Intracellular free calcium concentration in human taste bud cells increases in response to taste stimuli

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Abstract We examined changes of intracellular free calcium concentration $[Ca^{2+}]_i$ elicited by taste stimuli of sucrose, denatonium and NaCl in the taste buds of seven human fungiform papillae. In one taste bud we observed an increase in $[Ca^{2+}]_i$ induced by only NaCl. In another bud an increase of $[Ca^{2+}]_i$ in response to both NaCl and sucrose was found. The Ca^{2+} responses to NaCl and sucrose occurred in differential areas within the one taste bud. In the other five fungiform papillae $[Ca^{2+}]_i$ was not changed by the taste stimuli. These results suggest that an increase of $[Ca^{2+}]_i$ participates in taste transduction mechanisms for sucrose and NaCl, and that taste cells in one taste bud may respond to differential stimuli.

Key words: Taste; Human fungiform papilla; Intracellular calcium; Fura-2

1. Introduction

Gustatory transduction mechanisms for taste stimuli seem to differ in species of animals [1]. In rat taste cells, sugars increase the concentration of cyclic adenosine monophosphate (cAMP), but synthetic sweeteners elevate the concentration of inositol 1,4,5-trisphosphate (IP_3) [2]. A similar mechanism has been proposed in gerbil taste buds by intracellular Ca^{2+} measurement [3]. On the other hand, in hamster taste cells both sugars and synthetic sweeteners may induce a cAMP-PKA cascade that blocks a resting K^+ conductance, resulting in a depolarization of the taste cells [4]. Nothing is known about intracellular taste responses in the human taste buds. In this study we attempted to examine taste transduction mechanism using taste buds in the human fungiform papillae.

2. Materials and methods

2.1. Lingual preparation

A fresh lingual tissue was obtained when the macroglossia of a 22-year-old female was surgically reduced. Neither oral radiation therapy nor chemotherapy had been administered to the patient. There existed fifteen fungiform papillae on the surface of the lingual tissue located in the center of the human tongue. Elastase (1 mg/ml, Boehringer Mannheim, Germany) dissolved in mammalian physiological saline (MPS) was injected between the dorsal epithelial and muscular layers of a piece of the lingual tissue containing seven fungiform papillae. After incubating the tongue in MPS for 30 min at 32°C, the dorsal lingual epithelium was peeled off from the underlying muscle. Each of seven fungiform papillae had one taste bud because one taste pore was observed in each papilla. The seven fungiform papillae were employed for the measurement of intracellular Ca^{2+}.

2.2. Histology

In order to examine the structural preservative state of taste buds in the fungiform papilae, eight fungiform papilae were assigned to histological observation. The tissues were fixed by a mixture of 1% glutaraldehyde and 2% paraformaldehyde, postfixed by 1% osmic acid, and then observed by light (Zeiss, Axiophoto), scanning-electron (Hitachi, S-520) and transmission-electron (Hitachi, H-800) microscopes using conventional techniques.

2.3. Solutions and taste stimulation

MPS contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose, 10 sodium pyruvate and 10 HEPES (adjusted to pH 7.4 with Tris). Original concentrations of taste stimuli used were 500 mM in sucrose, 100 μM in denatonium and 1 M in NaCl. These were all prepared in MPS. The dorsal (mucosal) surface of the lingual epithelial sheet containing seven fungiform papillae was pinned on an experimental chamber coated by silicon rubber except for a hole (∅: 8 mm) at the bottom of the chamber (Fig. 1), and soaked in 120 μl of MPS. As soon as 40 μl of MPS was sucked from the experimental chamber using a fine pipette, an equal amount of a taste solution was applied to the chamber with another fine pipette. In this procedure the concentrations of the taste solutions prepared were diluted. The final stimulus concentrations were 167 mM in sucrose, 33 μM in denatonium and 0.426 M in NaCl. The Ca^{2+} measurement was avoided during the solution exchange.

2.4. Fluorescence recordings

The lingual epithelial sheet was exposed to 10 μM fura 2-acetoxymethyl ester (AM) with 0.02% cremophor EL for 60 min while bubbling with O_2 at 30°C, and subsequently washed by MPS six times.

For recording of fluorescence signals and construction of video images from the lingual epithelium, the experimental chamber containing the lingual epithelial sheet was mounted on the stage of an inverted microscope (DIAPHOT 300, Nikon, Japan) (Fig. 1) and the fungiform papillae were imaged with a 20× objective lens (Fluor 20, Nikon). Excitation light was provided by a 100-W Xenon lamp. Images were recorded with silicon intensified target (SIT) camera (C2400-08, Hamamatsu, Japan) and digitized to 8 bits per pixel

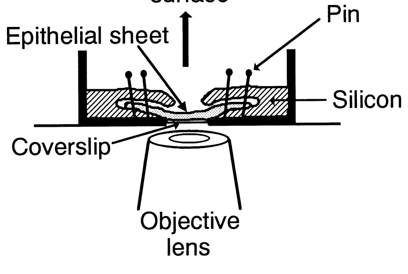

Fig. 1. Schematic drawing of the experimental set up.

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with Image Processor (Argus-50, Hamamatsu). Changes in fluorescence intensity ratios from excitation at 340- and 380-nm light pulses was measured to determine intracellular Ca^{2+} concentration. The fluorescence images were obtained from the horizontal plane of the fungiform papilla 50–60 μm away from the taste pore through a coverslip (∅: 8 mm) adhered to the edge of the hole at the bottom of the chamber. The imaging data were stored on a magnetic optical disk. Calibration was made with a calibration buffer kit (Molecular Probes Inc., Eugene, OR, USA). All experiments were carried out at a room temperature of 23–26°C.

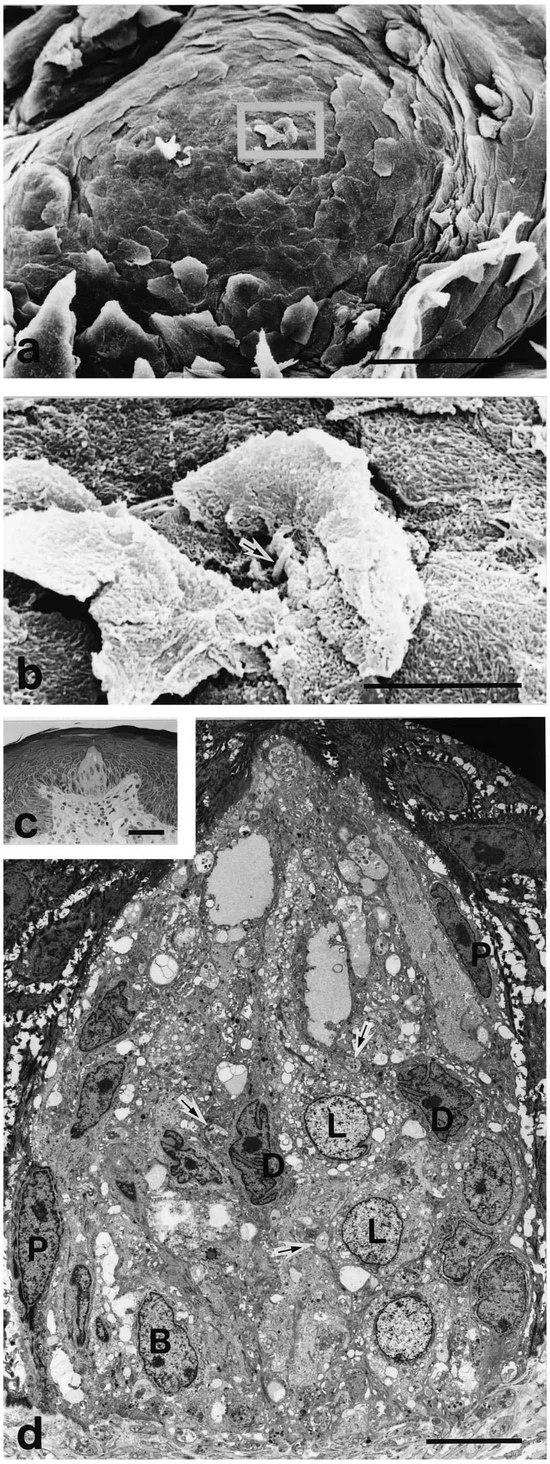

Fig. 2. a: Scanning electron micrograph of a human fungiform papilla. Bar = 100 μm. b: High magnification of the area outlined in a. Note the terminal microvilli of taste bud cells protruding from the taste pore (arrow). Bar = 10 μm. c: Light micrograph of a longitudinal, semithin (1 μm) section of a human fungiform papilla containing a taste bud. Bar = 50 μm. d: Transmission-electron micrograph of the taste bud of a semiadjacent ultrathin (0.1 μm) section of c, showing distinct cell types, dark (D), light (L), basal (B) and peripheral cell (P). Several nerve fibers (arrow) within the taste bud can be seen. Bar = 10 μm.

3. Results

In seven of eight fungiform papillae used for histological examinations, there were 1–3 taste buds on the top of a papilla. No taste buds were found in the one remaining fungiform papilla.

As shown in the electron-microscopic photographs in Fig. 2, the structures of microvilli in the taste pore region and the types of taste bud cells were similar to those in the mammalian taste buds [5–7]. Therefore, the structures of human taste

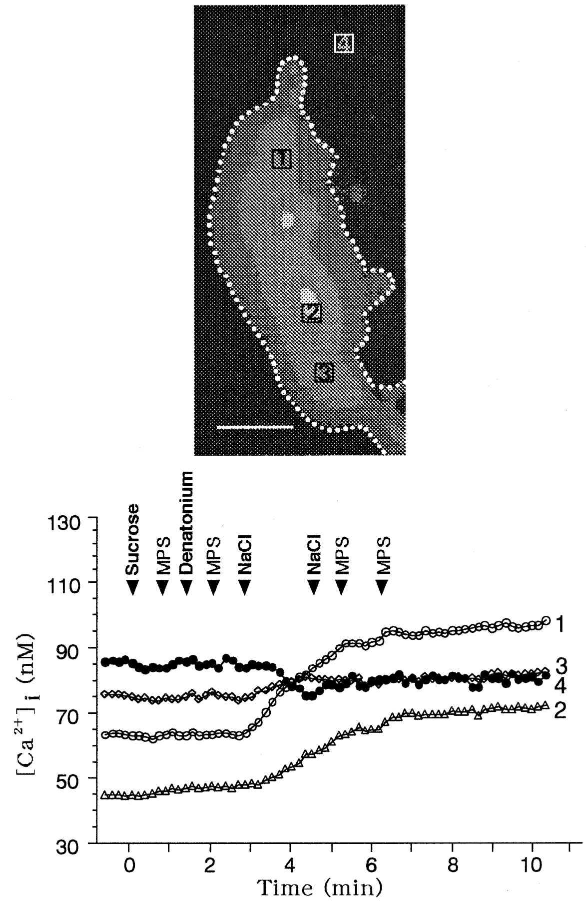

Fig. 3. Effects of NaCl stimulus on $[Ca^{2+}]_i$ in localized areas of the human taste bud. Upper part: Image of a fura-2/AM-loaded taste bud. Excitation at 380 nm. An outline of the taste bud is shown by a dotted line in this and the next figures. Small windows numbered denote 2.3×2.3-μm squares in this and the next figures. Bar in this and the next figures denotes 10 μm. In three regions inside the taste bud (window no. 1: ○; 2: △; 3: ◇) and one region outside the taste bud (window no. 4: ●), $[Ca^{2+}]_i$ was measured. Lower part: Time courses of $[Ca^{2+}]_i$ in four windows in the upper part of the figure. Arrows show timings of solution changes in this figure and the next figure.

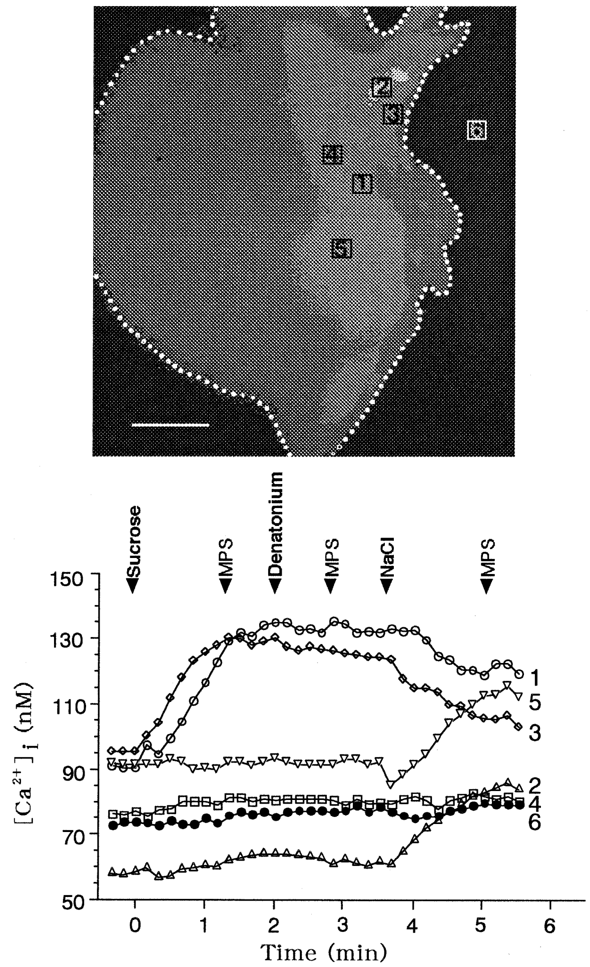

Fig. 4. Effects of sucrose and NaCl on $[Ca^{2+}]_i$ in localized areas of the human taste bud. Upper part: In five regions inside the taste bud (window no. 1: ○; 2: △; 3: ◇; 4: □; 5: ▽) and one region outside the taste bud (window no. 6: ●), $[Ca^{2+}]_i$ was measured. Lower part: Time courses of $[Ca^{2+}]_i$ in six windows in the upper part of the figure.

buds employed in the present study were preserved well for the experiments.

Seven fungiform papillae were used for the measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). As shown in Fig. 3, in the taste bud of one fungiform papilla, application of 0.426 M NaCl to the bud increased the $[Ca^{2+}]_i$ at least in two areas (windows nos. 1 and 2) of the bud by 29 nM (window no. 1) and 19 nM (window no. 2). The rise in the $[Ca^{2+}]_i$ continued for a long period of time and did not fall by physiological saline rinse. However, 167 mM sucrose or 33 μM denatonium did not change the $[Ca^{2+}]_i$ in any areas of the bud.

In Fig. 4, gustatory stimulation of another fungiform papilla with 167 mM sucrose increased the $[Ca^{2+}]_i$ at least in two areas (windows nos. 1 and 3) of the bud by 41 and 33 nM, respectively, but stimulation with 0.426 M NaCl inversely reduced the increased level of the $[Ca^{2+}]_i$ in the same areas. However, in two different areas (windows nos. 2 and 5) where no change in the $[Ca^{2+}]_i$ was elicited by 167 mM sucrose, 0.426 M NaCl induced the $[Ca^{2+}]_i$ rise by each 23 nM. 33 μM denatonium did not increase the $[Ca^{2+}]_i$ in all tested areas of the bud.

In any areas of taste buds in the other five fungiform papillae we could not observe the $[Ca^{2+}]_i$ increase by 167 mM sucrose, 33 μM denatonium and 0.426 M NaCl (data not shown). In all seven fungiform papillae which were employed for the measurement of intracellular Ca^{2+}, we could not observe the $[Ca^{2+}]_i$ increase by MPS.

4. Discussion

As gustatory transduction mechanisms are different among mammalian species [1], it is important for elucidating human taste transduction mechanisms to study human taste buds. In the present experiments, we studied $[Ca^{2+}]_i$ rises in response to sweet, salty and bitter stimulation. Akabas et al. [8] have reported that the intracellular Ca^{2+} level of an isolated taste cell in rat taste bud of the circumvallate papilla is increased by denatonium of a bitter substance. We also observed the $[Ca^{2+}]_i$ increase elicited by NaCl or sucrose in differential small areas within one taste bud. These results suggest that some cells in one taste bud may respond to a stimulus, but other cells in the same bud may respond to other stimuli. These results contradict the claim of von Békésy [9]. He classified human fungiform papillae into two groups: the sour-salty group and the sweet-bitter group. The present results were consistent with the properties of every single human taste papilla which is sensitive to several of the four taste qualities [10]. It has been supposed that sweet responses in taste cells are caused by two transduction mechanisms. One mechanism is mediated by cAMP, while another one is due to IP_3 [2,3]. Sucrose is thought to increase only the cAMP level in a taste cell. The cAMP can block K^+ conductance, resulting in the depolarization that activates the voltage-gated Ca^{2+} channels. Therefore intracellular Ca^{2+} increase in response to sucrose might be elicited by extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels.

Na^+ ions of NaCl stimulus are able to enter taste cells through apical cation channels resulting in the depolarization of the taste cells [11–15]. This process is not concerned with the second messenger. We observed $[Ca^{2+}]_i$ increase elicited by NaCl. This increase also could be due to the activation of the voltage-gated Ca^{2+} channels. The early process in transduction mechanism may be different between sweet and salty tastes. We could not observe $[Ca^{2+}]_i$ increase by denatonium. This result might show that there was no denatonium-sensitive taste cell in the fungiform papillae located in the center of the human tongue.

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