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A novel pungency biosensor prepared with fixing taste-bud tissue of rats

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ABSTRACT

A novel taste biosensor based on ligand–receptor interaction was developed through fixing taste-bud tissues of SD rats to a glassy carbon electrode. Using the sodium alginate-starch gel as a fixing agent, taste-bud tissues of SD rats were fixed between two nuclear microporous membranes to make a sandwich-type sensing membrane. With the taste biosensor, the response current induced by capsaicin and gingerol stimulating the corresponding receptors was measured. The results showed that the lowest limit of detection of this biosensor to capsaicin was 1×10^{-13} mol/L and the change rate of response current was the highest at the concentration of 9×10^{-13} mol/L, indicating that the capsaicin receptor was saturated at this point. The lowest limit of detection of this biosensor to gingerol was 1×10^{-12} mol/L, and the gingerol receptor was saturated when the concentration of gingerol was 3×10^{-11} mol/L. It was demonstrated that the interaction curves of capsaicin and gingerol with their respective receptors exhibited high correlation (R^2: 0.9841 and 0.9904). The binding constant and dissociation constant of gingerol with its receptor were 1.564×10^{-11} and 1.815×10^{-11} respectively, which were all higher than those of capsaicin with its receptor (1.249×10^{-12} and 2.078×10^{-12}). This study, for the first time, made it possible to quantitatively determine the interaction of the taste receptor and pungent substances with a new biosensor, thus providing a simple approach for monitoring pungent substances and investigating the mechanism of ligand–receptor interaction.

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1. Introduction

Taste, as an important physiological sense, can help specifically recognize and distinguish various taste substances. The taste-transduction process is a neurophysiological process, wherein some chemical information is converted to a second messenger molecule, thus causing the depolarization and Ca^{2+} release of taste cells. In other cases, the taste substance itself also act as cell signals (e.g., Na^+, K^+ and H^+) to induce action potential in taste-receptor cells. When the intercellular electrical signal reaches the threshold value, the nervous excitation can occur and be transmitted to the taste center in the brain so as to produce taste after multiple transductions (Yarmolinsky et al., 2009; Chandrashekar et al., 2006).

In the stripped taste mucosal epithelial tissue with taste buds, the taste receptor cells (TRCs) can retain their normal function well, and can be effectively activated by the taste substances in the invariant biological microenvironment. As a result, a new electrochemical biosensor with taste-bud tissues as the sensitive components can be developed through simulating the nerve-transduction mechanism of the taste (Fohlerova et al., 2007; El-Ali et al., 2006; Liu et al., 2006). Pungency is traditionally considered as a pain sense. Recently, the discovery of the pungent substance receptors, e.g., capsaicin receptor TRPV1 (Ramsey et al., 2006), has indicated that pungency is also a normal taste-transduction process. Although the qualitative and quantitative determination of the pungent substances has been conducted through various chemical methods (Ravishankar et al., 2000; Massa et al., 2006; Prescott and Julius, 2003), there have been few reported feasible methods to be used in the measurement of pungency up to now.

In most of the traditional studies of investigating the mechanism of ligand–receptor interaction, it is essential to purify the receptors first. However, when the receptors leave the cell membrane, the docking interactions of the membranes and the effects of certain intracellular cofactors do not exist any more. As a result, the real interaction mechanism of the receptor with its ligand cannot be accurately elucidated through the traditional method (Floriano et al., 2006; Walters and Hellekant, 2006; Omelyan and

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Kovalenko, 2013).

In this study, a new pungency biosensor was developed with taste-bud tissues as the taste susceptor. Because it precisely simulated the ligand–receptor interaction environment in organisms, the biosensor could be used to elucidate the interaction mechanism of pungent substances with their receptors more accurately. Moreover, the binding constant and dissociation constant of ligand–receptor could be determined to investigate the kinetics with the biosensor (Nunez et al., 2012; Hughes et al., 2012), which could help to further explain the interaction mechanism of pungent substances with their receptors.

2. Experimental

2.1. Materials and apparatus

The CHI660E electrochemical workstation was purchased from Shanghai Chenhua Instruments, Inc (China). Nuclear microporous membrane (0.2 μm pore size, Circles, 25 mm) was obtained from Whatman Inc. (The United Kingdom).

Capsaicin and gingerol were obtained from Sigma (USA). Soluble starch was from Tianjin Yingda Sparseness & Nobel Reagent Chemical Factory (China). Sodium alginate was from Tianjin Guangfu Fine Chemical Institute (China). collagenase II and dispase II were obtained from Sigma (USA). All the other chemicals are of analytical grade. Deionized water was from a Millipore Milli-Q Water System (Millipore Inc.), and was used in all of the experiments.

2.2. Collection of taste-bud tissues

The solution of 1 mg/mL collagenase II and 3 mg/mL dispase II (prepared with calcium-free Tyrode's solution) was used to isolate the rat tongue epithelium, and preheated in an incubator at 37 °C for 15 min before experimentation. After the SD rats were sacrificed by decapitation, the tongues (from the tip to foliate papillae) were removed and washed with the solution for anatomy (137 mM NaCl, 10 mM HEPES, 1.5 mM NaH_2PO_4, 4.5 mM KH_2PO_4, 17 mM glucose and 22 mM sucrose; pH 7.2–7.4) to clean up the blood on the surface. 0.3–0.5 mL of the preheated solution (collagenase II and dispase II) was injected into the space between the tongue epithelium and its muscle layer. The tongues were then placed in the solution for anatomy and incubated at room temperature for 6–8 min. The tongue epithelium was gently stripped from the muscle layer, and immediately stored in the iced solution for anatomy.

2.3. Preparation of the taste-tissue sensor

The starch solution was prepared with dissolving proper amount of soluble starch in 1.0% glutaraldehyde (m/v). After being heated at 80 °C and stirred for 30 min, the solution was kept overnight at room temperature to make starch and glutaraldehyde fully crosslink. Then, the solution was mixed with 2.0% sodium alginate solution at a ratio of 1:1 to obtain sodium alginate-starch gel (Gao et al., 2009). After 10 μL of the gel solution was applied evenly over the two polycarbonate microporous membranes, the processed taste-bud epithelial tissue (10 mm^2) was placed on the center of one microporous membrane, which was then overlapped with the other microporous membrane.

The prepared sandwich-type membrane was immersed into 5.0% $CaCl_2$ solution for 10 s, thereby causing the gelatinization of sodium alginate solution to form a good fixed agent (Zactiti and Kieckbusch., 2009; Reiss et al., 1998; Bierhalz et al., 2014). In order to remove the remaining Cl^- and Ca^{2+}, the membrane was rinsed gently with 0.01 mol/L phosphate buffer (pH 7.0). Finally, the membrane was fixed to the surface of a glassy carbon electrode, and the taste-bud tissue should be coincided with the characterized electrode core. As shown in Fig. 1, the principle of the sensor was as follows: the binding of the pungency substance to the receptor would induce taste cell depolarization and Ca^{2+} release to generate action potentials, and the electrical signal was conducted to the central nervous system via specific nerves to produce the taste. This developed biosensor simulated this progress and conducted the electrical signal to the computer via the electrodes,

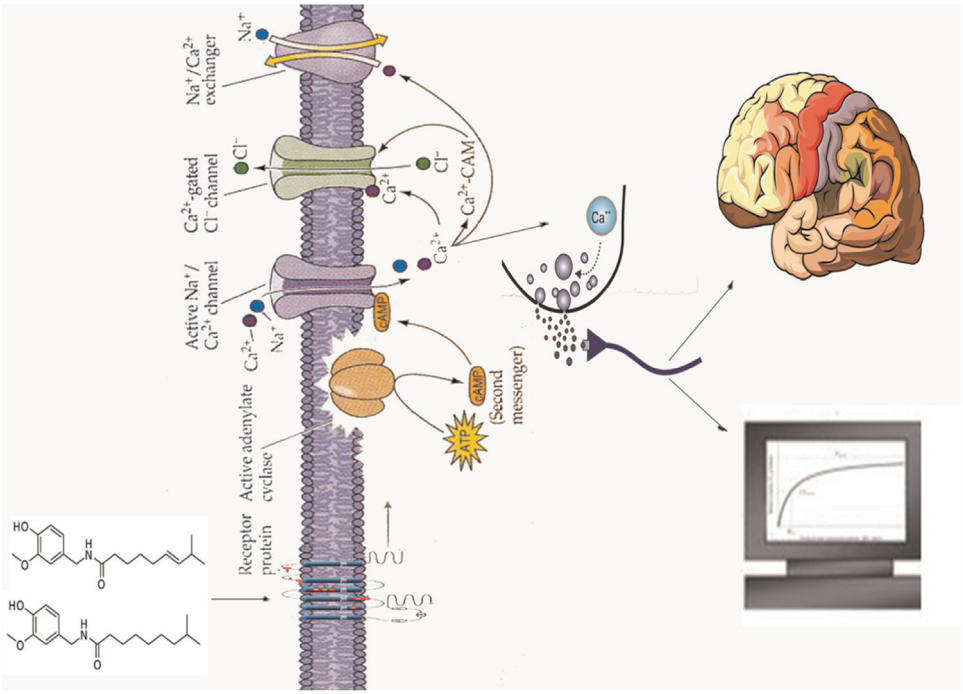

Fig. 1. Principle and scheme of the immobilized taste-bud tissue biosensor.

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