A serotonin-sensitive sensor for investigation of taste cell-to-cell communication

Peihua Chen, Wei Zhang, Peng Chen, Ziyu Zhou, Cheng Chen, Junsong Hu, Ping Wang

Taste receptor cells are the taste sensation elements for sour, salty, sweet, bitter and umami sensations. It was demonstrated that there are cell-to-cell communications between type II (sour) and type III (sweet, bitter and umami) taste cells. Serotonin (5-HT) is released from type III cells, which is the only type of taste cells that has synaptic process with sensory afferent fibers. Then, taste information is transmitted via fibers to the brain. During this process, 5-HT plays important roles in taste information transmission. In order to explore a sensor to detect 5-HT released from taste cell or taste cell networks, we develop a 5-HT sensitive sensor based on LAPS chip. This sensor performs with a detection limit of $3.3 \times 10^{-13} \text{M}$ and a sensitivity of 19.1 mV per concentration decade. Upon the stimuli of sour and mix (bitter, sweet and umami) tastants, 5-HT released from taste cells could be detected flexibly, benefit from the addressability of LAPS chip. The experimental results show that the local concentration of 5-HT is around several nM, which is consistent with those from other methods. In addition, immunofluorescent imaging technique is utilized to confirm the functional existence of both type II and III cells in a cluster of isolated taste cells. Different types of taste cells are labeled with corresponding specific antibody. This 5-HT sensitive LAPS chip provides a potential and promising way to detect 5-HT and to investigate the taste coding and information communication mechanisms.

© 2010 Published by Elsevier B.V.
as close as possible. Otherwise, 5-HT reaching biosensor cell would be insufficient.

Other kinds of sensors for neurotransmitter detection, such as ion-selective electrodes (Ueda et al., 2006) and microelectrode biosensors with corresponding enzyme (Kueng et al., 2004) were reported to have good performance. However, their structures are not suitable for a single taste cell or taste cell networks. LAPS (light addressable potentiometric sensor) chip has a planar surface, which is convenient for taste cells culture. Moreover, benefit from MEMS technology and addressability of LAPS, the recording spot can be chosen flexibly with laser scanning, which solves the problem of the spatial limitation.

In this work, we develop a 5-HT-sensitive sensor based on LAPS chip. Firstly, the performance of this sensor, such as sensitivity and detection limit is measured. Then, 5-HT released from taste tissue with taste stimuli is detected to confirm its feasibility. Afterwards, upon sour tastant, 5-HT released from taste cells can be detected extracellularly. When applied mix (bitter, sweet and umami) tastants, 5-HT could still be captured and read out from a cluster of taste cells. Finally, in order to confirm that there are both functional type II and type III taste cells, immunofluorescent imaging technique is utilized.

2. Materials and methods

2.1. Reagents and LAPS chip

We utilized LAPS chip with electrolyte-insulation-semiconductor (EIS) structure to detect 5-HT. N-type silicon wafers ($\varphi = 1.5\text{ in}$) with specific resistance of 10–15 $\Omega \text{ cm}$ were employed. A layer of 30 nm SiO$_2$ was thermally grown at 1000 $\circ$ C. Then, Si$_3$N$_4$ with 60 nm thick was deposited using plasma enhanced chemical vapor deposition (PECVD). A 1 $\mu$m layer of aluminum was sputtered on the backside of the wafer to create an ohmic contact. Platinum wire served as the ground reference. A petridish with a 5 mm-diameter hole in the center was attached to the LAPS chip for cell and tissue culture.

A PVC-based 5-HT sensitive membrane was fabricated and deposited on the surface of LAPS chip. The compositions of this membrane were adopted from publication, including ion-exchanger: KTpClPB 0.5 mg, solvent mediator: tris (2-ethylhexyl) 2-ethylhexyl phosphate 60 mg, and PVC 30 mg (Katsu and Hirodo, 2000). The three materials were dissolved in tetrahydrofuran 1 ml. Then, 10 $\mu$l of this mixture was poured into the hole of chamber on LAPS chip. After the solvent evaporation at room temperature, the resulting PVC membrane deposited on the LAPS chip was about 200 $\mu$m in thickness. Finally, the membrane was conditioned overnight in a solution of 1 mM serotonin hydrochloride (Sigma), pH = 7.4.

2.2. LAPS system and measurement

The LAPS measurement system was shown in Fig. 2(a). A modulated light (wavelength 543.5 nm, power 5 mW, 4 kHz) was focused on the surface of LAPS with diameter 10 $\mu$m. The characteristic $I$–$V$ curve of LAPS chip was scanned with parameter settings: the initial voltage $–4500\text{ mV with step 20 mV, scanning delay 200 ms and data points 200.}$ Photocurrent was recorded by the electrodes of potentiostat (model 273A, EG&G Princeton Applied Research) and the lock-in amplifier (model SR830 DSP, Stanford Research Systems). A 16 bit data acquired card and the software of LABVIEW were employed to control the data collection, analysis and storage. A local surface potential changes produced by 5-HT would be reflected by the shift of $I$–$V$ curve ($\Delta V$), as shown in Fig. 2(b).

Firstly, tyrode solution was applied to the LAPS chamber and $I$–$V$ curve was measured. Then, we switched to the tastant stimulus solution, $I$–$V$ curve was scanned and recorded for around 5 min. All measurement was performed at room temperature about 22 $\circ$ C. Standard error (SE) and Student’s t-test were calculated for statistical analysis. Standard errors are based on different measurements from different cell culture upon the same solution.

2.3. Taste bud cells and taste tissue

Female Sprague–Dawley rats (around 200 g) were injected with 5-HTP (5-hydroxy-l-tryptophan, Sigma) (80 mg/kg) 1 h before sacrifice. For taste tissue experiment, taste epithelium blocks were isolated according to published procedures (Herness and Sun, 1995). Briefly, circumvallate papillae were excised and incubated in a cysteine-activated (1 mg/ml) papain/divalent-free bicarbonate-buffered solution (14 U/ml) for 3 h at 37 $\circ$C in 5% CO$_2$/95% air. The epithelium block was removed from muscle tissue, and then transferred to the extracellular solution in LAPS chamber deposited with 5-HT sensitive membrane.

For the taste cells experiment, we adopted the method from Kinna-mon (Kinna-mon et al., 1988). 1–1.5 ml tyrode’s solution containing collagenase (2 mg/ml) and elastase (0.25 mg/ml) was injected under the epithelium of the dissected tongue. After incubation for 30 min in divalent-free tyrode solution, lingual epithelium was peeled off from the underlying connective tissue. Circumvallate taste cells were removed from epithelium with a fine polished glass pipette by a gentle suction. Taste bud cells were cultured about 2 h to attach to the surface of the membrane on LAPS.

The composition of tyrode’s solution (mM) contains 126 NaCl, 5 KCl, 5 NaH2PO4, H2O, 10 glucose, 2 CaCl2, 2 MgCl2 (pH = 7.4, adapted with NaOH).
Fig. 2. The scheme for 5-HT detection. (a) LAPS measurement system. (b) I–V curve moves toward right after applying 5-HT. (c) Taste cells and taste epithelium (d) were cultured on 5-HT sensitive LAPS chip. Bar: 10 μm.

2.4. Immunofluorescent imaging

For 5-HT immunofluorescent imaging assay, SD rats were injected with 5-HTP 1 h before sacrifice. Taste cells from circumvallate papilla were excised and cultured on glass coated with cell-TAK (tissue adhesive from BD Biosciences) for 1–2 h. After adhesion, taste cells were fixed in fresh 4% PFA (paraformaldehyde) in 0.1 M PBS (phosphate buffered saline; pH = 7.3) for approximately half an hour. After fixing, taste cells were washed three times totally for 15 min in PBS at room temperature. Then, taste cells were blocked in 5% normal goat serum and 0.3% Triton X-100 in 0.1 M PBS for 1–2 h at room temperature.

For single labeling assay, taste cells were incubated in primary antibody (for 5-HT, rabbit polyclonal anti-serotonin, diluted to 1:100, Sigma; for α-gustducin, rabbit polyclonal anti-α-gustducin, diluted to 1:200, Santa Cruz Biotechnology) in 0.1 M PBS overnight at 4 °C. Washed three times in PBS, taste cells were exposed to secondary antibody Cy3 conjugated to goat anti-rabbit IgG (diluted to 1:200, Jackson Lab) in 0.1 M PBS (pH = 7.3) for 1–2 h at room temperature. After washing three times in PBS, taste cells were sealed with 90% glycerin in PBS and observed with confocal laser scanning microscope (LSM510, Carl Zeiss).

For double labeling assay, the procedure was the same as above. However, taste cells were incubated simultaneously with...
two primary antibodies in different species: rabbit polyclonal anti-serotonin (diluted to 1:100) and mouse monoclonal anti-IP_{3}R3 (inositol 1,4,5-trisphosphate receptor type 3, diluted to 1:50, BD). Subsequently, taste cells were incubated with secondary antibodies: Cy3 conjugated to goat anti-rabbit IgG (diluted to 1:200) and FITC conjugated to goat anti-mouse IgG (diluted to 1:100, Jackson Lab).

3. Results and discussion

3.1. Characteristics of 5-HT sensitive LAPS chip

Firstly, in order to confirm the stability of 5-HT sensitive LAPS chip and measurement system, we tested working potentials in normal tyrode solution with a long period of time (> 30 min). The shifts of the working potentials compared with the first one at each time point were shown in Fig. 3(a). The time interval was 40 s. The data indicated that it worked stably after several hundred of seconds. When the system stabled, the mean of the voltage shifts was 3.2 ± 0.3 mV (mean ± SE), indicated that the change of log[5-HT] was around 0.17 ± 0.02. In addition, it was reported that this 5-HT sensitive membrane is stable in sour stimuli with pH 2–9 and is greatly inhibitory to Na^{+}, K^{+} and quaternary ammonium ions (Katsu and Hirodo, 2000; Ueda et al., 2006).

Afterwards, we tested the characteristics of 5-HT sensitive LAPS chip with different concentrations of 5-HT (pH = 7.4), around 10^{-11}–10^{-7} M and normal tyrode solution without 5-HT. Firstly, normal tyrode solution was applied and I–V curve was measured, from which we got the base working point. Then, solutions with different concentrations of 5-HT were tested. The working point potential shifted towards right compared with the base working point, and increased as the concentration of 5-HT increased. The relation between concentrations of 5-HT and the shifts of working potential compared with the base working potential was shown in Fig. 3(b). The abscissa denotes the logarithm values of the concentrations of 5-HT. Linear function was used to quantify the sensitivity of this 5-HT sensitive LAPS chip, which was 19.1 mV per concentration decade (r = 0.997). The linear equation was \( V = 19.1 \times \log[5-HT] + 239.2 \), where \( V \) denoted voltage shift, and [5-HT] denoted the concentration of 5-HT. The detection limit was 3.3 \times 10^{-13} M, which was a theoretic value defined as the intersection point of the extrapolated linear region of the calibration curve with abscissa. According to the results from Huang's group, it can be estimated that the local concentration of 5-HT released from taste cells upon stimulus was around 10^{-9} M, which was in the linear range of 5-HT sensitive LAPS chip. In conclusion, the performance of this sensor could be employed for the detection of 5-HT released from taste cells and taste epithelium.
3.2. Detection of 5-HT released from taste bud cells and taste tissue

The molecules of molecular biology and Ca2+ imaging indicated that type III taste cells could release 5-HT upon sour stimulus, which encodes taste information. And with the stimuli of bitter, sweet or umami tantants, type II taste cells release ATP onto type III taste cell, inducing 5-HT release. Therefore, if we detect 5-HT when applying mix stimuli, including MgSO4 (bitter), sucrose (sweet) and monosodium glutamate (umami), it could indicate that there might be a taste information communication between type II and III cells. With this knowledge, we designed to detect 5-HT released from taste cells upon sour tantast and from taste cell clusters upon mix tantast. Firstly, in order to confirm its feasibility, we cultured taste epithelium blocks on the surface of 5-HT sensitive PVC membrane. The structure of this epithelium was loosened to make serotonin outflow, which could be captured by 5-HT sensitive LAPS chip more easily.

Upon sour stimulus with pH 2, the characteristic I–V curve shifted towards right compared with the one before stimulus. The mean shift of the working potential was 79.4 ± 15.2 mV, which indicated that the local concentration of 5-HT released upon sour stimulus was around 4.3 × 10^{-9} M (Fig. 3(c)). In taste epithelium, the connections between type II and III taste cells were still retained. When applying the mix stimuli for type II cells, we found that the I–V curve also shifted to the right, demonstrating that 5-HT released. Statistical results showed that the mean working potential shift was 79.3 ± 20.2 mV and the corresponding concentration of 5-HT was 4.3 × 10^{-9} M (Fig. 4(d)). Thus, the data indicated that the mean local concentration of 5-HT released from taste epithelium was around several nM, which was comparable with that from biosensor cells (Huang et al., 2005). Therefore, this 5-HT sensitive LAPS chip should be reliable.

Thus, we tested taste cells and taste cell clusters cultured on LAPS chip. The experimental procedure was the same as above. The mean I–V curve shifts were 81.8 ± 19.6 mV (sour) and 97.8 ± 14.6 mV (mix), respectively, demonstrating that the local concentration of 5-HT were 5.8 ± 10^{-9} M (sour) and 4.0 ± 10^{-8} M (mix) (Fig. 3(c) and (d)). They both were very significantly larger than control, which was tested without taste cells or tissues on the chip. The data suggest that taste cells should transmit information with neurotransmitter 5-HT. Meanwhile, there should be taste information communications between type II and type III cells.

3.3. Immunofluorescent imaging

In order to confirm that there were type II and type III cells and they both functionally existed in a cluster of isolated taste cells, immunofluorescent imaging technique was utilized. Inositol 1,4,5-triphosphate (IP3) was reported to be an essential second messenger in taste cells (Clapp et al., 2001). And α-gustducin (Goα) and 5-HT immunoreactivities were just present in type II and type III taste cells, respectively (Clapp et al., 2008; Kaya et al., 2004). Thus, we took α-gustducin and IP3 R3 as the specific immunocytochemical markers for type II cells, and 5-HT for type III cells to discriminate these two types.

Several isolated taste cells were shown in Fig. 4(a) and (d). However, it was hard to identify. Thus, 5-HT and α-gustducin antibodies were used, respectively, as shown in Fig. 4(b) and (e). After overlapping, we obtained Fig. 4(c) and (f), which demonstrated that taste cells with blue arrows were type III and type II cells, respectively. Taste cells with red arrows should be the other types. In Fig. 4(g), IP3 R3 immunoreactivity was observed with GFP (green). 5-HT immunoreactivity was obtained with IgG (red) in Fig. 4(h). Fig. 4(i) denoted the overlap imaging of Fig. 4(g) and (h). It clearly showed that in a cluster of taste cells, type II and type III cells both functionally existed.

The above data demonstrated that there were functional type II and type III taste cells in isolated cells. Meanwhile, we could obtain a cluster of taste cells with both type II and type III cells. Therefore, this 5-HT sensitive LAPS chip with flexibility provides a promising method to investigate taste cell-to-cell communication mechanism.

4. Conclusion

In this work, we develop a 5-HT sensitive LAPS sensor for serotonin detection from taste cells and taste epithelium. Its flexibility, addressability, planar structure and good performance are suitable for investigation of taste cell-to-cell communication. Immunofluorescent imaging assay proves its feasibility and ensures that there are both functional type II and III taste cells in isolated taste clusters. Results indicate that serotonin should play essential roles in taste information transmission. This 5-HT sensitive LAPS sensor opens up a new direction for neurotransmitter detection and the investigation of taste information communication mechanisms.

Acknowledgements

This work was supported by the grants from the National Natural Science Foundation of China (Grant Nos. 60725102, 30970765) and the National Basic Research Program of China (Grant No. 2009CB320303).

References