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Depression of Gustatory Receptor Potential in Frog Taste Cell by Parasympathetic Nerve–Induced Slow Hyperpolarizing Potential

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Abstract

Parasympathetic nerve (PSN) innervates taste cells of the frog taste disk, and electrical stimulation of PSN elicited a slow hyperpolarizing potential (HP) in taste cells. Here we report that gustatory receptor potentials in frog taste cells are depressed by PSN-induced slow HPs. When PSN was stimulated at 30 Hz during generation of taste cell responses, the large amplitude of depolarizing receptor potential for 1 M NaCl and 1 mM acetic acid was depressed by ~40% by slow HPs, but the small amplitude of the depolarizing receptor potential for 10 mM quinine–HCl (Q-HCl) and 1 M sucrose was completely depressed by slow HPs and furthermore changed to the hyperpolarizing direction. The duration of the depolarizing receptor potentials depressed by slow HPs prolonged with increasing period of PSN stimulation. As tastant-induced depolarizing receptor potentials were increased, the amplitude of PSN-induced slow HPs inhibiting the receptor potentials gradually decreased. The mean reversal potentials of the slow HPs were approximately −1 mV under NaCl and acetic acid stimulations, but approximately −14 mV under Q-HCl and sucrose stimulations. This implies that when a slow HP was evoked on the same amplitude of depolarizing receptor potentials, the depression of the NaCl and acetic acid responses in taste cells was larger than that of Q-HCl and sucrose responses. It is concluded that slow HP–induced depression of gustatory depolarizing receptor potentials derives from the interaction between gustatory receptor current and slow hyperpolarizing current in frog taste cells and that the interaction is stronger for NaCl and acetic acid stimulations than for Q-HCl and sucrose stimulations.

Key words: basic taste stimuli, gustatory efferent synapse, parasympathetic nerve, receptor potential, slow hyperpolarizing potential

Introduction

Taste cells in frogs are innervated by efferent nerve fibers, and the sensitivity of the taste cells are modulated by activities of efferent nerve fibers (Sato et al. 2002, 2004, 2005). Existence of efferent synapses on taste cells has been morphologically suggested by electron microscopical studies (Nomura et al. 1975; Düring and Andres 1976; Yoshie et al. 1996). We have found that electrical stimulation (ES) of the parasympathetic nerve (PSN) efferent fibers running along the glossopharyngeal (GP) nerve produces a slow hyperpolarizing potential (HP) in taste cells of the frogs (Sato et al. 2002, 2004, 2005). This slow HP response has the following characteristics: 1) latency of ~7 s, 2) increase in input resistance, 3) reversal potential of approximately −14 mV, 4) blocking by substance P neurokinin-1 antagonist, and 5) mimic by substance P. Therefore, the slow HPs are regarded as an inhibitory postsynaptic potential.

When a taste cell in frogs is beforehand hyperpolarized by a PSN-induced sustained slow HP, the amplitudes of depolarizing receptor potentials superimposed on the slow HP are slightly increased for NaCl and sucrose but not for acetic acid and quinine–HCL (Q-HCl) (Sato et al. 2005). This enhancement comes from an increase in a motive force for cation entry across the gustatory receptive membrane because the taste cell membrane is beforehand hyperpolarized (Sato et al. 2005).

Suppression of excitatory postsynaptic potentials by inhibitory postsynaptic potentials in synapses among neurons of the central nervous system is a basic principle in the information processing (Eccles 1964, 1973). In the present study, we attempted to investigate the suppressive effects of PSN-induced short slow HPs superimposed on gustatory receptor potentials in frog taste cells. The results showed that

depolarizing receptor potentials of frog taste cells in response to 4 basic taste stimuli were all greatly depressed when the slow HPs were evoked during generation of the receptor potentials.

Materials and methods

Preparation

Bullfrogs (*Rana catesbeiana*) weighing 380–640 g were used in the experiments. All experiments were performed under the Guidance of Animal Experimentation in Nagasaki University. The animals were anesthetized by an intraperitoneal injection of 50% urethane–Ringer solution at a dose of 2–4 g/kg body weight (b.w.). Care was taken to keep the lingual blood circulation normal as long as possible. Both GP nerves were separated free from the surrounding connective tissues, cut centrally, and immersed into mineral oil. To eliminate the spontaneous twitches of the tongue muscles, both hypoglossal nerves were severed. The tongue was pulled out from the mouth as long as possible and pinned on a silicon rubber plate in the experimental chamber. All experiments were carried out with a room temperature of 23–26 °C.

Recording and stimulation

Intracellular recordings from taste cells in the fungiform papillae were made with a glass microelectrode of 30–70 MΩ. The fungiform papillae located at the apical and middle portions of the tongue were used. The recording methods and criteria were the same as previously described (Sato et al. 2002, 2004). Input resistance of the taste cells was measured by injecting constant hyperpolarizing current pulses at 1 Hz into the cells using a bridge circuit. In order to stimulate the PSN fibers in the GP nerve, repetitive pulses of 0.1 ms in duration and 15 V in strength were applied to the distal part of the cut nerve. Because the maximal amplitude of slow HP is induced by PSN stimulation at 30 Hz (Sato et al. 2002, 2005), PSN was stimulated at 30 Hz in most of the experiments.

To remove a large physicochemical junction potential generated between GP nerve–induced lingual saliva and lingual superficial solution (Sato et al. 2000), atropine sulfate (Sigma-Aldrich, St Louis, MO) was intravenously (i.v.) injected at 1 mg/kg b.w. This potential, which disturbs an intracellular analysis of slow HPs, was completely eliminated for >6 h.

Gustatory cells in the taste disk of the fungiform papillae were stimulated by 0.3–1 M NaCl, 0.3–1 mM acetic acid, 3–10 mM Q-HCl, and 0.5–1 M sucrose. The last 2 chemicals were dissolved in 0.1 M NaCl to eliminate the membrane hyperpolarization of Ringer-adapted taste cells induced by water as a solvent. Inhibitory effect of 0.1 M NaCl on Q-HCl and sucrose responses is weak (Sato and Sugimoto 1979; Okada et al. 1992). The tongue surface was continuously adapted to a frog Ringer solution by flowing it at a rate of 0.05 ml/s, and taste stimuli were flowed at the same rate soon after suspending the Ringer flow.The ionic composition of frog Ringer solution was 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 mM 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid. The pH was adjusted by a tris [tris (hydroxymethyl)aminomethane] buffer.

Identification of taste disk cells by dye injection

After intracellular recording of the membrane potentials of taste disk cells, single cells were iontophoretically stained by methylene blue as a vital staining dye. A 4% methylene blue solution dissolved in 0.1 M KCl was filled in microelectrodes whose electrical resistance was 80–120 MΩ. For iontophoretical staining of a cell, positive constant pulses of 3 ms in duration and 0.5–1 μA in strength were applied at 200 Hz to a microelectrode 20–60 s.

A dye-injected fungiform papilla was cut by a pair of fine scissors, mounted on a glass slide in a 50% glycerin solution, and slightly pressed with a coverslip. The preparation was inspected with a light microscope at 400× magnification.

Statistics

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

Results

Identification of taste disk cells

The taste disk located at the top of the fungiform papillae in bullfrogs is 200–300 μm wide and 60–70 μm deep, has 3 layers: superficial layer (SL), intermediate layer (IL), and basal layer and composed of 6 types of cells: types Ia (mucus cell), Ib (wing cell), Ic (glialike cell), II (receptor cell), III (receptor cell), and IV (basal cell) cells (Jaeger and Hillman 1976; Osculati and Sbarbati 1995).

As soon as a microelectrode tip put on the surface of a mucus cell at the SL of taste disk (SL in Figure 1B) was vertically moved a little, the first step-potential change suddenly appeared (① in Figure 1A). This is the resting potential of the mucus cell. Then, while the microelectrode tip was slowly advanced, the second step-potential change (②) suddenly appeared as a result of penetration of the microelectrode tip into the cell body of a wing cell at the upper portion of IL passing through the mucus cell. At the time of appearance of the second step-potential, the moved distance of a microelectrode tip was 29 μm as shown in the right ordinate. Further advancement of the microelectrode induced the third step-potential change (③). At this time, the microelectrode tip reached the 39 μm depth of the taste disk, indicating that the microelectrode tip impaled another cell (types Ic, II, or III cell) at the lower portion of the IL passing through the cell body of a wing cell. This cell was a type III cell judging

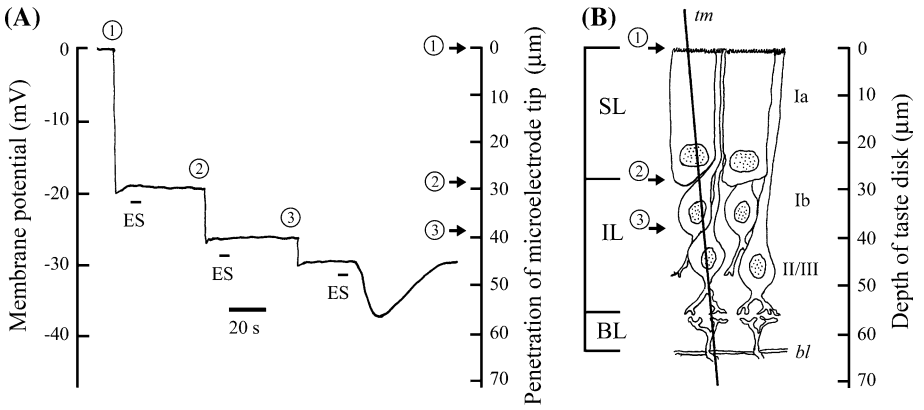

Figure 1 Identification of taste disk cells in bullfrog. **(A)** Appearance of 3 step-potential changes of membrane potentials in taste disk cells after penetration of a microelectrode through taste disk. First, second, and third step-potential changes in membrane potentials of a taste disk appeared after penetration of microelectrode into mucus cell (①), wing cell(②), and receptor cell (③), respectively. Amplitude of membrane potential is shown in left ordinate, and distance of movement of microelectrode tip is shown in right ordinate. Bars below trace show ES of PSN efferent fibers at 30 Hz. **(B)** Drawing of cell arrangement of taste disk in bullfrog. Drawing was based on iontophoretical staining of taste disk cells with methylene blue after recording of step-potential change in membrane potential, moved distance of microelectrode tip impaling taste disk, and histological preparation of fungiform papillae. BL: basal layer. Ia, Ib, and II/III: types Ia cell, Ib cell, and II/III cell in taste disk. tm: estimated track of movement of a microelectrode tip. bl: basal lamina. Right ordinate shows depth of taste disk. ①, ②, and ③ correspond to those of right ordinate in (A).

from the cell staining mentioned below. Stable recording of the membrane potential from a taste disk cell seemed to be due to the penetration of a microelectrode into the cell body region. When a microelectrode tip probably impaled a thin dendrite or proximal process of a cell, the membrane potential gradually or suddenly reduced and the tip became out of the cell.

As shown in horizontal bars below the membrane potential traces (Figure 1A), ES of the GP nerve induced a slow HP in neither mucus cell nor wing cell, but a large slow HP in a receptor cell. In 15 trials of taste disk penetration by microelectrodes, the slow HP was found in 13 of 15 taste receptor cells (87%) penetrated, but in neither 15 mucus cells nor 15 wing cells penetrated. The resting potentials measured from the 3 step-potential changes in the taste disk cells were -18 ± 2 mV ($n = 15$) in mucus cells, -27 ± 2 mV ($n = 15$) in wing cells, and -31 ± 1 mV ($n = 15$) in taste receptor cells.

After recording the membrane potential from each taste disk cell, a methylene blue dye was iontophoretically injected. The mucus cells having a cylindrical shape were histologically found in 6 of 7 injected cells, and wing cells having sheetlike processes at the dendrite were seen in 5 of 7 injected cells. The dyes of one injected mucus cell and 2 injected wing cells were lost. At the lower portion of IL, 20 cells were injected and 13 type III cells having a thin dendrite and 2 type II cells having a thick dendrite were found. The dyes of 5 injected receptor cells were also lost during taste disk isolation. No type Ic cells were stained. One of the reasons is probably due to the nonspheroidal cell body of Ic cells. Figure 1B was drawn using the methylene blue–stained cells, the step-potential changes in membrane potentials of taste disk cells the positions of impaling microelectrode tips and the histological preparation of bullfrog taste disks. In all experiments mentioned below, identification of taste receptor cells in the taste disks was based on 3 step-potential changes of the membrane potentials.

Characteristics of slow HP

Stimulation of PSN fibers in GP nerve induces a slow HP in frog taste cells (Sato et al. 2002, 2004, 2005). Figure 2A shows 2 types of slow HPs with a relatively rapid (a) and late (b) time course. The input resistance was increased during generation of slow HPs. Resting potentials of the taste cells that induced a slow HP by PSN stimulation were -32 ± 1 mV ($n = 70$) (Figure 2B). The amplitude of slow HPs from the resting potential level evoked by 30-Hz stimulation of PSN for 5 s was -9 ± 1 mV ($n = 70$) (Figure 2C), and the increase in input resistance during generation of slow HPs was $229 \pm 8\%$ ($n = 66$) (Figure 2D). When PSN was stimulated at 30 Hz for 5 s, the latency of slow HPs was 8 ± 0 s ($n = 70$) (Figure 2E) and the peak time was 21 ± 1 s ($n = 70$) (Figure 2F). The shorter the latency of slow HPs, the shorter the peak time. The fall time of slow HPs was dependent on stimulation time of PSN. When stimulating at 30 Hz for 5 s, the fall time was 74 ± 4 s ($n = 58$).

Depression of gustatory receptor potential by slow HP

When a slow HP evoked by PSN fiber stimulation appeared during generation of depolarizing receptor potentials in taste cells, the amplitude of the receptor potentials was suppressed. Figure 3 shows an example of depression of depolarizing receptor potentials elicited by 1 M NaCl and 10 mM Q-HCl when PSN was stimulated at 1–30 Hz. The depression percentage of the receptor potentials increased with

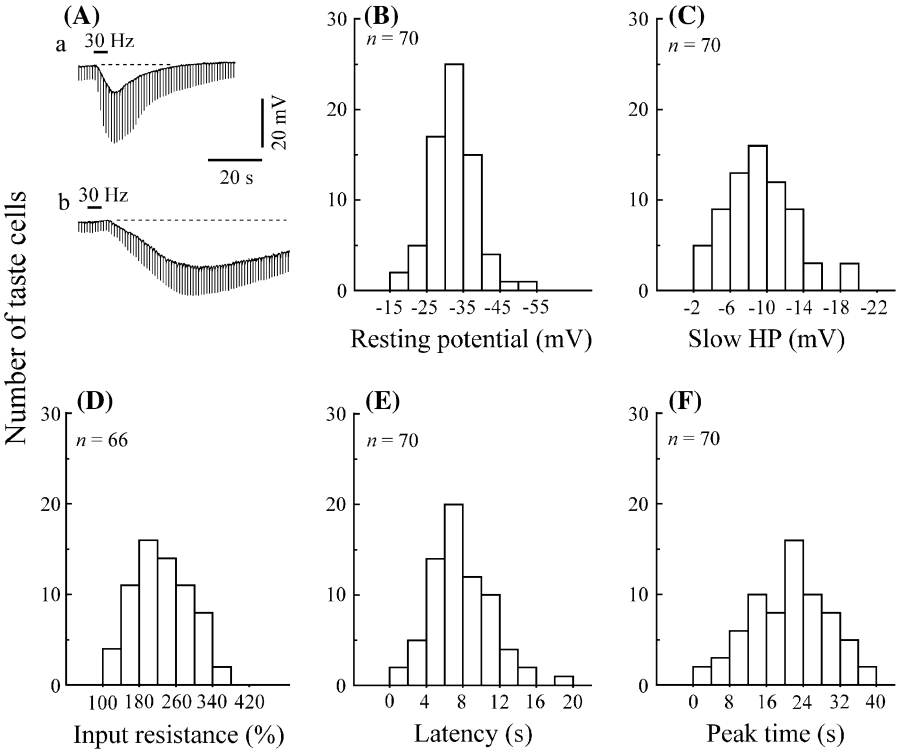

Figure 2 Characteristics of slow HPs in frog taste cells induced by ES of PSN in GP nerve. **(A)** Slow HPs representing a relatively rapid (a) and late (b) time course. To measure input resistance of a taste cell, constant hyperpolarizing pulses are superimposed on membrane potential. Resting potentials were −32 mV (a) and −32 mV (b). **(B)** Histogram of resting potentials of taste cells inducing slow HPs. **(C)** Histogram of slow HPs induced by PSN stimulation. **(D)** Histogram of input resistances increased during generation of slow HPs. Input resistance of taste cells at rest was taken as 100%. **(E)** Histogram of latencies of slow HPs. **(F)** Histogram of peak time of slow HPs. PSN was stimulated at 30 Hz for 5 s. Means and standard errors mean were −32 ± 1 mV (n = 70) for resting potential (B), −9 ± 1 mV (n = 70) for slow HP (C), 229 ± 8% (n = 66) for input resistance (D), 8 ± 0 s (n = 70) for latency (E), and 21 ± 1 s (n = 70) for peak time (F).

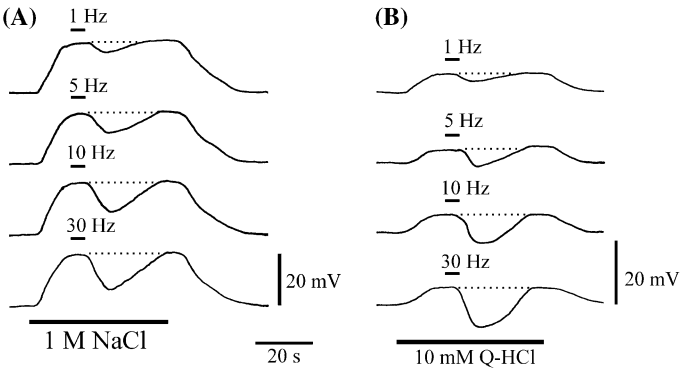

Figure 3 Depression of depolarizing receptor potentials in taste cells by slow HPs induced by PSN stimulation. **(A)** 1 M NaCl. **(B)** 10 mM Q-HCl. Short horizontal bars above traces show stimulus frequencies (1–30 Hz) applied to PSN and long horizontal bars below traces denote taste stimuli applied to tongue. Resting potentials were −30 mV (A) and −31 mV (B).

increasing frequency of PSN stimulation. Figure 4 illustrates a gradual decrease in the depolarizing receptor potentials for 1 M NaCl (A), 1 mM acetic acid (B), 10 mM Q-HCl (C), and 1 M sucrose (D) with increasing amplitudes of slow HPs induced by PSN stimulation at 1–30 Hz. In all experiments of Figure 4, the stimulation time was for 5 s with pulses of 0.1 ms in duration and 15 V in strength. The large amplitude of 1 M NaCl- and 1 mM acetic acid–induced depolarizing receptor potentials was depressed by 41 ± 14% (n = 6) and 38 ± 6% (n = 6), respectively, by 30 Hz stimulation of PSN (Figure 4A,B).

On the other hand, the small amplitude of 10 mM Q-HCl– and 1 M sucrose-induced depolarizing responses was depressed 80–100% (n = 8) by 5 Hz stimulation of PSN and was completely depressed 100% by the stimulation at >10 Hz. Furthermore, the membrane potential of taste cells sensitive to Q-HCl and sucrose was hyperpolarized beyond the resting potential to −3 ± 1 mV (−75 ± 25% [n = 8] of 10 mM Q-HCl response and −100 ± 23% [n = 8] of 1 M sucrose response) by a slow HP induced by 30 Hz PSN stimulation (Figure 4C,D).

The effects of a stimulation time of PSN on a depressed period of the gustatory receptor potential were examined with 1 M NaCl and 1 M sucrose. As the time of repetitive stimulation of PSN was increased, the depression duration of gustatory receptor potentials was gradually prolonged (Figure 5A,B). When PSN was stimulated for 5 s at 30 Hz, the duration of 1 M NaCl/1 M sucrose-induced receptor

potential depressed by a slow HP was 90 ± 5 s (n = 6). When prolonged for 10 and 15 s, the receptor potentials were depressed 1.6 ± 0.3 (n = 6) and 2.7 ± 0.4 (n = 6) times longer than the depression time elicited by 5-s stimulation, respectively.

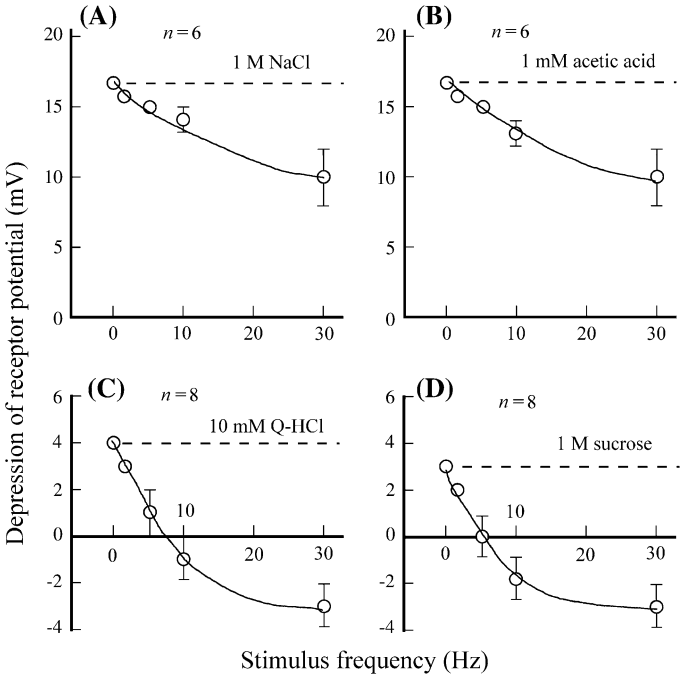

Figure 4 Effects of stimulus frequencies applied to PSN on depression of receptor potentials induced by 4 basic stimuli. **(A)** 1 M NaCl. **(B)** 1 m M acetic acid. **(C)** 10 mM Q-HCl. **(D)** 1 M sucrose. 0 in ordinate indicates mean amplitude of resting potentials of −29 ± 1 mV (n = 6) (A), −32 ± 2 mV (n = 6) (B), −31 ± 1 mV (n = 8) (C), and −32 ± 2 mV (n = 8) (D). Horizontal dashed lines indicate mean amplitudes of receptor potentials of 17 ± 2 mV (n = 6) (A), 17 ± 1 mV (n = 6) (B), 4 ± 0 mV (n = 8) (C), and 3 ± 0 mV (n = 8) (D), when PSN was not stimulated. Horizontal bars in this and the other figures are standard errors mean.

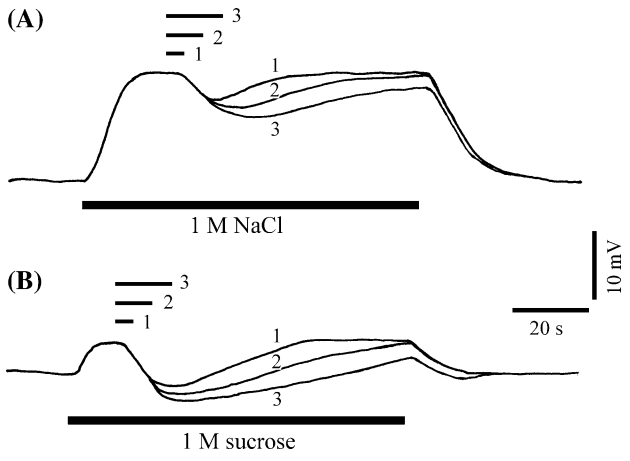

Figure 5 Time course of receptor potential depressions induced by different PSN stimulation time. **(A)** 1 M NaCl. **(B)** 1 M sucrose. Bars above traces show PSN stimulation at 30 Hz for 5 s (1), 10 s (2), and 15 s (3). Numerals on traces correspond to the numerals of the bars. Resting potentials were −31 mV (A) and −32 mV (B).

Relationship between amplitude of gustatory receptor potential and amplitude of slow HP

Whether the amplitude of slow HPs induced by PSN stimulation is influenced by the amplitude of gustatory receptor potential was examined. As shown in examples with NaCl and sucrose (Figure 6), decreasing the membrane potential by an increase in receptor potential reduced a slow HP in amplitude. Figure 7 summarizes the relationships between the amplitudes of receptor potentials for various concentrations of 4 basic taste stimuli and the amplitudes of PSN-induced slow HPs. When no taste stimuli were applied, the mean amplitudes of the slow HPs evoked at the resting potential level were −8 to 10 mV (n = 6–11) in NaCl-, acetic acid–, Q-HCl–, and sucrose-sensitive taste cells. There were no differences in the slow HPs among taste cells with different sensitivities ($P > 0.05$). This implies that each taste cell is innervated by an efferent fiber having similar physiological properties. As seen in Figure 6, slow HPs were evoked at the plateau level of receptor potentials. With any taste stimuli of NaCl (A), acetic acid (B), Q-HCl (C), and sucrose (D), the amplitude of slow HPs gradually decreased as the amplitude of receptor potentials was increased with increasing stimulus concentration. Figure 8 represents the relationships between the plateau level of depolarizing receptor potentials induced by NaCl (0, 0.5, 1 M), acetic acid (0, 0.3, 1 mM), Q-HCl (0, 3, 10 mM), and sucrose (0, 0.5, 1 M) and the amplitude of slow HPs evoked on the plateau level in 4 taste cells. By extrapolating the experimental points, the reversal potentials of slow HPs induced by PSN stimulation could be measured. It is seen that the reversal potential under Q-HCl and sucrose stimulations is different with that under NaCl and acetic acid stimulations. Table 1 gives the mean reversal potentials of slow HPs when the membrane potential of taste cells was changed by receptor potentials induced by 4 basic taste stimuli. The reversal points for slow HPs were classified into 2 groups. One was −1 mV obtained

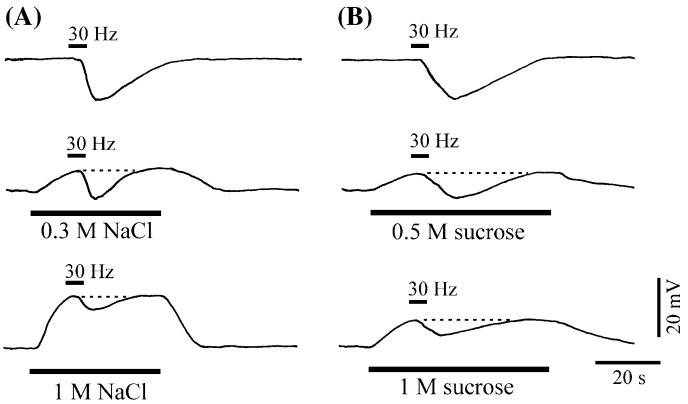

Figure 6 Slow HP–induced depression of receptor potentials for different concentrations of taste stimuli. **(A)** NaCl. **(B)** Sucrose. In top trace, no taste stimulus was applied to tongue. Bars above traces denote PSN stimulation at 30 Hz for 5 s. Resting potentials were −32 mV (A) and −31 mV (B).

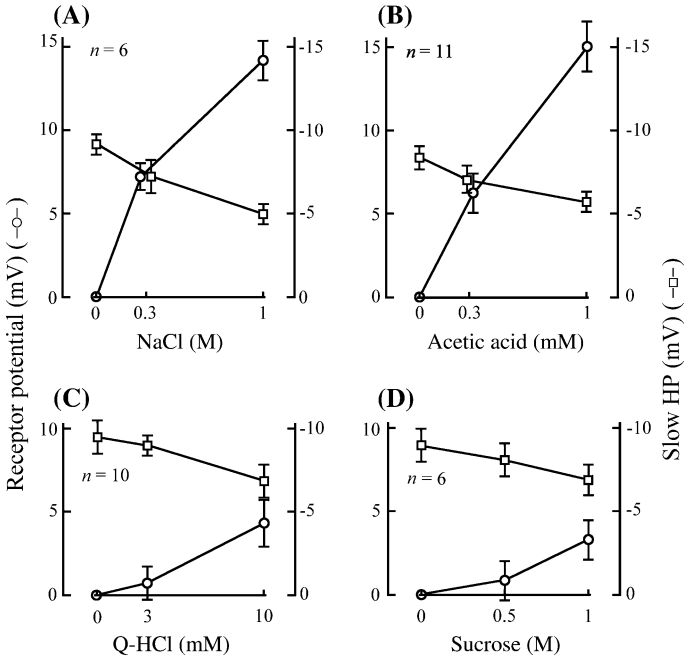

Figure 7 Effects of concentrations of taste stimuli on amplitudes of receptor potentials and slow HPs. **(A)** NaCl. **(B)** Acetic acid. **(C)** Q-HCl. **(D)** Sucrose. All slow HPs were induced by PSN stimulation at 30 Hz for 5 s. Resting potentials were −32 ± 2 mV (n = 6) (A), −30 ± 1 mV (n = 11) (B), −29 ± 2 mV (n = 10) (C), and −31 ± 1 mV (n = 6) (D).

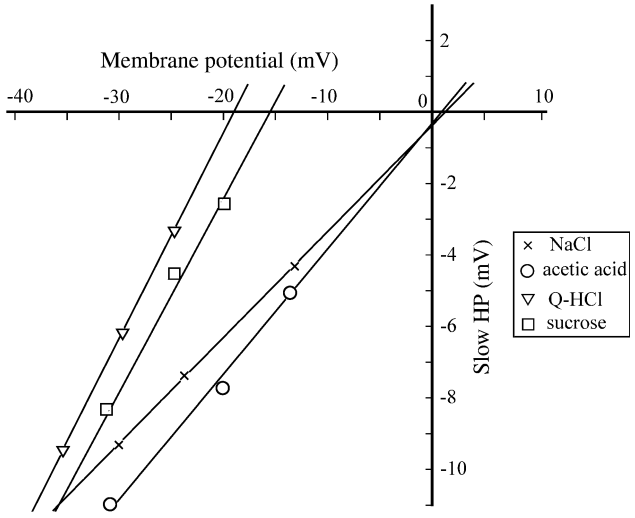

Figure 8 Relationship between membrane potential level of taste stimulus–induced receptor potentials and amplitude of PSN-induced slow HPs. Taste stimuli used were NaCl (0, 0.5, 1 M), acetic acid (0, 0.3, 1 mM), Q-HCl (0, 3, 10 mM), and sucrose (0, 0.5, 1 M), and PSN stimulation was at 30 Hz for 5 s. Four straight lines through experimental points were obtained from 4 different taste cells. Resting potentials were −30 mV (O, ×), −31 mV (□), and −35 mV (∇).

under stimulation with NaCl and acetic acid, and the other was −12 to 16 mV under stimulation with Q-HCl and sucrose. Because there is no difference between the reversal potentials under Q-HCl and sucrose stimulations (P > 0.05, n = 6–11), the mean reversal potential under both stimulations was calculated as −14 ± 2 mV (n = 17).

Table 1 Reversal potential of PSN-induced slow HP measured under tastant-induced receptor potential level

Taste stimuli	Reversal potential (mV)	Range (mV)	n
NaCl	−1 ± 4	−8 to 10	5
Acetic acid	−1 ± 3	−10 to 9	8
Q-HCl	−16 ± 2	−26 to −8	11
Sucrose	−12 ± 3	−18 to −1	6

When the membrane potential of taste cells is changed only by injecting electric currents, the reversal point of slow HPs induced by PSN stimulation is −13 ± 2 mV (Sato et al. 2002). This is equivalent to −14 ± 2 mV (P > 0.05, n = 6–17) of reversal potential under Q-HCl and sucrose stimulations, but not to −1 ± 3 mV (P < 0.01, n = 6–13) of reversal potential under NaCl and sucrose. In Figure 8, the slow HPs induced by PSN stimulation are rapidly reduced with a decrease in membrane potential induced by Q-HCl and sucrose but are relatively slowly reduced with that induced by NaCl and acetic acid. These data suggest that depression percentage of tastant-induced receptor potentials by slow HPs is larger under stimulation with NaCl and acetic acid than under stimulation with Q-HCl and sucrose. Figure 9 clearly shows these relations. When the amplitude of receptor potentials was >6 mV, depression of receptor potentials in taste cells by slow HPs is significantly larger under NaCl and acetic acid stimulations than under Q-HCl and sucrose stimulations (P < 0.05, n = 4). However, when the receptor potential was <6 mV, its depression by slow HPs was not different among the 4 taste stimuli (P > 0.05, n = 4).

Discussion

Efferent innervation of frog taste cells has been physiologically suggested by recording slow HPs from taste cells following ES of C-fibers in the GP nerve (Sato et al. 2002, 2004). The efferent nerve fibers of the taste cells are PSN fibers running along the GP nerve (Sato et al. 2005). Tonic activities of PSN from the medulla oblongata continuously elevate the resting potential in the frog taste cells (Sato et al. 2005). During a sustained hyperpolarization of membrane potentials in the frog taste cells by PSN-induced slow HPs, the receptor potentials of taste cells induced by NaCl and sucrose are slightly enhanced in amplitude by an increase in motive force for cation movement (Sato et al. 2005). This enhancement of receptor potentials may be related to the presynaptic facilitation in generation of neural signals at gustatory afferent synapses (Sato et al. 2006). This mechanism is well known in the central nervous system (Mendell and Wall 1964; Hodge 1972).

Because PSN-induced slow HPs are obtained from ~85% of impaled taste cells (Sato et al. 2004), there is the possibility

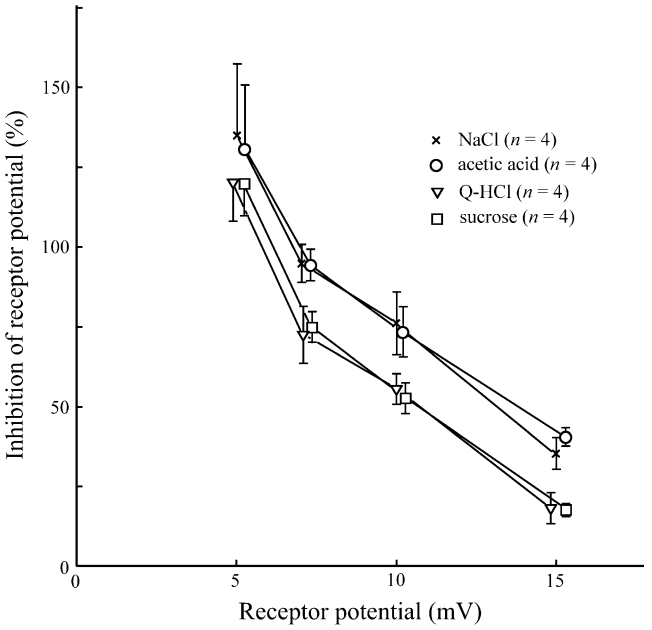

Figure 9 Inhibition percentage of receptor potentials by slow HPs. Receptor potentials were evoked by 0.3–1 M NaCl, 0.3–1 mM acetic acid, 3–10 mM Q-HCl, and 0.5–1 M sucrose. Slow HPs were induced by stimulation of PSN at 30 Hz for 5 s. Data for each stimulus were measured from the data of membrane potential–slow HP relation as in Figure 8 using 4 taste cells. Ordinate shows inhibition percentage of receptor potential by slow HP: (slow HP/receptor potential) × 100. Resting potentials were −30 ± 1 mV (n = 4) for NaCl, −32 ± 2 mV (n = 4) for acetic acid, −28 ± 2 mV (n = 4) for Q-HCl, and −31 ± 2 mV (n = 4) for sucrose.

that some data of slow HPs are induced from neighboring taste cells by the paracrine action of a transmitter released from a PSN terminal. If the paracrine action is effective, slow HPs will be recorded from wing cells extending the proximal processes near taste receptor cells. This is not the case as described in the present work (Figure 1A). After blocking neurotransmitter receptors in gustatory efferent synapses, no slow HPs are elicited (Sato et al. 2004). If paracrine action is very effective in generating slow HPs in taste cells, slow HPs may be sufficiently induced even after blocking the neurotransmitter receptors because the neurotransmitter is still released from PSN terminals. Therefore, paracrine action for slow HPs may be weak, and most slow HPs in frog taste cells are elicited postsynaptically.

In the present study, we showed that when slow HP pulses are superimposed on depolarizing receptor potentials in frog taste cells induced by basic taste stimuli, the receptor potentials are greatly depressed in amplitude. It is well known that depolarizing and spiking activities of neurons in the central nervous systems and cardiac and smooth muscles in the visceral organs are depressed by inhibitory postsynaptic/junctional potentials appearing on the neurons and muscles (Hutter and Trautwein 1956; Bülbring and Kuriyama 1963; Eccles 1964, 1973). The depression of depolarizing responses in taste cells by a slow HP resembles the inhibition of depolarizing responses in neurons and muscle cells by inhibitory postsynaptic/junctional potentials.

In the present study, ES at 30 Hz of cut parasympathetic efferent fibers of the frog depressed by ~40% 1 M NaCl- and 1 mM acetic acid–induced receptor potentials but completely depressed 1 0 mM Q-HCl– and 1 M sucrose-induced receptor potentials. The large reduction of Q-HCl– and sucrose-induced responses is due to the smaller amplitudes of receptor potentials evoked by Q-HCl and sucrose stimuli. These results suggest that gustatory neural responses induced by NaCl, acetic acid, Q-HCl, and sucrose may be greatly reduced by selectively stimulating PSN efferent fibers in the GP nerve. It has been suggested that some sweet amino acids such as D-phenylalanine and some bitter substances such as denatonium increase intracellular inositol triphosphate (IP_3) and release Ca^{2+} from the internal stores in mammalian taste cells and that the increased intracellular Ca^{2+} directly releases a transmitter from taste cells without generating a depolarization in the cells (Sato et al. 1994; Uchida and Sato 1997). If similar phenomena occur in frog taste cells, the gustatory neural responses for some nonsugar and bitter stimuli may not be depressed by stimulation of PSN efferent fibers in GP nerve.

As the amplitude of depolarizing receptor potentials is increased, the size of slow HPs elicited from the depolarized level by PSN stimulation gradually reduced. The estimated reversal potentials of slow HPs are approximately −1 mV when the membrane potential of taste cell is altered by NaCl- and acetic acid–induced receptor potentials but are approximately −14 mV when the membrane potential is altered by Q-HCl– and sucrose-induced receptor potentials. The 2 values are quite different. When the membrane potentials are altered only by pure electrical currents, the reversal potential of slow HP is −13 mV (Sato et al. 2002). This value is similar to the reversal point of slow HP obtained under Q-HCl and sucrose responses but is quite different from the reversal point of slow HP under NaCl and acetic acid responses.

As mechanisms underlying generation of receptor potentials in frog taste cells, we have proposed that cation channels in the apical receptive membrane of the taste cells are opened in NaCl and acid transductions (Miyamoto et al. 1988, 1993; Okada et al. 1994; Sato et al. 1995). The slow HPs in frog taste cells induced by PSN stimulation are generated by closing cation channels permeable to Na^+ and K^+ existing in the subsynaptic membrane of the proximal processes of taste cells (Sato et al. 2002). Although the space constant of taste cells are relatively small (Ewald and Roper 1992), cross talk will occur between a depolarizing receptor current induced in the apical receptive membrane of taste cell by taste stimulation and a slow hyperpolarizing current induced in the proximal membrane of the cell by PSN stimulation. The part of receptor currents in frog taste cells induced by NaCl and acid stimulations is carried by Na^+ (Miyamoto et al. 1988, 1993; Okada et al. 1994). Therefore, the reversal

potential of PSN-induced slow HP under NaCl and acid stimulations is likely to be shifted to the direction of the equilibrium potential of Na^+ as −1 mV (Table 1). As mechanisms of generation of the receptor potentials for Q-HCl and sucrose stimuli, we have proposed that a Cl^- pump at the receptive membrane is involved in Q-HCl transduction (Okada et al. 1988) and a H^+ entry from the apical receptive membrane is involved in sugar transduction (Okada et al. 1992). Because Na^+ and K^+ currents are not probably involved in a generation of the receptor potentials for Q-HCl and sucrose stimulations, the reversal potential (−14 mV) of PSN-induced slow HPs under Q-HCl and sucrose stimulations is equivalent to the reversal potential (−13 mV) of the slow HPs measured by intracellularly injected electrical currents. In conclusion, the percentage of depression of the depolarizing receptor potentials in frog taste cells by PSN-induced slow HP is larger under NaCl and acid stimulations than under Q-HCl and sucrose stimulations. This comes from a difference in reversal points of slow HPs when frog taste cells are stimulated with the 2 groups of taste stimuli.

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