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Biosensor recording of extracellular potentials in the taste epithelium for bitter detection

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ABSTRACT

The sense of bitter taste provides critical information about ingestion of toxic and noxious chemicals. In this study, a novel biosensor, mimicking biological responses to bitter compounds, was developed to measure and evaluate bitters. We used 32-channel microelectrode arrays (MEAs) with the diameter of 30 μm as a multi-channel recording platform, and employed intact taste epithelium of rats as a biological sensing element. Electrophysiological activities of epithelium which preserved native state of taste cell population were measured and analyzed through the multirecording system. We found that administrations of different bitter stimuli such as quinine, denatonium and cycloheximide significantly evoked specific responses respectively, and electrophysiological signal characteristics, such as firing rates, amplitudes and power spectrum, have a visible increase with concentrations of bitternesses. The investigation of taste epithelium with cellular potential measurement based on MEAs represents a fast and reliable biosensor for recognizing and distinguishing bitter tastants.

1. Introduction

Human beings and animals have sensitive and versatile taste systems to distinguish between sweet, sour, bitter, salt and umami tastes [1,2]. Of these basic tastes, bitter perception has a particularly important role: many kinds of poisons are bitter, and virtually all animal species show a behavioral aversion to such substances [3–5]. Therefore, the electronic tongue for bitter detections, mimicking the biological function, has received great attention for its high-potential applications of food safety, pharmaceutical industry, and environment monitoring [6–8]. These electronic tongues, usually employing electrochemical, optical, mass and enzymatic sensors, show specificities to different bitter taste-causing compounds. However, the electronic tongue is not as perfect as the biological gustatory system in performance of sensitivity and specificity, which benefit from the well-evolved receptors and information coding logic for bitter taste.

Taste transduction begins with interactions between tastants and taste receptor cells (TRCs), which are assembled into basic taste sensing organs called taste buds. To bitter, taste is mediated by a family of approximately 30 highly divergent G-protein-coupled receptors (GPCRs) that selectively expressed in TRCs [2,9,10]. Interactions between bitternesses and specific GPCRs elicit second messenger cascades, activate a member of the transient receptor potential (TRP) channel family named TRPM5, and depolarize TRCs. Chemical signal is converted into action potential of TRCs then electrical signal is delivered to afferent nerves to encode and decode, ultimately the taste information about bitter forms in the brain [11,12].

Electrical signals can be measured with electrophysiological detection methods, and analyzed in time domain and frequency domain respectively to build biosensors for recognizing and distinguishing bitter taste components. The conventional patch clamp technology is a standard detection method for cellular electrophysiology research with the quality of large information content and high resolution. Nevertheless, due to its invasive characteristic, low-throughput nature and operational complexity, and even great efforts were made to build other automated and easy-to-use electrophysiological detection platforms with high-throughput [13–17].

Microelectrode arrays (MEAs), in a noninvasive and long-term manner, can monitor electrophysiological activities of taste cells from multiple network sites with high spatial and temporal resolution, and thus are a promising detecting method to cellular electrophysiology research. In recent years, a number of studies

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have reported that cells were cultured on surface of MEAs *in vitro* for various applications in pharmacology, toxicology, developmental biology and basic research [18–21]. However, unlike biological tissue with three-dimensional formation and intrinsic interaction between neighboring cells, cell networks cultured on MEAs were limited to a two-dimensional environment and lose inherent cell-to-cell communications by means of biochemical and mechanical cues [22–24]. Cellular signal transduction of cell networks was altered into a two-dimensional plane, and such networks can lose specific properties of living tissue. Consequently, taste epithelium can be employed as a biological sensitive element to keep biological structure and properties of taste buds, maintaining advantages of *in vitro* experiment such as controllable experiment condition.

Cell and tissue based biosensors are special devices that employ immobilized living cells and tissues as sensing elements, combined with sensors or transducers to produce responses, such as electrical signal, through interactions between stimulus and cells or tissues [25–27]. In our present study, we managed to combine taste epithelium with MEAs for a bioelectronic tongue of taste buds. Taste epithelium was stripped from tongue of rats with the mixture of collagenase and proteolytic enzymes, and fixed on the surface of MEAs. Electrophysiological signals, elicited by TRCs expressing bitter receptors and transmitted were measured through the multi-channel recording system with MEAs. Ultimately, the multi-channel signals were analyzed to reveal the spatial and temporal information of periphery taste sensing system for bioelectronic tongue.

2. Materials and methods

2.1. Design and preparation of MEAs

MEAs were composed of an array of electrodes where intact taste epithelium was fixed for detecting electrical signal produced by taste buds (Fig. 1). The fabrication, preparation and exact measurement methodology of MEAs were described detailedly in our previous olfactory sensing work [28]. The standard MEAs, with a 6×6 array of gold microelectrodes, were 30 μm in diameter and 200 μm in inter-electrode spacing, which avoided the electric interference between the neighboring electrodes effectively and helped us obtain more channels of signals from a small tissue. A plastic ring was fixed onto each MEAs chip to allow addition of liquid stimuli and buffer solution applied to rinse. Prior to experiments, platinum black was electrodeposited onto exposed electrodes to improve the signal-noise-ratio of the chip. The petri dish with diameter of 15 mm was fixed around the chip by epoxy and a spiral platinum wire was used as the reference electrode.

2.2. Isolation and fixation of taste epithelium

Sprague–Dawley rats weighing about 250 g were provided by the Laboratory of Animal Research Center of Zhejiang Province, China. After being anesthetized by intraperitoneal injection of 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 collagenase and protease. The isolated epithelium (about 5 mm × 5 mm), from the tongue root on which many circumvallate papillae, was rinsed with PBS and placed with taste pores side up on the surface of MEAs. The epithelium was then fixed by a plastic ring-shaped frame covered with a tightly stretched piece of mesh. The natural structures of taste buds were preserved with the basic receptor cells population maintained integrated.

2.3. Electrical signals recording

For tissue recording, the MEA measurement is mainly based on Hodgkin–Huxley (H–H) theory of the action potential and the solid–electrolyte interface model of electric double layer [28]. We applied the USB-ME16-FAI system from Multi-channel Systems (MCS, Reutlingen, Germany) for synchronously recording signals from 16 channels. The amplifier employed has a bandwidth from 1 Hz to 3 kHz and a gain of 1200. The whole recording system was placed in a shielding box to avoid external electromagnetic interference. Noise level was maintained at about 20–40 μV peak-to-peak referred to the electrodes. A MC RACK software (MCS, Reutlingen, Germany) was used to display and store up signals in real time with the sampling rate of 20 kHz.

2.4. Taste experimental protocol

In our experiment, the concentrations for quinine, denatonium and cycloheximide were chosen from 10 μM to 100 mM. Experiments were divided into two phases. To investigate specific response to different bitternesses, quinine, denatonium and cycloheximide with different concentrations were added to stimuli epithelium in turns. The same bitternesses at increasing concentrations were delivered into the MEAs chamber to explore the dose-dependent characteristic of biosensor.

Before stimulations, native electrophysiological activities of the epithelium in PBS were recorded for 150 s and used as control condition. Then, bitter stimuli were added into the bath solution in the MEAs chamber and the electrophysiological signal of epithelium was also recorded for 150 s. After an administration of bitter compounds, PBS was injected into the MEAs chamber automatically and repeated twice to wash out the bitternesses totally. In order to rule out the influence of residual tastants and make electrodes and tissue return to a stable state, the minimum interval between the taste injections was 300 s. All recordings were performed at room temperature (18–25 °C).

2.5. Mathematical statistics

The data, such as amplitude, duration and firing rate, were acquired from signals of different experiments respectively, and given as mean ± standard deviation of n samples. For each series, data were normalized to the mean value at the highest concentration or the largest response signal. Mean values were also statistically compared using the Student's t-test. Responses were significant different from each other if $P < 0.05$.

3. Results and discussion

3.1. Multi-channel recording of the taste epithelium

Extracellular potentials of taste buds in epithelium were recorded by MEAs (Fig. 1). In the figure, signal of denatonium at 10 μM was given as a typical multirecording. Electrical signals could be monitored simultaneously from multiple network sites of taste epithelium and analyzed in parallel with 16 channels. The extracellular potentials were recorded with mixture of two kind of waveforms (i.e. the enlarged display of signal in channel 04 to denatonium in the figure). One category was waveforms with large amplitudes of several hundred microvolt and long durations of hundreds milliseconds, and other waveform showed low amplitudes of about 50 μV and short durations of ten milliseconds. The measured extracellular potentials showed similar shape. Typically, the initial stage in each event was a rapid downstroke to a minimum field potential amplitude followed by a repolarization to positive peak and a return to baseline. The distinct components of potentials