-dependent manner, while the frequency of signals revealed different results. In the low concentration of saccharin, cluster firing signals with high frequency were recorded periodically in short duration, while during the higher concentrations of 10 mM and 15 mM, long-time sustained signals were detected with visible dose-dependent fires. Although the frequency of signals to saccharin with low concentration was higher than that with other concentrations, the total signal firing did not reach high intensity as it fired transiently and irregularly.

As the basic characteristics of signals, especially amplitude, represented the response intensity to different stimuli, the comparison results of basic characteristics implied the relationship between concentrations and the intensities of sweetness. It is evident that both natural sugar and artificial sweetener displayed evident increasing trends with concentrations in amplitude, revealing the dose-dependent increase of sweetness intensity. It is in accordance with our sensory evaluation that a sweetener with high concentration tastes sweeter than that with low concentration. These results

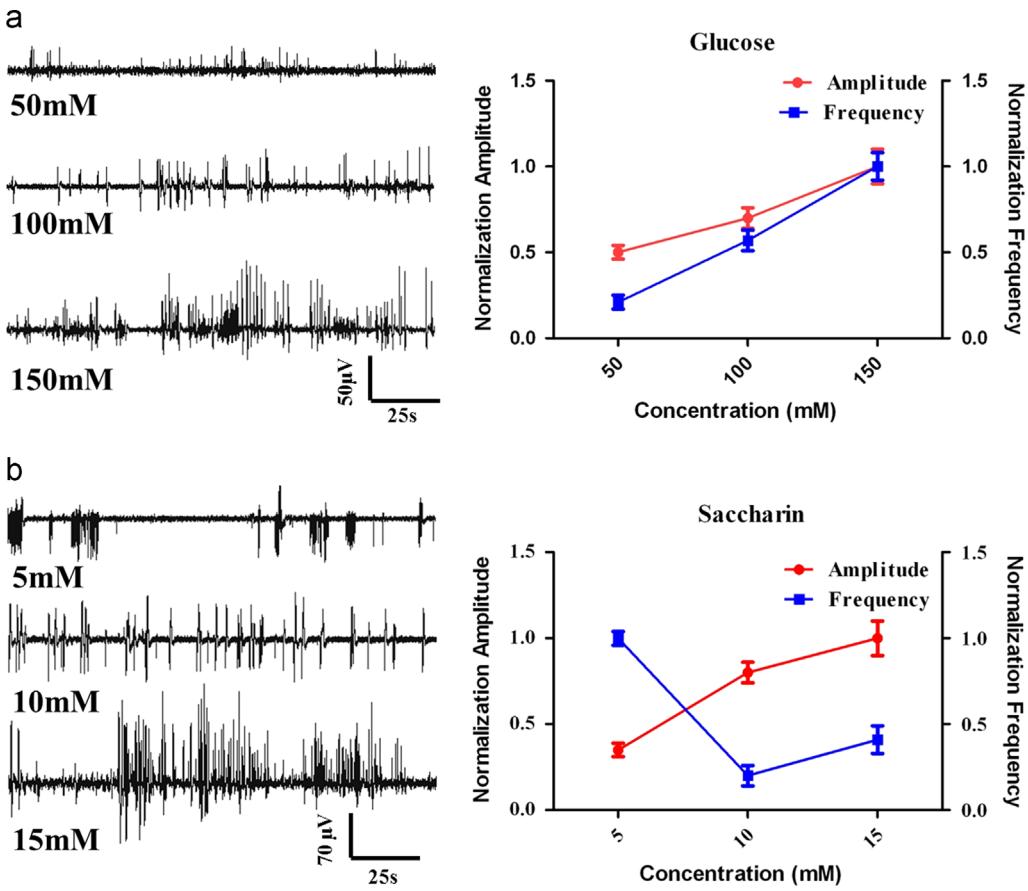

Fig. 5. Analysis of sweeteners with different concentrations. Long-time signals of glucose (a) and saccharin (b) respectively, with different concentrations and a mathematical statistic on normalized amplitude and frequency. Mean ± sd. ($n \geq 20$) are shown.

demonstrated that the biosensor reflected a dose-dependent behavior in electrophysiological activities to sweeteners at increasing concentrations.

3.5. *Mixture sweeteners experiment*

After the stimulations of four individual sweeteners, we added mixture sweet stimuli and same real sample of foodstuff to the biosensor (Fig. 6). Two mixture sweet stimuli were used, one is a mixture of four sweeteners (glucose, sucrose, saccharin, and cyclamate) and the other one is a mixture of two natural sweeteners. After the stimulation of four sweeteners, we recorded a kind of complex signal, which is hard to be recognized and discriminated. Considering the interactions of the four sweeteners, we thought that the mixture stimulation may cause the signal variability, which needs to be further investigated. However, the potentials were recorded after the mixture stimulation of two natural sweeteners; some of them presented a regular signal response pattern. For example, the result of the mixture of glucose and sucrose, which has the same functional group, is shown in the figure. It is obvious that the signals of this mixture stimulation presented two patterns of signal responses, with the small spikes similar to the typical waveform of glucose and the large ones similar to those of sucrose. Besides, the responding time–frequency distributions of two waveforms are also similar with those of individual glucose and sucrose stimulation. That is to say, our taste epithelium-MEA biosensor is able to discriminate sweeteners with a similar functional group while hard to recognize the mixture of more than two kinds of sweeteners. More mixture sweeteners cannot be recognized effectively, which may result from the interactions of signals to these sweeteners.

Besides above stimulations, we also tried applying our sensor to actual sweetened foodstuff detection, for example, the beverages of Coca-Cola and Coca-Cola Zero. We found that the signals caused by Coca-Cola were larger both in amplitude and firing frequency than those caused by Coca-Cola Zero. In another aspect, although our sensor could distinguish between Coca-Cola Zero and Coca-Cola, it could hardly recognize specific sweetener from one beverage. This may be owing to the complexity of actual sweetened beverages components and the diversity of the sweeteners included. Hence, our sensor can be used to contrast the sweetness of sweetened beverages and preliminary explore the impact caused by beverages on physiological taste, but can hardly identify the various components of beverages respectively. Improved membrane sensitivity and advanced pattern recognition should be developed for real samples such as foodstuffs.

During the analysis of different sweeteners individually, we found that the responses to artificial sweeteners were quite different from those of natural sugars in both time and frequency domains. In the perception of artificial sweeteners, the taste epithelium biosensor responded to the low concentration with intensive spike firing and broad frequency distribution, while sparse signals with low frequency distribution were released to natural sugars with high concentration. Actually, it has been studied with assays for IP_3 and with Ca^{2+} imaging that stimulation with the non-sugar sweeteners such as saccharin and others rapidly increased the cellular content of IP_3 greatly, while sucrose, but not saccharin, increased the content of cAMP of tissue preparation (Bernhardt et al., 1996). In the analysis of sweeteners with different concentrations, we found that the dose-dependent phenomenon existed in both natural and artificial sweeteners, presenting good applications of concentration evaluation with our biosensor.

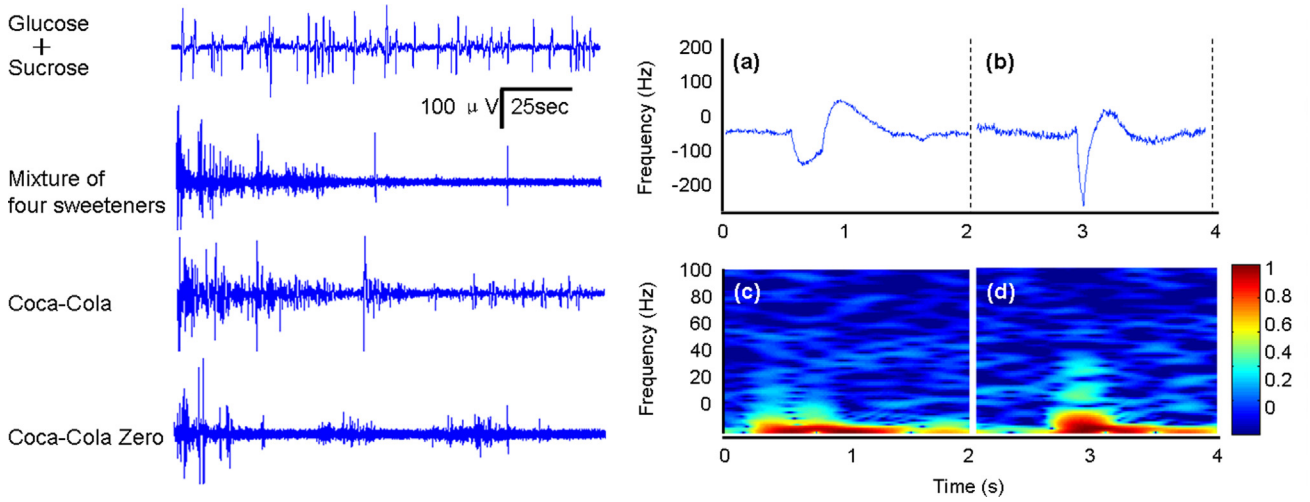

Fig. 6. Response signals under mixture sweet stimuli and real samples of Coca-Cola and Coca-Cola Zero, with the sonogram analysis of typical waveforms of glucose and sucrose in the mixture.

Artificial sweeteners, labeled as low calorie and nonnutritive, have established a significant commercial market in our daily life. Therefore, the sensing and their perception process, and the differences between natural and artificial sweeteners are gathering more and more attention. Many techniques have been applied for sweetener study, and biosensors are thought to be one of the most promising platforms. Our epithelium biosensor realized the rapid detection and analyzing of different sweeteners, providing the basic spatial and temporal information of different sweeteners. However, the signals from taste epithelium are more complex and need special signal process, which may be different from those directly elicited by sweet stimuli. At the same time, for more complex sweeteners which are commonly used in the industry, the response of the biosensor was also more complex with different kinds of stimulated receptors. The biosensor will be improved if we can extract the specific receptors for sweet perception, and the signals will be directly used to characterize different sweeteners in the next step.

In this study, we realized the detection and analyzing of different sweeteners by means of action potential in taste epithelium. With the activity of taste epithelium preserved, several assays were performed with our biosensor for rapid detection of different sweet stimuli, and the sensor is recyclable with good reproducibility to different stimuli when the taste stimulus was thoroughly washed out from the chamber by standard perfusate after each taste recording. The electrophysiological information of signals to different sweeteners was detected, which played an important role in the molecular understanding of the mechanism of sweet taste signal transduction. Therefore, the signal results of our study provided the basic information for the exploration of the mechanism of sweet taste perception and different transduction pathways in natural and artificial sweeteners recognitions. Besides, the study can be applied to explore the effects of other substances, such as drugs, on the sweet perception. For further research, we want to explore methods to improve our biosensor to obviously discriminate mixtures of sweeteners with different functional groups. And then we can apply this technique to distinguish sweeteners included in food and drinks and detect their impacts on taste.

4. Conclusion

The epithelium biosensor was used to detect and evaluate sweeteners in an efficient way. Both natural and artificial sweeteners were evaluated by the electrophysiological activities. When stimulated by different sweeteners, the recorded signals presented different potential patterns and signal characteristics in both time and frequency domains, while the sweetener with different concentrations revealed obvious dose-dependent results in the intensity of sweetness. The multi-channel results indicated a specific region of taste epithelium mediating the sweet sensing, revealing the unique spatial expression. With function units well preserved and successfully recorded, the biosensor analysis of sweet taste provides a promising approach for sweetness measurements.

Acknowledgments

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