Biosensors and Bioelectronics 54 (2014) 385–392

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Biosensor analysis of natural and artificial sweeteners in intact taste epithelium

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ARTICLE INFO

Article history:
Received 31 August 2013
Received in revised form
4 November 2013
Accepted 6 November 2013
Available online 15 November 2013

Keywords:
Biosensor
Natural sugar
Artificial sweetener
Taste epithelium
Microelectrodes

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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http://dx.doi.org/10.1016/j.bios.2013.11.020

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