Sensors and Actuators B 223 (2016) 461–469

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Detection of bitterness *in vitro* by a novel male mouse germ cell-based biosensor

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 24 August 2015
Accepted 24 August 2015
Available online 30 September 2015

Keywords:
Cell-based bitter biosensor
Male mouse germ cells
Bitter taste receptor
Cell-based impedance sensor (CIS)

ABSTRACT

Bitter detection has attracted extensive attention in industries and researches due to pharmacological and/or food safety issues. Common electronic tongues constructed with electrochemical, optical or mass sensors are often subjected to the drawback of low-specificity in detecting a wide range of bitter compounds with diverse chemical structures and functions. However, biological gustatory components present unique specificity to bitter compounds, which may provide a promising approach in bitter detection. The activation of G protein coupled receptors (GPCRs) by ligands will affect cell morphology in a specific manner, which can be monitored by cell-impedance sensor. Thus cells expressing bitter receptors (a subfamily of GPCRs) can be used as sensing material in specific bitter detection. In this study, we explored the feasibility of utilizing male mouse germ cells in testis and cell-based impedance sensors (CIS) to build a cell-based bitter biosensor. Male mouse germ cells express bitter receptor T2Rs, which can sensitively and specifically respond to bitter compounds. To verify bitter sensing capacity of the germ cells, calcium responses of germ cells to three bitter compounds were examined prior to cell impedance response measurement. Furthermore, a bitter receptor blocker, probenecid, was used to verify the specificity of T2R-mediated impedance responses in germ cells. Cell-impedance response profiles of germ cells to four bitter compounds were investigated by analyzing the response intensity under various concentrations. Finally, impedance responses to five basic tastes were examined to evaluate the performance of cell-based bitter biosensor in bitter detection. The results revealed that this hybrid bitter biosensor could respond to those bitter compounds in a dose-dependent manner. The detection threshold for quinine was 0.125 mM, lower than our previous cell-based bitter biosensor. The calcium imaging tests and use of probenecid confirmed that T2R activation contributed to the cell impedance responses of this cell-based biosensor. Moreover, this germ cell-based cell could specifically detect bitter compounds among five taste stimuli, which may provide a promising and valuable approach for detection of various bitter compounds.

1. Introduction

Bitter perception is one of five basic tastes in mammals. In the natural environment most of the poisons taste bitter, which usually arouse an unpleasant emotion in animals. Thus animal could distinguish and avoid those bitter poisons when preying, which play a crucial role in their survival and evolution. Bitter substance detection and identification have wide application in the fields such as pharmaceutical industry, food industry and biosafety [1–4]. In these applications of bitter detection, various types of electronic tongue are the core components, which usually employ electrochemical, optical and mass sensors. Though these sensors have some merits in chemical detection, they still have some shortages in detecting the bitter taste compounds, which are a wide range of chemicals with various chemical structures and distinct

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http://dx.doi.org/10.1016/j.snb.2015.08.105
0925-4005/

pharmacological functions. For instance, most electrochemical detection approaches rely much on redox-active interactions, which are insufficient in specificity and selectivity in detecting bitter compounds with diverse chemical structures [5–7]. Optical sensor [8], mass sensors such as quartz crystal microbalance (QCM) [9,10] and surface acoustic wave (SAW) [11] also suffer the disadvantage of low specificity due to their non-specific sensing property. However, biological gustatory system provides a novel and promising approach to this issue with a group of bitter receptors sensitively and specifically responding to those bitter compounds. Therefore, bionic sensors constructed with biological gustatory components have attracted much attention in detecting bitter compounds [12–15].

In mammalian, bitter perception is initiated by the interaction between bitter compounds and taste receptor cells in the taste buds of the tongue. In particular, bitter taste is mediated by a subfamily of G-protein-coupled receptors (GPCRs), T2Rs (taste receptor, type II), which are ~40 highly specialized membrane receptors for bitter signal transduction [16,17]. T2Rs' activation by specific ligands could induce a series of intracellular and extracellular responses including molecular and electrophysiological changes [18,19]. With their unique physiological activity and specificity, cells expressing T2Rs can be useful and sensitive elements to construct cell-based biosensors.

In the previous studies of taste cell-based biosensor, one of the issues that impede the further application of these biosensors is how to acquire enough taste receptor cell quantity. In the taste bud, bitter taste receptor cells take up only a small part (~10–15%) of taste bud cells in the taste epithelium. Recent studies have revealed, T2Rs are not only expressed in taste-related tissue but also were found in other non-taste tissues such as nasal cavity, airway epithelium, brain and mouse testis [20–23]. Germs cells in the testis are in large quantity and easy to obtain. Therefore male germ cells became our choice of cell source for bitter sensing.

In order to detect the T2Rs activation in germ cells, cell-impedance sensor (CIS) was used as secondary sensor to establish a bitter taste biosensor. Cell-impedance sensing technology is widely used in drug screening and cellular toxicity [24–26]. Recently, studies have reported that the addition of the agonist for different GPCRs will affect cell morphology in a very specific manner depending on the pathways being activated [27,28]. The morphological changes can be monitored by cell-impedance sensor, which reflects the specific GPCR signaling that are activated by the ligand [29–31]. Thus the CIS could be used to monitor germ cellular responses induced by bitter receptor activation. In the bitter receptor signaling, it is known that T2R activation triggers a series of intra- or extracellular molecular events including the generation of inositol triphosphate (IP_3), the Ca^{2+} release from endoplasmic reticulum Ca^{2+} stores, the ion channel opening and depolarization of membrane potentials. These cellular responses may change the cell morphology as well as the local ionic environment, thus altering the impedance. Compared with our previous cell-based biosensor in electrophysiological study [13,32,33], the readout of CIS is integral response from certain number of cell population, which are more repeatable and representative than extracellular potentials from individual cells and/or uncertain number of cell population. Besides, CIS is label-free and non-invasive to cells, which better reveals the genuine cellular responses and allows for long term monitoring and recording.

In this study, male mouse germ cells and CIS were integrated to build a cell-based bitter biosensor. To testify the effectiveness of cell-impedance measurement in monitoring cellular responses induced by GPCR activation in our study, HEK293 cells' impedance responses to isoproterenol (ISO) were determined. HEK293 cells endogenously express G protein coupled receptors (β2 adrenergic receptor), which can be specifically activated by ISO. Calcium responses of germ cells to three bitter compounds were examined prior to cell impedance response measurement to verify bitterness sensing capacity of the germ cells. A bitter receptor blocker, probenecid, was employed to verify the specificity of T2R-mediated responses in germ cells. Finally, impedance responses to five basic tastes were examined to evaluate the sensitivity and specificity of cell-based bitter biosensor. All the details are discussed in the following sections.

2. Materials and methods

2.1. Reagents and animals

Four bitter compounds with diverse chemical structures (Fig. 1a): denatonium, N-phenylthiourea (PTC), 6-propyl-2 thiouracil (PROP), quinine were selected as bitter stimuli in the present study. Denatonium is a standard bitter substance in the study of bitterness perception. It is recognized by eight distinct bitter receptors in humans, including hT2R4 and hT2R43 [34]. Mouse mT2R8 has also been reported to respond to denatonium as well. PTC is a bitter compound that it either tastes very bitter or virtually tasteless to an individual, depending on the genetic makeup of the taster. Therefore it is commonly used to define taste genetic variation of humans. PTC is reported to functionally activate human hT2R38. However, its receptor in mice has not yet been revealed. PROP, a compound chemically related to PTC, is also a probe for genetic variation in bitter taste, although PROP is safer with less sulfurous odor. Quinine is an alkaloid isolated from the bark of the cinchona tree [35]. It is also used as a standard substance in the study of bitter perception [16,36]. Agonists of quinine has been reported to increase intracellular calcium levels or electrophysiological excitability [37]. Interestingly quinine is auto-fluorescent. Thus the calcium fluctuation could not be detected by fluorescent imaging techniques.

Bitter compounds and bitter blocker probenecid were purchased from Sigma–Aldrich (St. Louis, MO). Other tasting compounds (glucose, NaCl, monosodium glutamate (MSG)) and HCl solution were purchased from Sinopharm Chemical Reagents Co. Ltd (Shanghai, China). Poly-L-lysine and laminin were purchased from Sigma–Aldrich (St. Louis, MO). Experiments with animals were performed according to the protocols approved by the Institutional Animal Care and Use Committee of the Monell Chemical Senses Center.

2.2. Mouse germ cell isolation

For each experiment, one wild-type adult male mouse (around 8 weeks old) was anesthetized by 25% (w/w) urethane. Two testes were immediately removed and transferred into HS medium containing (mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 30 HEPES, 10 glucose, 10 lactic acid and 1 pyruvid acid (pH adjusted to 7.4 with NaOH). After washing twice in the HS medium, the testes were minced into small pieces and transferred into 1 ml HS medium containing 5 mg/ml bovine serum albumin (BSA). The germ cells were dissociated and collected from upper portion of the solution by pipette. And the tissue clumps were removed by low speed centrifuge. Thus the germ cell suspension was obtained.

2.3. Mouse germ cell immobilization

For cell impedance sensing assay, attachment between cells and electrodes is critical to determining the cell status. Germ cells are not so easy to get attached as cell lines are. In order to enhance and accelerate the germ cell-electrode attachment, the plates were precoated with poly-lysine (1 mg/ml) followed by laminin (5 μg/ml). The germ cell suspension obtained in Section 2.2 was seeded into

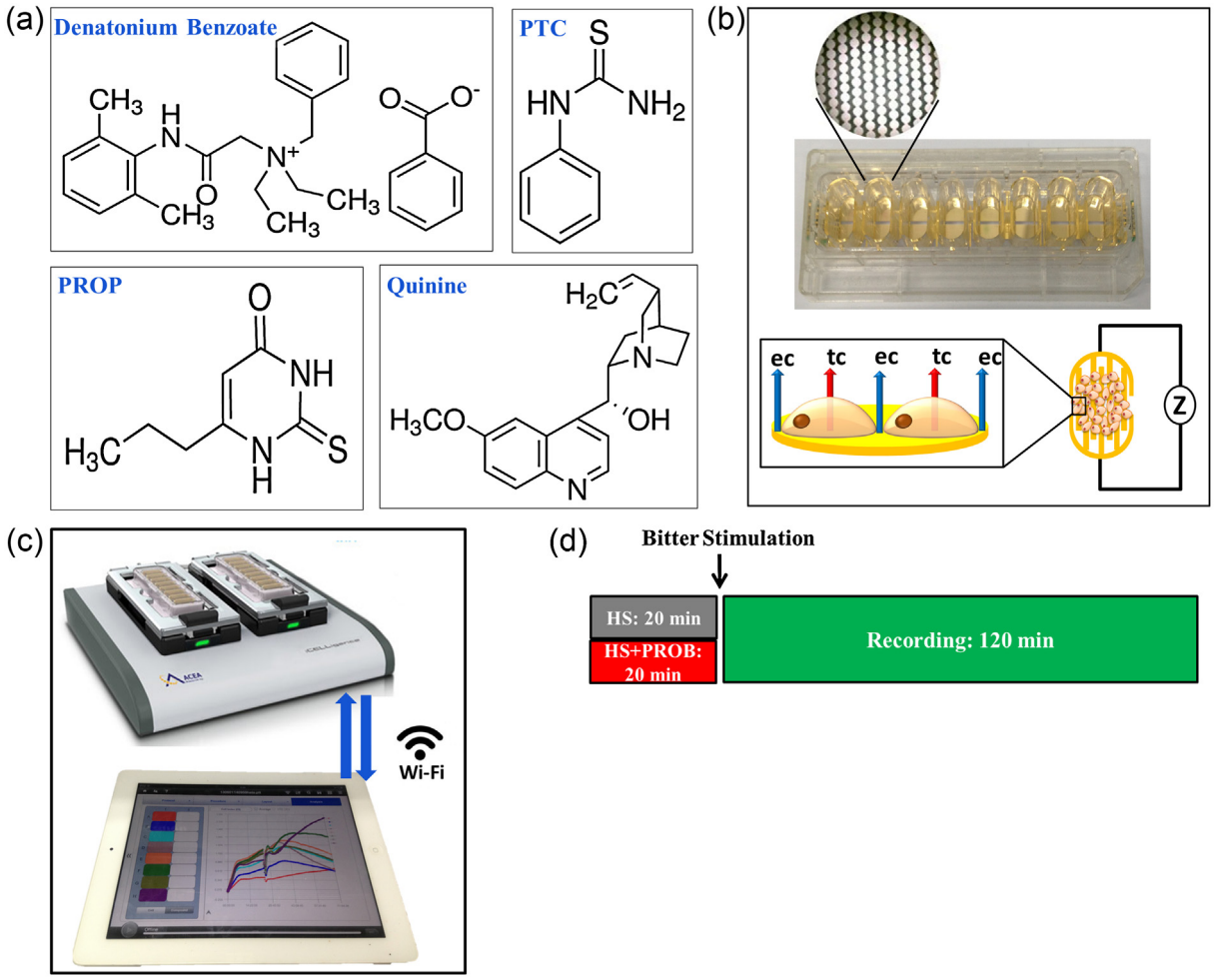

Fig. 1. A. The chemical structures of the four bitter compounds: denatonium benzoate, PTC, PROP, quinine. B. The 8-well CIS chip and enlarged electrode with "circle on line" layout (upper panel). Schematic diagram of impedance measurement (lower panel). ec, extracellular current; tc, transcellular current. Frequency is fixed at 10 kHz to capture the cellular status characteristics from both intracellular and extracellular currents. C. The CIS recording system. The iPad controls the recording and setting of the CIS recording device through Wi-Fi signal. D. The experimental diagram of each trial for probenecid-untreated group and probenecid-treated group. The gray rectangle indicates the probenecid-untreated group in which germ cells were incubated in HS solution before bitter stimulation. The red rectangle indicates the probenecid-treated group in which germ cell were incubated in HS containing 0.5 mM probenecid before bitter stimulation. After the addition of bitter solution as stimulus, the impedance changes were measured in both groups.

two E-plates (ACEA Biosciences Inc., China). Subsequently, the plates were centrifuged at the speed of 500 rpm for 5 min and loaded on the iCelligence system (ACEA Biosciences Inc., China) in the incubator at 37 °C before impedance measurement. The germ cells were freshly isolated and were used only once in each trial in order to ensure the consistency and repeatability of the response.

To verify the immobilization performance, HEK293 cells were also immobilized in the same way as germ cells did. HEK293 was transfected with G protein chimera Gα16gust44 in order to enhance the cellular responses including morphological changes, which ultimately enhance the cell-impedance responses. Parental HEK293 cells were used as control. Using the similar immobilization method, HEK293/Gα16 cells and control cells were quickly immobilized onto the electrode surface. The cell density was 1.8×10^5 cells/well. The impedance responses to ISO were recorded and monitored.

2.4. Calcium imaging with germ cells

The calcium imaging procedure has been described in detail in our previous paper [38]. In brief, after cell dissociation, germ cells were loaded with 5 μM Fura-2-acetoxymethyl ester (Fura-2/AM) and 80 μg/ml pluronic F127 (Molecular Probes, Eugene, Oregon) and transferred onto coverslip for at least 1 h at room temperature. The coverslip with spermatogenic cells were mounted in a recording chamber and superfused with HS medium or HS containing taste compounds via a valve controller (VC-8, Warner Instruments, Hamden, CT). Calcium responses were recorded as previously described [39]. Cells were excited at 340 and 380 nm, and signals at 510 nm were captured by a cooled CCD camera. Bitter stimulation duration was 30 s, and perfusion rate was 0.8 ml/min. The changes in fluorescence ratio were recorded for regions of interest drawn on the cells.

2.5. Cell-based impedance sensor and recording system

The cell-based impedance sensor (CIS) consisted of 8 wells in each plate. The electrodes in each well were made of thin gold film using lithographical methods and coated with poly-lysine and laminin prior to experiments. The layout of electrode was designed as "circles on line" in order maximize the coverage area of each well (Fig. 1b, upper panel), which allows for maximal sensitivity for the detection of the cells with relatively uniform distribution of the electric filed. The electrodes covered ~75% of the total area of

the well. Germ cells were 100–150% confluent in each well. The cell electrode impedance was mainly determined by cell quantity, adhesion and some dynamic changes such as morphology and migration. To quantify the cell-electrode impedance, a parameter termed cell index (CI) was derived as

$$CI = \max_{i=1,\ldots,N} \left(\frac{R_{cell}(f_i)}{R_b(f_i)} - 1 \right)$$

where $R_b(f)$ and $R_{cell}(f)$ are the frequency-dependent electrode resistances (a component of impedance) without or with cells in the presence, respectively. High-frequency current (>40 kHz) will penetrate the cell membrane, which is mainly affected by cell coverage. Low-frequency currents (<2 kHz) flow to the cell and the gap between electrodes, which is mainly determined by the changes in the space between or under cells (Fig. 1b, lower panel). The working frequency of the CIS in our study was 10 kHz in order to acquire the cellular characteristics from both intracellular and extracellular currents. Thus, CI was a quantitative measure of the cell status in real-time. Under same physiological conditions, more cells attached to electrodes led to a larger $R_{cell}(f)$ value and thus a larger CI value. For the same cell quantity in the well, changes in cell status that alter the cell-electrode contact will be also reflected by changes in impedance. The iCelligence system for this CIS was shown in Fig. 1c. The iPad (lower panel) (Apple Inc., USA) was used to control the recording and setting of recording device (upper panel) through Wi-Fi signal.

2.6. Tastant stimulation and bitter receptor blocker inhibition

Before sacrificing mice for each experiment, stock solutions of tastants at 10 fold concentrations (bitter, sweet, umami, sour and saline) were freshly made with distilled H_2O from stock solutions. The stimulating solutions were then incubated in 37 °C for over half an hour before being applied to the cells. Germ cells in 180 μl HS medium (with BSA) in each well were placed in E-plates. After centrifuge, the plates were loaded in the recording system to start recording. After 20 min incubation to reach stability, taste stimulating solutions of 20 μl were added to each well. As variations in cell morphology are consequences of changes in underlying cytoskeletal organization and dynamics, which usually take a few minutes to hours for the whole process [28,29], the impedance responses to taste stimulation were recorded for 120 min. For probenecid treated group, germ cells in 180 μl HS containing 0.5 mM probenecid were placed in the wells and 20 μl bitter solutions were added. The diagrams for normal bitter stimulated group without probenecid treatment and probenecid-treated group were shown in Fig. 1d.

For HEK293/Gα16 cells stimulation, various doses of ISO ligand solutions (20 μl) were added immediately after plates were loaded. The impedance responses were recorded for 120 min. All the impedance measurement was conducted in the incubator at 37 °C.

2.7. Data analysis

To quantify the CI response under various concentrations, ΔCI curves were obtained by normalizing the CI values of at certain point as 1, and regulate other CI values based on the normalized point. Impedance response intensity was defined by the CI values at certain time point when CI reached peak or at 70 min after bitter stimulation when peak was not observed. The response intensity was calculated by subtracting CI value of the control group (buffer added) from the CI value of bitter treated groups. The response characteristics of this biosensor were determined by the dose-dependent response curves. To illustrate the specificity of the biosensor, comparisons were made between bitter blocker-treated and -untreated groups. The dose-dependent response curves from probenecid-treated and -untreated groups were normalized as following equation:

$$\text{Normalized Response Intensity}_i = \frac{\text{Response Intensity}_i}{\text{Response Intensity}_{highest}}.$$

where *highest* indicate at highest stimulus concentration. Thus, the normalized response intensity was 1 at highest concentration stimulation.

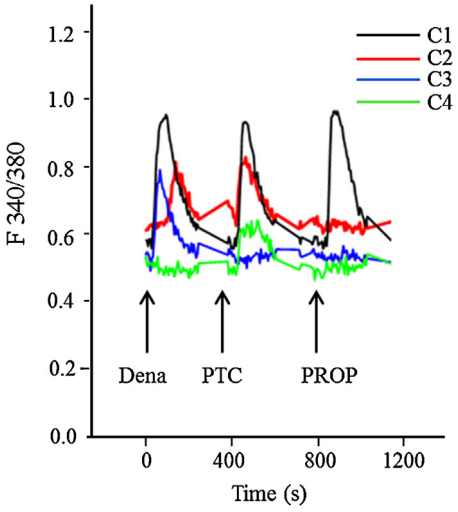

Fig. 2. Activation of germ cells by bitter compounds increases intracellular calcium concentrations. Typical calcium response traces of four individual germ cells to denatonium, PTC, PROP. Germ cells were loaded with calcium-sensitive dye Fura-2/AM.

3. Results

3.1. Calcium responses of germ cells to bitter compounds

In order to verify that bitter compounds could activate bitter taste receptor-mediated signaling pathways in the germ cells, calcium responses were measured by fluorescence imaging. Bitter compounds denatonium, PTC, PROP increased intracellular calcium release in germ cells. And the response profiles of individual germ cells were different: some responded to more tastants at variable intensities than others did (Fig. 2). Statistically, from 25 germ cells, 16 (64%) cells responded to denatonium; 9 (36%) cells to PTC; 8 (32%) cells to PROP. This observation is similar to that of taste bud cells from the oral cavity [17]. The difference in response profiles is believed to be due to the differences in expressed receptor gene subsets and/or their expressional levels in these cells. The calcium imaging results confirmed that germ cells were able to respond to these bitter compounds by activating T2Rs, thus providing biological bases for a cell-impedance biosensor.

3.2. Cell impedance response of HEK293 cells to ISO

To verify the cell-impedance measurement in monitoring cellular responses by GPCR activation, impedance responses of HEK293 to ISO were monitored and characterized. HEK293 cells were quickly immobilized onto cell-impedance sensor surface in the same way as germ cells in order to evaluate the immobilization performance. The immobilization took apart within less than 10 min, which is much faster than HEK293 cells would need for attaching in untreated surfaces, and thus clearly showed the high efficiency of our coating. Our results showed ISO stimulation led to a concentration-dependent impedance response of HEK293/Gα16

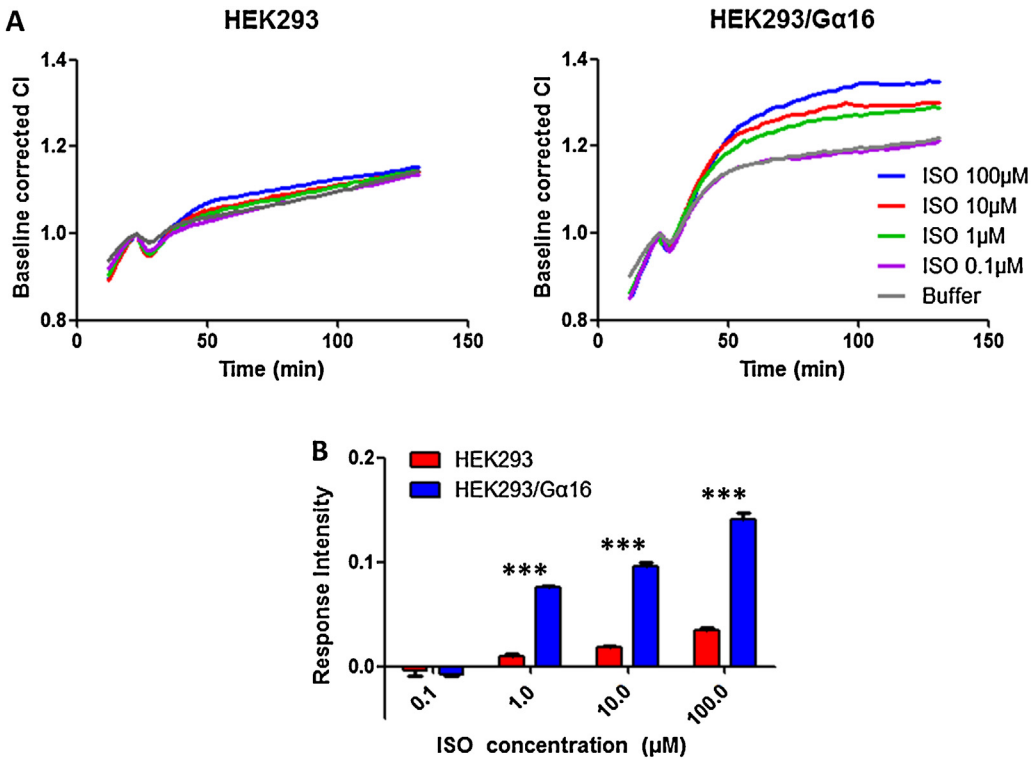

Fig. 3. Cell-impedance sensor can be used in cellular responses induced by GPCR activation in HEK293 cells. A. Cell index curves of control HEK293 cells (left panel) versus HEK293/Gα16 (right panel) in responses to ISO. B. Quantitative analyses of response intensity of HEK293 and HEK293/Gα16 cells at each ISO concentration. ***$p < 0.001$ (two tailed t-test). The bars represent standard deviations.

cells (Fig. 3a, right panel), which are consistent with the previously reported reports [31]. Immediately following ISO addition, the impedance response briefly decreased below 1 before quickly reverting to a positive slope. Subsequently, the impedance rose sharply at first, followed by a slow ascending phase. The CI values at 70 min post-stimulation were acquired as the impedance response intensity at each concentration, which should reflected morphological changes of HEK293 cells induced by GPCR activation. As comparison, the impedance response of the untransfected control HEK293 cells was too weak to observe (Fig. 3a, left panel). The response intensity of transfected cells were significantly greater than those of untransfected HEK293 cells when ISO was over 1 μM ($p < 0.001$) (Fig. 3b). The result confirmed both the successful introduction of Gα16 into HEK293 cells and the significant responses recorded by the impedance system, implying the immobilization method was effective in forcing cell attachment, which was presumed to be feasible for germ cell attachment as well. More importantly, the results demonstrated effectiveness of cell-impedance measurement in monitoring cellular responses in GPCR signaling.

3.3. Impedance response of mouse male germ cells to bitter compounds

Having proved the feasibility of impedance measurements for monitoring cellular responses brought by GPCR activation, we investigated the impedance responses of mouse male germ cells to four bitter compounds: denatonium, PTC, PROP, quinine. The results of cell-impedance detection in Fig. 4a (left panel) showed that denatonium firstly induced an obvious decrease immediately after its addition, which has been proved to be attributable to the compound's own property without interaction with cells. After sharp decrease, impedance rapidly increased and surpassed the baseline. In the experiment, the impedance responses between 156, 313, 625, 1250 mM were often not so distinguishable. However, the impedance intensity values were acquired from at least three independent experiments. The impedance response intensity still presented a dose-dependent relation (Fig. 4a, right panel).

For PTC, the impedance slightly decreased in first several minutes after bitter compound addition, but it increased afterwards. When the concentrations were lower (<1 mM), the responses were almost vanished and indistinguishable, which was consistent with calcium results [38]. The CI values at 70 min post-stimulation at each concentration were selected and the response intensities were calculated. In Fig. 4b (left panel) the result of cell-impedance sensing showed that PTC stimulation also induced a dose-dependent increase in impedance.

PROP is a bitter compound chemically related to PTC. In the cell-impedance detection, response profile to PROP is also similar to those of PTC (Fig. 4c, left panel). There was an initial fast increase period in 40 min after stimulation, followed by a slow increase. When the concentrations were lower (<0.4 mM), the responses were very weak and were indistinguishable between 0.08, 0.016 and 0.003 mM. Dose-dependent relations were also observed in response intensity (Fig. 4c, right panel). It is observed that the overall detection threshold to PROP was 2 order lower than PTC, which is also consistent with the psychophysical data indicating that humans perceived greater bitter intensity of PROP than that of PTC [40].

For the detection of quinine, the cell-impedance results also revealed that germs cell responded to quinine in a dose-dependent manner (Fig. 4d). Because quinine itself is highly fluorescent, it seems difficult to use calcium imaging to characterize the cellular responses to this compound. Since the cell-impedance method measures the electrical properties instead of fluorescence intensity, auto-fluorescence observed in many organic bitter-tasting compounds does not interfere with this measurement, which is an advantage of this method over the calcium imaging. The detection

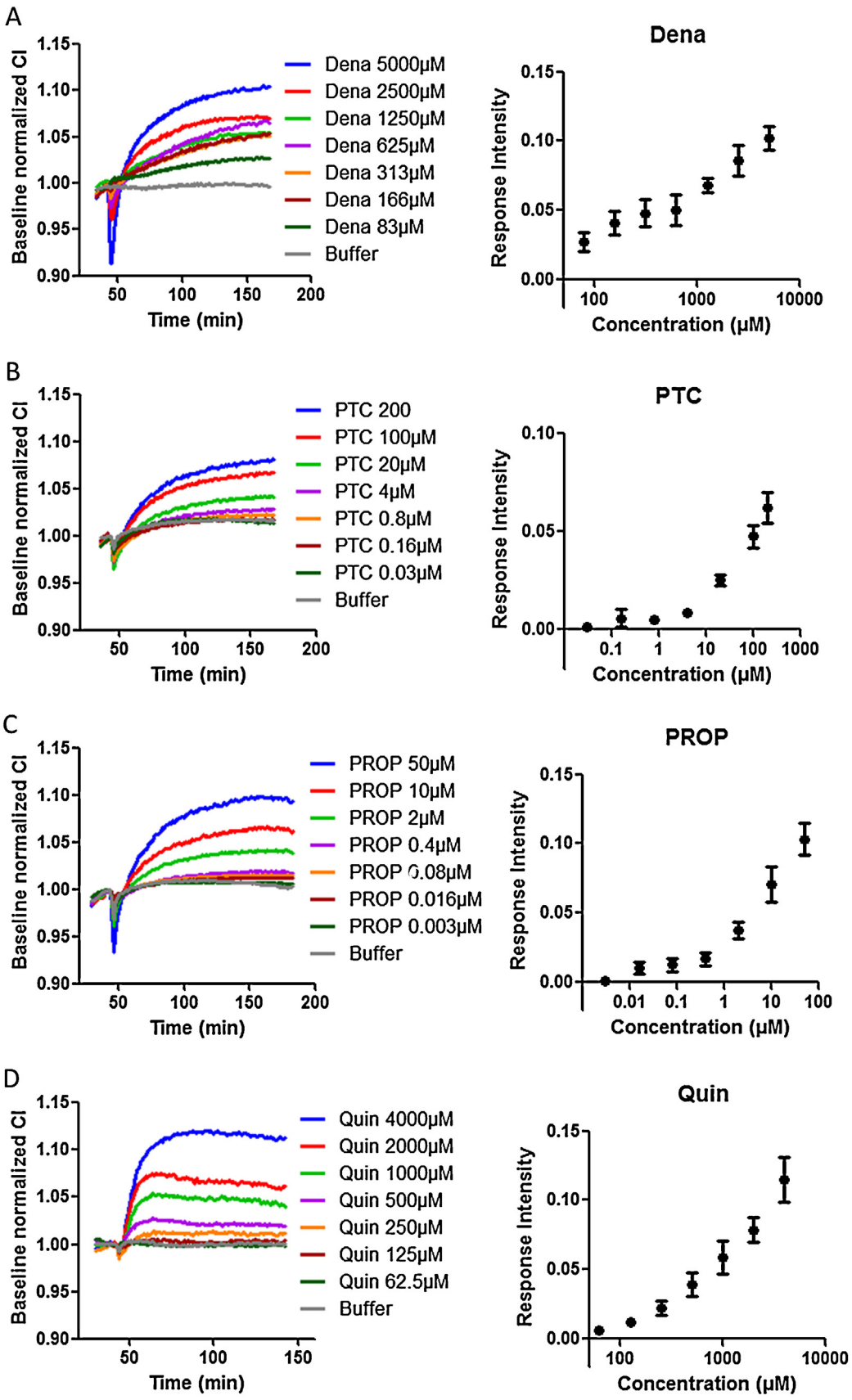

Fig. 4. Activation of germ cells by bitter compounds leads to a dose-dependent increase in cell-impedance response. Typical cell-impedance CI curves of germ cells responding to denatonium (Dena) (A), PTC (B), PROP (C), and quinine (Quin) (D) at various concentrations. Baselines were normalized to 1. Response intensity of germ cells to bitter stimuli displayed dose-dependent relations. Data were acquired from at least 3 independent experiments. The bars represent standard deviations.

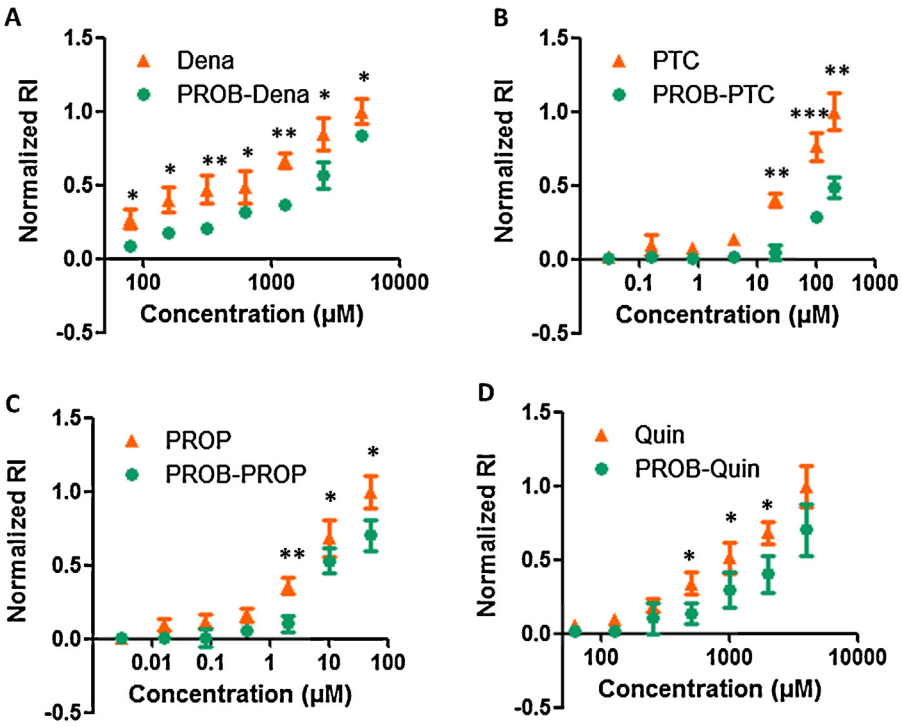

Fig. 5. Germ cell impedance responses to denatonium (A), PTC (B), PROP (C), and quinine(D), could be inhibited by a specific bitter receptor blocker, probenecid (PROB). Normalized RI (*Y*-axis) represents the normalized response intensity. Orange triangles represent the impedance response amplitudes four bitter compounds without probenecid treatment. Green circles represent the probenecid-treated impedance responses to the bitter stimulation. All of the response amplitudes were normalized. Data were acquired from at least 3 independent experiments. For the same bitter stimulation, the response amplitudes of probenecid-treated and -untreated trials were compared by two tailed *t*-test. $^{*}p < 0.1$; $^{**}p < 0.01$; $^{***}p < 0.001$. The bars represent standard deviations.

threshold for quinine was 0.125 mM, which was consistent with sensory sensitivity of human beings in Polish standard [41]. It is also superior in sensitivity to our previous cell-based bitter biosensors [32]. However, compared with other sensors in quinine detection such as piezoelectric quartz crystal sensor [42] and optical sensor [43], our biosensor is less sensitive due to the biological interactions between quinine and bitter receptors, which requires certain concentration to activate receptors. Quinine could activate T2R4, 10, 14, 40, etc., whose effective concentrations were all around 0.01 mM in cell-based assay [34]. Thus the detection threshold of cell-based biosensors is not likely lower. Besides, all the data were collected from at least 3 independent experiments, demonstrating the repeatability of this cell-based biosensor. The cell-impedance results along with the calcium imaging results demonstrated the feasibility of the cell-impedance measurement in monitoring cellular responses of germ cells to various bitter compounds.

3.4. Inhibition of mouse male germ cells' responses to bitter tastants by a bitter blocker

To further validate that the impedance responses to bitter tastants were mediated by T2Rs and to determine whether the impedance method could also be applied to the characterization of taste inhibitors, we examined the effect of a bitter blocker, *p*-(dipropylsulfamoyl) benzoic acid (probenecid), which is known to directly interact with taste receptors rather than downstream signaling molecules [44]. Probenecid has been reported to inhibit hT2R16, hT2R38 and hT2R43 human bitter taste receptors [44]. To characterize the inhibitory efficacy of probenecid, dose–response curves to four bitter substances were generated in the absence and presence of 0.5 mM probenecid (Fig. 5). In the presence of probenecid, denatonium-induced response intensity was significantly lower than in the absence of probenecid (Fig. 5a). Probenecid also remarkably weakened the PTC-induced impedance response intensity. When PTC concentration was 0.02 mM, probenecid almost abolished PTC-induced response (Fig. 5b). For PROP response, the inhibitory effect was significant when PROP concentrations were high. When quinine was lower than 2 mM, the inhibitory effect was significant. Table 1 showed the response characteristics of this cell-based biosensor with and without probenecid treatment. The detection limit (DL) was reduced and detection ranges (DR) were narrowed for all the four bitter compounds. The maximal response intensity (response intensity at highest stimulus concentration) was also lowered in the presence of probenecid. The inhibitory effects of probenecid in these bitter compound-induced responses confirmed that T2R activations were involved in the cell-impedance responses. Interestingly probenecid did not completely inhibit the responses. Possible reason is that it is known that one

Table 1
The response characteristics of the germ-cell-based biosensor for four bitter stimuli with and without the treatment of bitter receptor blocker, probenecid. DL, detecting limit; DR, detecting range; MRA, maximal response amplitude; the first three columns DL, DR and MRA indicate the original bitter stimulated groups without probenecid treatment. The last three columns P-DL, P-DR and P-MPA indicate the probenecid treated groups.

Bitters	DL(nM)	DR (uM)	MRA	P-DL(jiM)	P-DR(uM)	P-MRA
Denotonium	78	83–5000	0.102 + 0.009	156	156–5000	0.085 + 0.003
PTC	4	4–200	0.093 + 0.012	20	20–200	0.036 + 0.012
PROP	0.4	0.4–50	0.104 + 0.018	2	2–50	0.903 + 0.168
Quinine	62.5	62.5–4000	0.125 + 0.024	250	250–4000	0.088 + 0.022

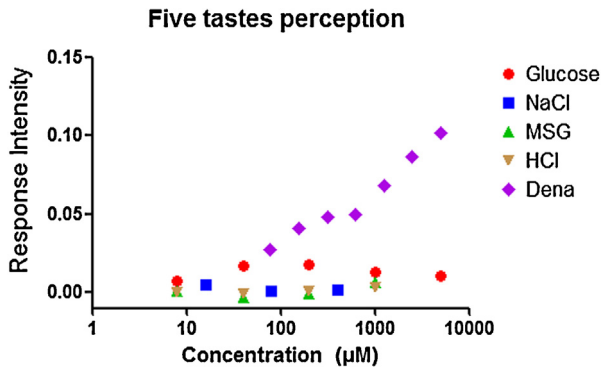

Fig. 6. This germ-cell-based biosensor is specifically responsive in bitter detection. Sweet (glucose), umani (MSG), salt (NaCl) and sour (HCl) taste stimuli did not elicit recognizable impedance responses. Only bitter (denatonium) stimulus evoked obvious dose-dependent responses. All the data points were mean values from more than 3 independent experiments.

bitter compound can stimulate many different T2Rs and vice versa [16,34]. Some of which may not be affected by probenecid. The underlying molecular mechanism remains to be studied.

3.5. Germ cells respond to bitter tastants with high sensitivity and specificity

To determine whether this germ cell-based biosensor is specific to bitter sensing among five basic tastes, representative stimuli of five basic tastes were tested. For a CIS, highly concentrated stimuli (e.g., >10 mM), especially some strong electrolytes, influence the impedance results by greatly changing the conductance of medium. We observed an initial sharp decline of CI immediately after the addition of high concentrations of taste stimuli, including sweet (glucose), umami (MSG), (NaCl) and sour (HCl), which might have covered the actual cellular responses. When concentrations of these compounds were relatively low (<10 mM), NaCl, HCl, MSG, glucose stimuli did not stimulate any detectable responses whereas denatonium was still able to evoke impedance responses in a dose-dependent manner (Fig. 6). Besides, this germ-cell-based biosensor could also respond to other three bitter compounds (Fig. 4). Thus the results indicate that this germ-cell-based biosensor can specifically detect bitter compounds but do not respond to other taste substances. These results proved that this germ cell-based cell could selectively detect bitter compounds among five taste stimuli, which provided a promising and valuable approach for bitter detection.

4. Conclusion

In the present study, mammalian male germ cells were first used as sensing elements to establish a cell-based biosensor for bitter-tasting compound detection. Calcium imaging results confirmed the T2R activation in those male germ cells. By immobilizing germ cells on CIS, the dose-dependent cellular responses evoked by T2R activation were detected. These cellular responses could be specifically suppressed by probenecid, indicating the specific contribution of T2R activation to the generation of these cell-impedance responses. Furthermore, this germ-cell-based biosensor was able to detect bitter stimuli but did not respond to other taste stimuli (sweet, umami, sour and salt tastes), further proving its specificity and sensitivity in bitter detection. In addition, cell-impedance signal is an integrated effect induced by large numbers of cells, which renders this germ cell-based biosensor better stability. At present, the impedance response intensity of germ cells is not as strong as that of cell lines. In future work, better adhesion and immobilization would be made to increase the attachment of cells and electrode. The overall results suggest that this novel germ cell-based biosensor is promising and valuable in detecting various bitter compounds.

Acknowledgement

This work was supported by Major International Cooperation Project of Natural Science Foundation of China (No. 61320106002), Marine Public Welfare Project of China (No. 201305010), and Natural Science Foundation of China (No. 31228008), High-level Personnel Training Project of Cooperation Improvement of America and Oceania Region (No. 20142029).

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