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Electrical Properties and Gustatory Responses of Various Taste Disk Cells of Frog Fungiform Papillae

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Abstract

We compared the electrical properties and gustatory response profiles of types Ia cell (mucus cell), Ib cell (wing cell), and II/III cell (receptor cell) in the taste disks of the frog fungiform papillae. The large depolarizing responses of all types of cell induced by 1 M NaCl were accompanied by a large decrease in the membrane resistance and had the same reversal potential of approximately +5 mV. The large depolarizing responses of all cell types for 1 mM acetic acid were accompanied by a small decrease in the membrane resistance. The small depolarizing responses of all cell types for 10 mM quinine–HCl (Q-HCl) were accompanied by an increase in the membrane resistance, but those for 1 M sucrose were accompanied by a decrease in the membrane resistance. The reversal potential of sucrose responses in all cell types were approximately +12 mV. Taken together, depolarizing responses of Ia, Ib, and II/III cells for each taste stimulus are likely to be generated by the same mechanisms. Gustatory depolarizing response profiles indicated that 1) each of Ia, Ib, and II/III cells responded 100% to 1 M NaCl and 1 mM acetic acid with depolarizing responses, 2) approximately 50% of each cell type responded to 10 mM Q-HCl with depolarizations, and 3) each approximately 40% of Ia and Ib cells and approximately 90% of II/III cells responded to 1 M sucrose with depolarizations. These results suggest that the receptor molecules for NaCl, acid, and Q-HCl stimuli are equivalently distributed on all cell types, but the receptor molecules for sugar stimuli are richer on II/III cells than on Ia and Ib cells. Type III cells having afferent synapses may play a main role in gustatory transduction and transmission.

Key words: basic taste stimuli, fungiform papilla, gustatory transduction, taste disk cell, taste response profile

Introduction

The fungiform papillae are scattered all over the dorsal tongue surface in the frogs. Only 1 taste disk of approximately 200–400 μm in diameter is located at the top of the papillae. The cell types and functions in the taste disks of the fungiform papillae have been investigated by light microscopy since the middle of the 19th century, and several different cell types have been found (Jaeger and Hillman 1976). Recent electron microscopic studies have established that the taste disk in the frogs is composed of 3 layers: superficial, intermediate, and basal and that the cell types in the disk are composed of 6: types Ia cell (mucus cell), Ib cell (wing cell), Ic cell (glia-like cell), II cell (receptor cell having a microvilli-bearing dendrite), III cell (receptor cell having a rod-like dendrite), and IV cell (basal cell) (Richter et al. 1988; Witt 1993; Osculati and Sbarbati 1995; Li and Lindemann 2003). The cell bodies of Ia cells as cylindrical cells are located at the superficial layer. The cell bodies of Ib cells are located at the upper part of intermediate layer, but the cell bodies of Ic and II and III cells are placed at the lower part of intermediate layer. The cell bodies of IV cells are located at the peripheral side of the basal layer. The types Ia, Ib, and Ic cells are epithelial ones; the first 2 have a function of mucous secretion, and the last one has a supportive function. The types II, III, and IV cells are neuroepithelial cells, and the first 2 and the last 1 are thought to be chemosensitive and mechanosensitive, respectively (Osculati and Sbarbati 1995).

The knowledge of functions of these taste disk cells in the frogs is fragmental. Type Ia cells are responsive to 4 basic taste stimuli with depolarization (Sata and Sato 1990; Sata et al. 1992). The II/III cells of gustatory receptors respond to various chemicals with hyperpolarizing and depolarizing responses (Sato and Beidler 1975; Akaike et al. 1976; Sato 1980). The III and IV cells are innervated by afferent nerve fibers, but type II cells are not (Düring and Andres 1976;

Osculati and Sbarbati 1995). Recently, our work suggests that II/III cells are innervated by efferent fibers of the parasympathetic nerve (PSN) (Sato et al. 2005).

Voltage-gated Na, K, and Ca channels are found in II/III cells of frog taste disks (Kashiwayanagi et al. 1983; Avenet and Lindemann 1987; Miyamoto et al. 1991; Fujiyama et al. 1994; Okada et al. 1994; Takeuchi et al. 2001; Suwabe and Kitada 2004). The Ib cells also have voltage-gated Na and K channels, but Ia cells do not (Takeuchi et al. 2001; Suwabe and Kitada 2004). The Ib and II/III cells generate spike potentials, but Ia cells do not (Takeuchi et al. 2001; Suwabe and Kitada 2004).

In the present studies, we attempted to examine the differences in electrical properties and tastant-induced depolarizing response profiles among various taste disk cells of Ia, Ib, II, and III cells in the bullfrog.

Materials and methods

Preparation

All the experiments were carried out using adult bullfrogs (*Rana catesbeiana*) weighing 320–680 g under the Guidance for Animal Experimentation of Nagasaki University. Animals were anesthetized by intraperitoneal injection of a 50% urethane–Ringer solution at a dose of 1.5–4.0 g/kg body weight. Care was taken to keep the lingual blood circulation normal as long as possible. Both the glossopharyngeal nerves (GPNs) were separated from surrounding connective tissues and kept intact. When contribution of GPNs to taste disk cell responses was investigated, GPNs were transected centrally to cut the PSN fibers innervating taste receptor cells (Sato et al. 2006). The hypoglossal nerves were cut bilaterally to remove spontaneous contractions of the tongue muscles. The GPNs were immersed into mineral oil, and the tongue was pulled out from the mouth and pinned on the surface of a silicon rubber plate. All the experiments were carried out at room temperature of 23°C–25°C.

Electrical recordings and stimulations

Intracellular recordings were made from taste disk cells of the fungiform papillae located at the apical and middle loci of the tongue. The methods of the recordings were the same as previously described (Sato et al. 2002, 2004, 2005). Briefly, a 3-M KCl–filled microelectrode of 20–60 MΩ was vertically inserted into the central area of taste disk to penetrate a taste disk cell. The tip of microelectrode was advanced easily in any layer of taste disk by rotating the micrometer equipped in a micromanipulator. In order to measure the membrane resistance of taste disk cells, stationary hyperpolarizing current pulses at 1 Hz were injected into the cells with a bridge circuit.

The tongue surface was always adapted to a frog Ringer solution flowing at 0.05 ml/s. In part of a Ringer solution–delivering tubing, a small port for injecting taste stimuli was built. As soon as a taste solution was injected into the port, the Ringer flow was stopped to remove a dilution of the stimulus solution. As taste stimuli, 0.5–1 M NaCl, 1 mM acetic acid, 10 mM quinine–HCl (Q-HCl), and 1 M sucrose were used. Because stimulating effects of Q-HCl and sucrose molecules on frog taste disk cells were weak, 10 mM Q-HCl and 1 M sucrose were prepared in 0.1 M NaCl to eliminate hyperpolarization of cell membrane by water as a solvent (Sato et al. 2005). Because of small responses for the Q-HCl and sucrose stimuli, these responses were recorded on a pen recorder with a high sensitivity of 0.5 mV/cn. The composition of a frog Ringer solution was 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 HEPES [4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid]. The pH was adjusted to 7.2 by a Tris [tris(hydroxymethyl)aminomethane].

Electrical stimulation of PSN in the distal portion of cut GPN was performed at 30 Hz with pulses of 0.1 ms in duration and 15 V in strength. Electrical stimulation of PSN induces a large physicochemical junction potential between a secreted salivary solution and a lingual adapting solution (Sato et al. 2000). This potential disturbs the analysis of physiological potentials induced in taste cells, so that atropine sulfate was injected intravenously at 1 mg/kg body weight to block the junction potential.

Identification of taste disk cell types

As mentioned in Introduction, the cell bodies at the central area of taste disk are arranged at 3 strata. The Ia cell bodies are located at the superficial layer; the Ib cell bodies are at the upper part of the intermediate layer; and the cell bodies of Ic, II, and III cells are at the lower part of the intermediate layer. Therefore, when the tip of microelectrode was inserted from the summit of taste disk into its inside, the resting potentials of cell bodies appeared as 3 step potential changes because the amplitude of resting potentials of the cell bodies was larger at the deeper part of taste disk (Sato et al. 2007). The first step potential came from a Ia cell body, the second step potential came from a Ib cell body, and the third step potential from a II or III cell body. This has been confirmed by the dye staining of taste disk cells after intracellular electrical recordings (Sato et al. 2007). The resting potential of Ic cell bodies is unknown because no electrical recordings from Ic cells have succeeded in spite of several trials of electrical recordings (Takeuchi et al. 2001; Suwabe and Kitada 2004; Sato et al. 2007). This is probably due to the complex structure of Ic cells wrapping II cells (Osculati and Sbarbati 1995). In this study, the criteria for identifying taste disk cell type were appearance of 3 step potential changes in resting potentials. No intracellular dye stainings of cells were performed after recordings.

Statistics

All data were expressed as means ± standard error of means. The level of significance was set at $P < 0.05$ at a Student's *t*-test.

Results

Resting potentials of taste disk cells

The stable recording of resting potentials was observed only when the tip of microelectrode was penetrated into the cell bodies of taste disk cells. When a microelectrode put at the top of a fungiform papilla was vertically inserted slowly through the taste disk, 3 step potential changes appeared in taste disk cells (Figure 1A, a and b). The trace (a) was obtained from a taste disk with the cut GPN and the trace (b) from another taste disk with the intact GPN. Sometimes, 4 step potential changes were seen. The hyperpolarizing pulse trains were superimposed on the membrane potential of taste disk cells to measure cell input resistance. As revealed by intracellular dye staining of taste disk cells (Sato et al. 2007), the layer structure of taste disk indicates that the first, second, and third step potentials derived, respectively, from Ia cell bodies at the superficial layer, Ib cell bodies at the upper part of intermediate layer, and II/III cell bodies at the lower part of intermediate layer. Figure 1B summarizes the mean amplitudes of resting potentials measured from the first to third step potentials before and after the PSN in the GP nerve was transected. There was no difference in the sizes of resting potentials in either Ia or Ib cells before and after transection of PSNs ($P > 0.05$, $n = 41$–73). However, the resting potential of II/III cells measured from the third step potential change was significantly larger in the intact state of PSN (-42 ± 1 mV, $n = 96$) than in the cut state (-31 ± 1 mV, $n = 43$) ($P < 0.05$). The larger amplitude of resting potentials in II/III cells in an intact state of PSN derives from the spontaneous tonic activities of PSN fibers via the medulla oblongata (Sato et al. 2006).

Input resistance of taste disk cells

When the input resistance of Ia cells at rest under the cut PSN was taken as 100, the input resistance of the types Ib and II/III cells was significantly larger than that of type Ia cells (Figure 2) ($P < 0.05$, $n = 15$). The input resistance of Ia and Ib cells was the same before and after the PSN was cut ($P > 0.05$, $n = 15$). Furthermore, the input resistance of type II/III cells with intact PSNs was 1.7 times larger than that of type II/III with cut PSNs. This comes from an increase in the membrane resistance, which was due to cation channels closed during spontaneously PSN-induced slow hyperpolarizing potentials in II/III receptor cells (Sato et al. 2002, 2004).

Depolarizing responses and membrane resistance changes

As shown in an example of Ib cell responses (Figure 3A), depolarizing potentials induced by 1 M NaCl, 1 mM acetic acid, and 1 M sucrose were accompanied with a decrease in the membrane resistance but the depolarizing response induced by 10 mM Q-HCl was accompanied with an increase in the resistance. This tendency was seen all types of taste disk cell. The amplitudes of depolarizing responses by the NaCl and acetic acid tended to increase in the order of Ia cell > Ib cell > II/III cell (Figure 3B,C). A significant difference was found only between Ia cell and II/III cell with the cut PSN ($P < 0.05$, $n = 11$–17). The amplitudes of depolarizing responses induced by 10 mM Q-HCl and 1 M sucrose were not different among types Ia, Ib, and II/III cells under cut PSNs (Figure 3D,E). The amplitude of membrane resistances during 1-M NaCl–induced large depolarizations decreased in the order of Ia cell > Ib cell > II/III cell under cut PSNs. During stimulation with 1 mM acetic acid and 1 M sucrose, the membrane resistance of all Ia, Ib, and II/III cells under cut PSNs slightly decreased but did not differ in amplitude among all cells ($P > 0.05$, $n = 11$–17). The membrane resistance of all types of cell during Q-HCl stimulation slightly increased but did not differ among the cell types ($P > 0.05$, $n = 11$–17). The depolarizing responses of II/III cells for 1 NaCl and 1 M sucrose were significantly larger under intact PSNs than under cut PSNs ($P < 0.05$, $n = 12$–17). Also, the membrane resistances of II/III cells

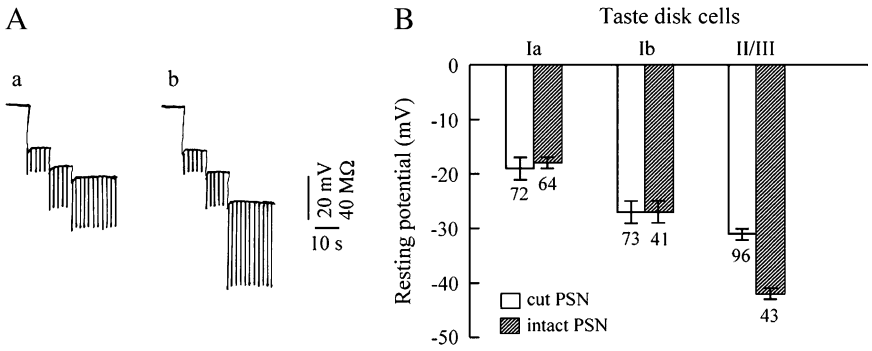

Figure 1 Resting potentials of taste disk cells in bullfrogs. **(A)** Three step potential changes in membrane potential across taste disk cells in fungiform papillae with cut **(a)** and with intact **(b)** PSN. A microelectrode was vertically inserted from top of fungiform papilla across taste disk. Vertically deflected pulses superimposed on membrane potential are hyperpolarizing potential trains for measuring membrane resistance. **(B)** Resting potentials of Ia, Ib, and II/III cells before and after cutting PSN. Vertical bars of columns are standard error of means, and numerals are number of taste disk cells tested.

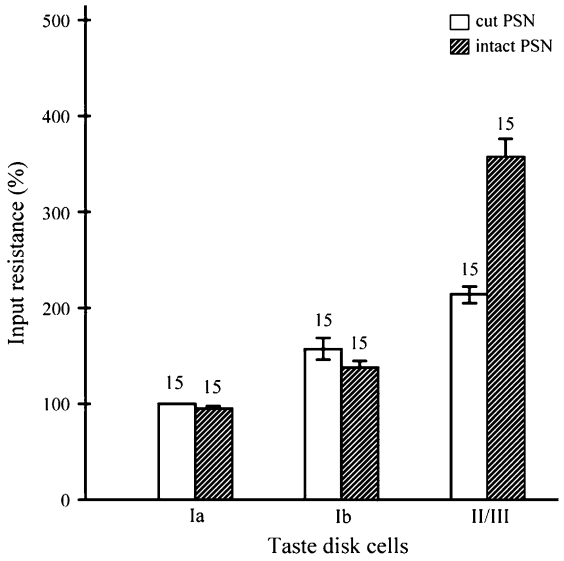

Figure 2 Input resistance of taste disk cells under cut and intact PSN. Input resistance of Ia cells under cut PSN was taken as 100. The other values are relative to this value. Input resistance of Ia cells was 21 ± 3 MΩ (n = 15). Vertical bars and numerals are standard error of means and number of taste disk cells sampled, respectively.

during the NaCl and sucrose stimulation significantly reduced largely under intact PSNs than under cut PSNs ($P < 0.05$, n = 12–17).

Reversal potential of taste disk cell response

The reversal potentials of the depolarizing responses in varying taste disk cells induced by 4 basic stimuli are shown in Figure 4. Reversal potentials in Ia, Ib, and II/III cells were obtained when stimulated with 0.5 M NaCl and 1 M sucrose. However, no reversal potentials were determined in Ia, Ib, and II/III cells when stimulated with 1 mM acetic acid and 10 mM Q-HCl. The mean reversal points for depolarizing responses are listed in Table 1. In our previous works (Miyamoto et al. 1988), the reversal potential of 1-mM HCl–induced depolarization in probably type III receptor cells was as large as approximately +80 mV. Although the sizes of depolarizing responses of II/III cells for 0.5 M NaCl and 1 M sucrose were larger under intact PSN than under cut PSN, the reversal potentials did not differ between the II/III cells with the intact and cut PSNs ($P > 0.05$, n = 3–4). The reversal points of 0.5-M NaCl– and 1-M sucrose–induced depolarizations did not differ among the Ia, Ib, and II/III cells ($P > 0.05$, n = 3–5).

Gustatory response profiles of taste disk cells by basic taste stimuli

Figure 5 illustrates examples of depolarizing responses when the 4 basic taste solutions stimulated types Ia, Ib, and II/III cells in taste disks. The time courses of all depolarizations were slow. Particularly, the time course of 1-M sucrose–

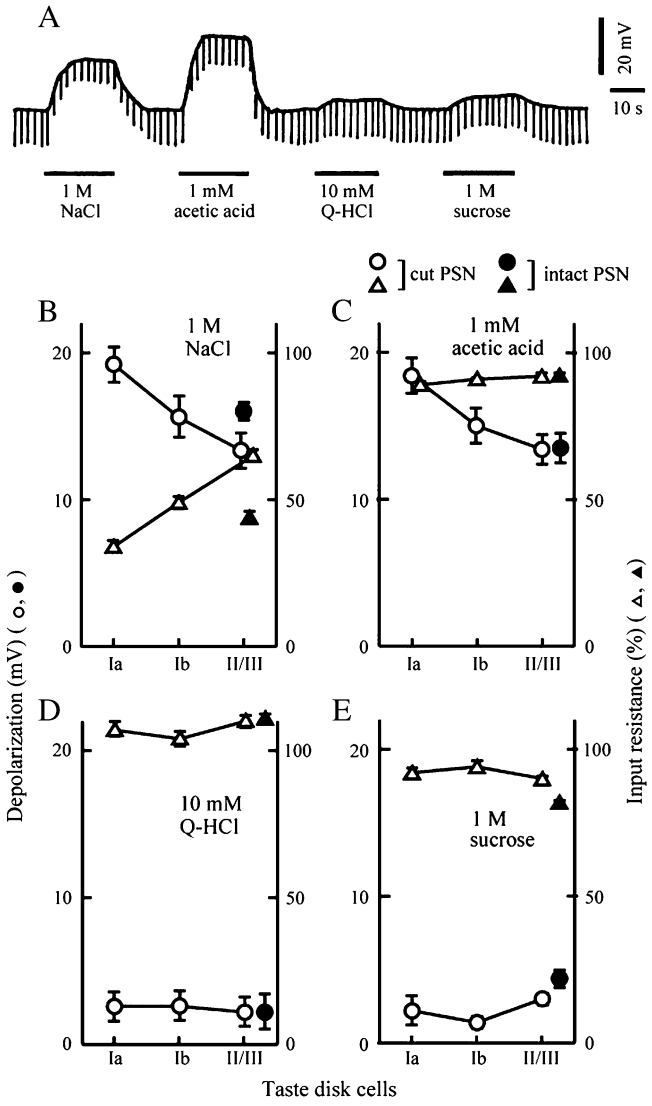

Figure 3 Amplitudes of depolarizing responses and changed input resistances in taste disk cells during gustatory stimulation. **(A)** Traces of depolarizing responses of a Ib cell for 1 M NaCl, 1 mM acetic acid, 10 mM Q-HCl, and 1 M sucrose. **(B–E)** Amplitudes of depolarizing responses and changed input resistances during stimulation with 1 M NaCl (B), 1 mM acetic acid (C), 10 mM Q-HCl (D), and 1 M sucrose (E). Input resistance of taste disk cells at rest was taken as 100%. Values of open circles and triangles (means of 11–17 cells) in each figure denote depolarizations and input resistance changes, respectively, recorded from Ia, Ib, and II/III cells under cut PSN, and values of filled circles and triangles (means of 12 cells) denote depolarizations and input resistance changes, respectively, recorded from II/III cells under intact PSN. Vertical bars are standard error of means.

induced response was much slower because of viscous nature in a 1 M sucrose solution. The depolarizations for NaCl and acetic acid were generally much larger than those for Q-HCl and sucrose in Ia, Ib, and II/III cells. Figure 6 shows gustatory response profiles in Ia, Ib, and II/III cells under cut PSNs (A–C) and in II/III cells under intact PSNs (D). When stimulated with 1 M NaCl and 1 mM acetic acid, all tested cells of types Ia, Ib, and II/III responded 100% to each

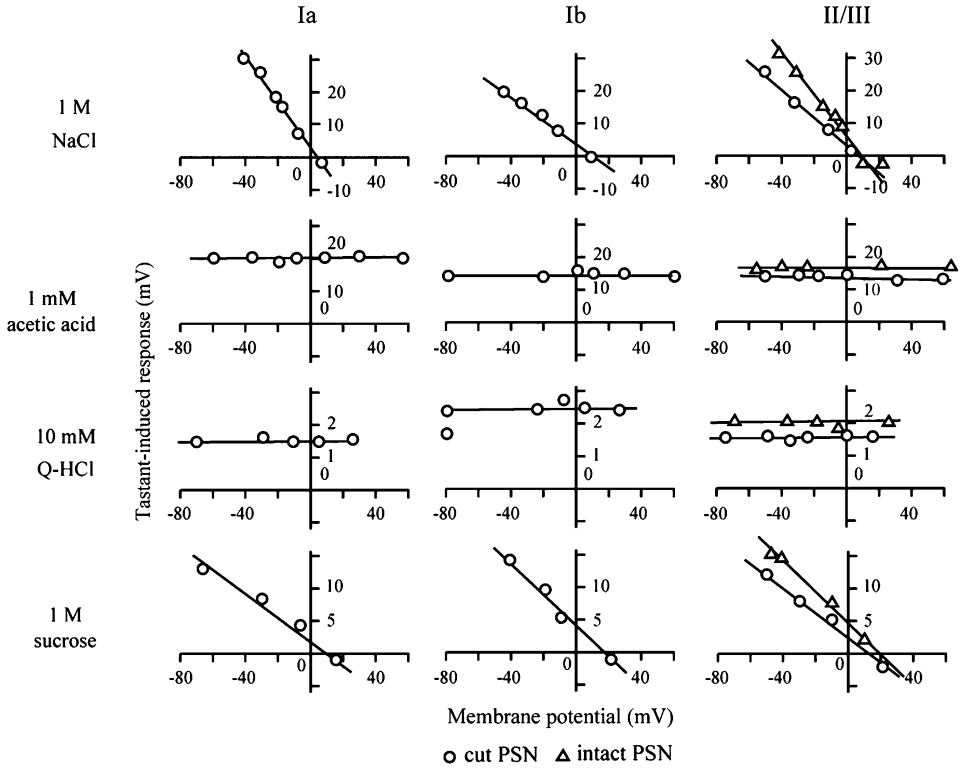

Figure 4 Relationship between membrane potential and gustatory response in Ia, Ib, and II/III cells. Straight lines were obtained through experimental points. Taste stimuli used were 0.5 M NaCl, 1 mM acetic acid, 10 mM Q-HCl, and 1 M sucrose. Values of circles were obtained from gustatory responses of Ia, Ib, and II/III cells under cut PSN, and values of triangles were obtained from gustatory responses of II/III cells under intact PSN. Membrane potential was changed by intracellularly injecting constant currents.

Table 1 Reversal potentials of taste disk cells for 4 basic taste stimuli

Stimuli	Ia (*n*)	Ib (*n*)	II/III (*n*)
0.5 M NaCl	4 ± 2 mV (5)	4 ± 2 mV (4)	5 ± 4 mV (4)
			5 ± 4 mV (3)*
1 mM acetic acid	∞ (2)	∞ (2)	∞ (2)
			∞ (2)*
10 mM Q-HCl	∞ (3)	∞ (2)	∞ (2)
			∞ (2)*
1 M sucrose	11 ± 8 mV (5)	13 ± 6 mV (3)	12 ± 5 mV (3)
			14 ± 6 mV (4)*

Most of the data were obtained from taste disk cells under cut PSN. Some data were obtained from II/III cells under intact PSN (denoted by asterisks). ∞ means unmeasured values.

stimulus. However, the response percentage of all the taste disk cells for 10 mM Q-HCl ranged from 45% to 55%. The response percentage for 1 M sucrose was 36–45% in Ia and Ib cells but was as large as 88–92% in II/III cells with intact and cut PSNs. These data suggest that the number of receptor molecules on taste disk cells for Q-HCl stimuli does not differ among Ia, Ib, and II/III cells. However, the number of receptor molecules for sucrose stimuli is much smaller in Ia and Ib cells than in II/III cells. The receptor molecules for 1 M NaCl and 1 mM acetic acid existed 100% in all cells of types Ia, Ib, and II/III.

Discussion

Gustatory response characteristics of taste disk cells

In the present study, we investigated the passive electrical properties and gustatory responsiveness of Ia, Ib, and II/III cells in the central area of the frog taste disks. Because the type IV cells (basal cells) in the taste disk are located at the periphery and do not reach the free surface of taste disk, these cells were out of study. The type Ic cells (glia-like cells) of relative small sizes have cell bodies at the lower part of intermediate layer in the taste disk and enclose the II receptor cells (Osculati and Sbarbati 1995). In identification of taste disk cell types with methylene blue, we could not find the Ic cells (Sato et al. 2007). Patch clamp studies with the slice preparation of frog taste disks also could not identify Ic cells (Takeuchi et al. 2001; Suwabe and Kitada 2004). These suggest that the stable electrical recording from the Ic cells is not easy because their cell bodies are not spheroidal. Morphological studies on taste disk cells at the apical

and middle regions of the frog tongue suggest that the ratio of Ic cell:II cell:III cell at the lower part of the intermediate layer of the taste disk is 1:1:8 (Osculati and Sbarbati 1995; Li and Lindemann 2003). These morphological and physiological data suggest that microelectrodes inserted into the lower part of intermediate layer of taste disk almost penetrate III cells.

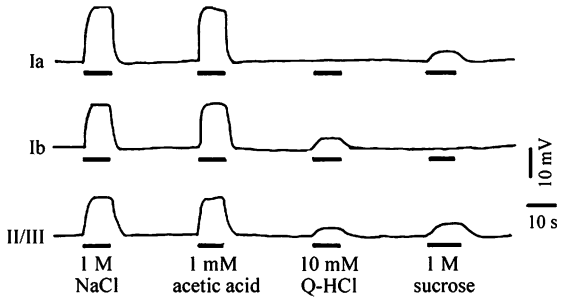

Figure 5 Depolarizing responses of Ia, Ib, and II/III cells for 4 basic taste stimuli. Each trace was obtained from different types of cell under cut PSN.

Depolarizing responses for the 4 basic taste stimuli were obtained from the Ia, Ib, and II/III cells. Large depolarizing responses of all cell types for 1 M NaCl stimulus were accompanied by a large decrease in the membrane resistance and had the same reversal potential of approximately +5 mV when stimulated with 0.5 M. Na^+ entry across the apical receptive membrane of type II/III receptor cells contributes to generation of depolarizing receptor potential in response to NaCl stimulation (Sato and Beidler 1975; Akaike and Sato 1976; Miyamoto et al. 1993; Sato et al. 1995), so that the same mechanisms will be involved in generating the depolarizing responses of Ia and Ib cells. Acid-induced

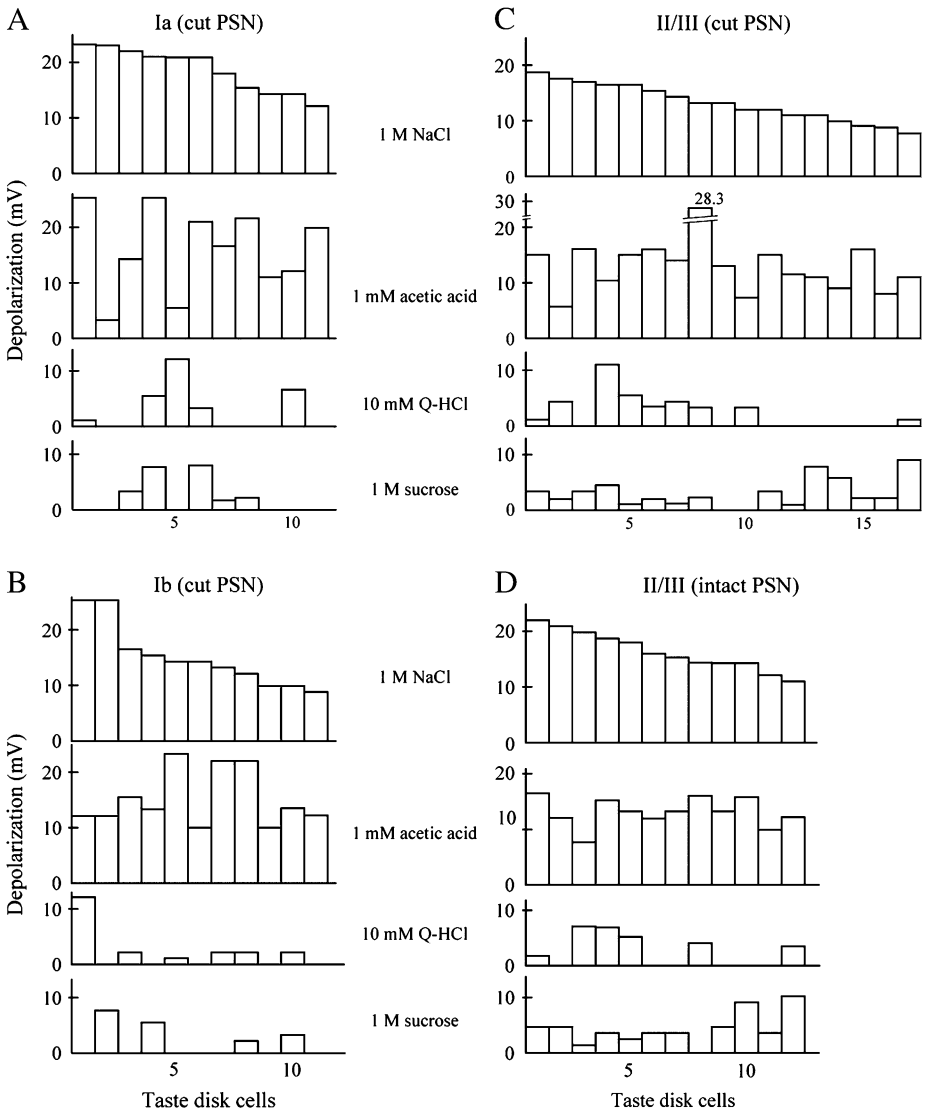

Figure 6 Gustatory response profiles of taste disk cells for 4 basic taste stimuli. **(A)** Ia cells under cut PSN. **(B)** Ib cells under cut PSN. **(C)** II/III cells under cut PSN. **(D)** II/III cells under intact PSN. Taste stimuli were 1 M NaCl, 1 mM acetic acid, 10 mM Q-HCl, and 1 M sucrose. Ordinates are amplitudes of depolarizations, and abscissae are number of taste disk cells tested. Cells are arranged in order of amplitudes of depolarizations for 1 M NaCl.

depolarizations of all Ia, Ib, and II/III cells of taste disk were also large and accompanied by a slight decrease in the membrane resistance. The largest depolarizing response for acetic acid appeared in Ia cells. This is not due to cell coupling among Ia cells but to the responsive properties of Ia cells themselves (Sata et al. 1992). The reversal potential of acid-induced depolarizing responses in all cell types was not measurable. Our previous work showed reversal potential of frog taste cells for 1 mM HCl stimuli is as large as approximately +80 mV, suggesting that the acid response is related to the entry of Na^+/Ca^{2+} across the receptive membrane (Miyamoto et al. 1988; Okada et al. 1994). Probably, reversal points of acid responses in all cell types will be determined by shifting the membrane potential to more positive direction and by changing an adapting solution on the tongue surface.

Q-HCl–induced depolarizations of all types of cell examined in the taste disk were as small as 5 mV or less and accompanied by a small increase in the membrane resistance, and the reversal potentials of their responses were not measurable. These same response properties among all cell types suggest the mechanism underlying generation of Q-HCl responses may be the same. Our previous work suggested that Q-HCl response of frog taste cells is related to a release of Cl^- from the apical receptive membrane with Cl^- pump (Okada et al. 1988). Takeuchi et al. (2001), who studied Q-HCl responses of frog taste disk cells with a slice preparation, have demonstrated that a large Q-HCl current is induced from Ib and II cells but not from Ia and III cells. The large inward current was 1000 pA, and the receptor potential was probably as large as several tens millivolts. These values are quite different from our data obtained from the intact tongue (Okada et al. 1988). It is estimated that the large Q-HCl responses of Ib and II cells might be due to Q-HCl stimulation of nonselective cation channels located at the dendrite membrane below the apical receptive membrane. No Q-HCl responses in Ia and III cells might be due to the absence of nonselective cation channels near the apical membrane. The 1-M sucrose–induced depolarizations in all types of cell were on average 2–3 mV and accompanied by a slight decrease in the membrane resistance, and the response-generating mechanism will be also the same in all cell types of taste disk. We have proposed that the main mechanism of generation of sugar responses is H^+ entry across the apical receptive membrane of frog taste cells (Okada et al. 1992).

Gustatory response profile of taste disk cells

Gustatory response profile of taste disk cells for the 4 basic taste stimuli showed that 1) each 100% of Ia, Ib, and II/III cells sampled responded to 1 M NaCl and 1 mM acetic acid with depolarizing responses, 2) each approximately 50% of Ia, Ib, and II/III cells responded to 10 mM Q-HCl with depolarizations, and 3) each approximately 40% of Ia and Ib cells and approximately 90% of II/III cells responded to 1 M sucrose with depolarizations. When stimulated with 1 M NaCl and 1 mM acetic acid, the amplitude of depolarizing responses of Ia cells was larger than that of Ib and II/III cells. The amplitudes of small depolarizing responses induced by 10 mM Q-HCl and 1 M sucrose did not differ among all types of cell. These imply that the numbers of receptor molecules for NaCl, acetic acid, and Q-HCl stimuli are equivalently distributed on all the taste disk cells, but the number of sucrose-binding receptors is larger on II/III cells than on Ia and Ib cells. The II and III cells in frog taste disk are considered to be taste receptors, but only III cells make synaptic contacts with gustatory afferent fibers (Düring and Andres 1976; Osculati and Sbarbati 1995).

In 1994, we examined gustatory responsiveness of nongustatory epithelial cells outside the taste disks in the filiform and fungiform papillae, the lingual ventral side, and the palate in frogs. Most of the nongustatory epithelial cells showed a multiple sensitivity for 4 basic taste stimuli (NaCl, acetic acid, Q-HCl, and sucrose) and the large amplitude of depolarizing responses (Sata and Sato 1994). Gustatory response characteristics of Ia and Ib cells in frog taste disks are similar to those of nongustatory epithelial cells outside the taste disk. This suggests that gustatory responses of Ia and Ib cells are due to electrical activities of these cells themselves but not due to the secondary responses transferred from II/III cells.

Functional gap junction channels are basically formed between homogenous cells, such as epithelial cells, myocardial cells, and smooth muscle cells of the same type (Ganong 2005; Simon et al. 2005). Dye coupling studies on frog taste disk cells suggest that gap junctions exist between homogenous cells such as Ia cells, Ib cells, and III cells (Sata et al. 1992). However, it is uncertain whether gap junction exists between heterogenous cells such as Ia–Ib cells, Ia–III cells, Ib–III cells, and II–III cells in frog taste disk. Although weak dye couplings between heterogenous cells in frog taste disk are shown (Sata et al. 1992), it is possible that the dye couplings between 2 heterogenous cells derive from a dye diffusion through the electrode pathway following withdrawal of a dye-filled microelectrode inserted into a taste disk cell. Patch clamp studies with sliced frog taste disks have revealed that no dye couplings exist between any pairs of Ia, Ib, II, and III cells excepting between Ia cells (Takeuchi et al. 2001; Suwabe and Kitada 2004). In mammalian taste bud, it has been thought that type II cells are regarded as gustatory transduction cells and type III cells are regarded as gustatory transmission cells (Huang et al. 2007). An ATP transmitter released from the pannexin 1 hemichannels of type II cells plays an important role in activating type III cells having afferent synapses. Because even Ia and Ib cells of nongustatory cells elicit depolarizing responses for basic taste stimuli, depolarizing responses recorded from II and III cells of frog gustatory cells are very likely to be their own responses. As suggested in mammalian taste bud, if the part of gustatory responses of III cells in the frog taste disk derives from activities of II cells, a transmitter released from

a II cell must diffuse up to many III cells approximately 10 μm apart from the II cell through the narrow extracellular space. It is concluded that type III cells in the frog taste disk play a main function in gustatory transduction and gustatory transmission because most of the taste receptor cells are composed of III cells having afferent synapses.

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