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Analysis of Slow Depolarizing Potential in Frog Taste Cell Induced by Parasympathetic Efferent Stimulation under Hypoxia

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Abstract

Strong electrical stimulation (ES) of the frog glossopharyngeal (GP) efferent nerve induced slow depolarizing potentials (DPs) in taste cells under hypoxia. This study aimed to elucidate whether the slow DPs were postsynaptically induced in taste cells. After a block of parasympathetic nerve (PSN) ganglia by tubocurarine, ES of GP nerve never induced slow DPs in the taste cells, so slow DPs were induced by PSN. When Ca^{2+} in the blood plasma under hypoxia was decreased to ~0.5 mM, the slow DPs reduced in amplitude and lengthened in latency. Increasing the normal Ca^{2+} to ~20 mM increased the amplitude of slow DPs and shortened the latency. Addition of Cd^{2+} to the plasma greatly reduced the amplitude of slow DPs and lengthened the latency. These data suggest that the slow DPs depend on Ca^{2+} and Cd^{2+} concentration at the presynaptic PSN terminals of taste disk. Antagonists, [D-Arg[1], D-Trp[7,9], Leu[11]]-substance P and L-703 606, of neurotransmitter substance P neurokinin$_1$ receptor completely blocked the slow DPs. Intravenous application of substance P induced a DP of ~7 mV and a reduction of membrane resistance of ~48% in taste cells. A nonselective cation channel antagonist, flufenamic acid, completely blocked the slow DPs. These findings suggest that the slow DPs are postsynaptically initiated in frog taste cells under hypoxia by opening nonselective cation channels on the postsynaptic membrane after substance P is probably released from the presynaptic PSN axon terminals.

Key words: frog taste cell, gustatory efferent synapse, hypoxia, presynaptic modulation, slow depolarizing potential

Introduction

The dorsal tongue surface in frogs has a great number of fungiform papillae in which single taste disks are located at the tops. The taste disk is innervated by somatosensory and gustatory afferent fibers and parasympathetic nerve (PSN) efferent fibers (Sato 1976; Sato et al. 1983, 2005; Inoue et al. 1992). Electron-microscopic studies have suggested that taste receptor cells have both afferent and efferent synapses (Nomura et al. 1975; Witt 1993; Yoshie et al. 1996). Electrophysiological studies revealed that electrical stimulation (ES) of glossopharyngeal (GP) efferent fibers induces a slow postsynaptic potential in frog taste cells (Sato et al. 2002, 2004, 2005), and the taste cell responses induced by gustatory stimuli are modulated by the slow postsynaptic potential (Sato et al. 2005).

Under a normal velocity of capillary blood flow (1–1.5 mm/s) in the tongue, ES of the PSN efferent fibers in the GP nerve of frogs induces slow hyperpolarizing potentials (HPs) alone in taste cells, which show the biophysical properties of slow postsynaptic potentials (Sato et al. 2004, 2005). Our studies (Sato et al. 2002, 2004) suggest that the slow HPs in frog taste cells are induced by closing nonselective cation channels on the proximal membrane of taste cells following a release of substance P from the presynaptic axon terminals. At gustatory disks of the frog fungiform papillae, PSN efferent fibers might make synaptic contacts with the proximal processes of taste cells (Sato et al. 2005). Because the activity of the efferent axon terminals produces a slow HP in taste cells as presynaptic cells, presynaptic facilitation (Mendell and Wall 1964; Ganong 2003) will occur at gustatory afferent synaptic transmission (Sato et al. 2006).

When the velocity of capillary blood flow in the frog tongue is lowered at <0.2 mm/s, venous O_2 tension (P_{O_2}) is estimated to be <4.5 mmHg (<20% of maximum) (Prosser and Brown 1965; Wilson 1979). In this situation, PSN stimulation induces a slow depolarizing potential (DP) alone in frog taste cells. This is a kind of slow postsynaptic potential (Sato et al. 2002). Since PSN-induced slow DPs appear in taste cells via an efferent synapse on the taste cells, presynaptic inhibition (Eccles 1973; Ganong 2003) will occur at gustatory afferent synaptic transmission.

As arterial P_{O_2} and blood pressure are decreased, the tissue respiration is gradually reduced and thereby tissue activities are damaged by O_2 deficiency. With decreasing P_{O_2} in blood, most of the O_2-sensitive ion channels are gradually inhibited to reduce the tissue activity, resulting in avoiding loss of metabolic energies under hypoxia (López-Barneo et al. 2001). It is tempting that the physiological properties and generative mechanism of slow DPs in frog taste cells are clarified for understanding switching mechanism between slow HP and slow DP under different P_{O_2} levels.

The main objective of the present study is to elucidate whether slow DPs are postsynaptically induced in frog taste cells by PSN stimulation under hypoxia. We examined the effects of 1) Ca^{2+} and Cd^{2+} and 2) neurotransmitter receptor antagonists and agonists on the slow DPs. The results suggest that the slow PDs are postsynaptically induced in frog taste cells under hypoxia by substance P probably released from the PSN terminals in the taste disk.

Materials and methods

Preparation, recording, and stimulation

All experiments were performed under the Guideline for Animal Experimentation of Nagasaki University. The experiments were carried out with the whole body in bullfrogs (*Rana catesbeiana*) anesthetized with 50% urethane solution at a dose of 1–3 g/kg body weight (b. w.). The methods of making experimental preparations, electrical recording from taste cells, and ES of the GP nerve were the same as previously mentioned (Sato et al. 2002, 2004, 2005). In briefly, changes in the membrane potential of taste cells in the fungiform papillae were intracellularly recorded with a microelectrode filled with 3 M KCl. The distal part of cut GP nerve was electrically stimulated at 30 Hz with pulses of 0.1-ms duration and 15-V strength to obtain the maximal slow DPs from a taste cell. The microelectrode was always advanced into the lower portion of intermediate layer of the taste disk to impale taste receptor cells. In order to avoid penetrating a basal cell, the microelectrode was advanced into the central area of the taste disks. The criteria of taste cell penetration were appearance of 3-step potential changes in the membrane potentials of the taste cells (Sato et al. 2005, 2006). Input resistance of taste cells was measued with a bridge by passing a constant hyperpolarizing current at 1 Hz into an impaled taste cell. All experiments were carried out at room temperature (23 °C–26 °C).

Drugs

$CaCl_2$, $CdCl_2$, ethylene glycol-bis(2-aminoethyl-ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), prazosin hydrochloride, DL-propranolol hydrochloride, (±)-SKF-83566 hydrochloride, metergoline, tropine 3,5-dichlorobenzoate, spiperone, calcitonin gene–related peptide (CGRP) fragment 8–37, vasoactive intestinal peptide (VIP) fragment 6–28, [D-Arg^{1}, D-Trp^{7,9}, Leu^{11}]-substance P acetate salt, L-703 606 oxalate salt, substance P acetate salt, flufenamic acid, tubocurarine chloride, and atropine sulfate salt were used. All drugs were purchased from Sigma–Aldrich Co (St Louis, MO).

The synaptic regions of taste cells in the taste disks were perfused via abundant capillary vessels in the fungiform papillae (Jaeger and Hillman 1976) by intravenously (i. v.) injecting a frog Ringer solution containing pharmacological drugs. The amount of injected Ringer solution was 2 ml/kg b. w. To check that an injected drug may reach synaptic area of taste disks, 4% methylene blue solution was i. v. injected at 4 ml/kg b. w. Taste disks of all the fungiform papillae in the tongue were stained blue within 1 min after the injection. Blue-stained nerve fiber terminals within the taste disks were found by inspecting the isolated fungiform papillae with a light microscope (400×). This suggests that i. v. injected drug easily reaches gustatory synaptic area. Ca^{2+} concentrations in the blood plasma were decreased by injecting EGTA at 23.9 mg/kg b. w. and increased by injecting $CaCl_2$ at 116 mg/kg b. w. Estimated low and high Ca^{2+} concentrations in the plasma were ~0.5 and ~20 mM, respectively, which were calculated from the amount of injected drugs and the plasma volume of frogs (4% of the b. w.) (Thorson 1964). Normal Ca^{2+} in the frog plasma is ~2 mM (Wilson 1979).

Experimental procedure

Strong repetitive ES of GP nerve produces a large slow potential on the lingual surface and taste disk cells. This derives from the physicochemical junction potential generating between a secreted saliva from lingual glands and a lingual surface solution (Sato et al. 2000). The junction potential disturbs an analysis of physiological slow DPs in taste cells. Therefore, soon after anesthesia of animal with a urethane-Ringer solution, atropine sulfate was injected i. v. at 1 mg/kg b. w. to completely block the junction potential. The injection effect lasted for >7 h.

The PSN fibers in the GP nerve innervate the taste disk of the frog fungiform papillae, and ES of the fibers induces slow HPs alone in frog taste cells under the normal velocity of capillary blood flow (1–1.5 mm/s) along the fungiform papillae (Inoue et al. 1992; Sato et al. 2002, 2004). On the other hand, when the velocity of capillary blood flow in the frog tongue lowered at <0.2 mm/s 3–5 h after a moderate anesthesia with urethane, ES of GP nerve induces slow DPs alone in frog taste cells under hypoxia (Sato et al. 2002). All the experiments were carried out under the capillary blood flow rate at <0.2 mm/s.

Comparison of control and test slow DPs was performed using the data obtained from 2 different taste cell groups of the same individual. The control data were collected from 10–30 taste cells for 90 min before a drug injection, and the test response data were collected from 10 to 30 taste cells for 90 min after a 30-min circulation of an injected drug.

Statistics

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

Results

Effect of block of PSN ganglia on slow DP

Slow DPs were observed in 169 of 195 taste cells (87%) tested in six frogs. No other types of taste cell response were observed in the remaining 26 cells. In the frog venous blood, P_{O_2} is estimated to be ~23 mmHg under a normal velocity of capillary blood flow and ~4.5 mmHg under a lowered velocity of capillary blood flow at 0.2 mm/s (Prosser and Brown 1965; Wilson 1979).

We examined whether ES of GP nerve elicits a slow DP in taste cells after the PSN ganglia (psng in Figure 1A) in the tongue under hypoxia were blocked by tubocurarine (1 mg/kg b. w.). Before the drug injection, a slow DP of ~5 mV accompanied by a decrease in the membrane resistance was induced in a taste cell by ES of GP nerve (Figure 1B [eft]). However, as shown in Figure 1B (right) and C, after the drug injection, no slow DPs were evoked in taste cells by ES of the GP nerve containing the sympathetic postganglionic and parasympathetic preganglionic fibers. This strongly suggests that slow DPs in taste cells are evoked only by PSN efferent fibers in the GP nerve but not by sympathetic efferent fibers.

Effect of Ca^{2+} concentration on slow DP

It is well known that a release of neurotransmitters is triggered by Ca^{2+} influx at the presynaptic axon terminals and modulated depending on Ca^{2+} concentration at the terminals (Katz and Miledi 1967). To test this possibility in generating slow DPs in frog taste cells, Ca^{2+} concentration in the taste disks was altered by changing the amount of Ca^{2+} in the blood plasma. As shown in Figure 2A, the amplitude of slow DPs in taste cells was significantly smaller ($P < 0.05$, n = 16–18) when Ca^{2+} concentration in the plasma was lowered from a normal level (~2 mM) to a low level (~0.5 mM) but was significantly higher ($P < 0.05$, n = 14–18) when Ca^{2+} was increased to a high level (~20 mM). The latency of slow DPs was significantly longer at a low Ca^{2+} level than at a normal Ca^{2+} level ($P < 0.05$, n = 15–16) but was significantly shorter at a high Ca^{2+} level than at a normal Ca^{2+} level ($P < 0.05$, n = 12–15) (Figure 2B). In this experiment, when Ca^{2+} concentrations were low, normal, and high, the input resistance of taste cells at rest was 48 ± 11 MΩ (n = 16), 53 ± 13 MΩ (n = 15), and 56 ± 8 MΩ (n = 14), respectively. No differences were found among the 3 values ($P > 0.05$, n = 14–16). Because the intracellular recording condition in taste cells was stable, the data in Figure 2 were physiological.

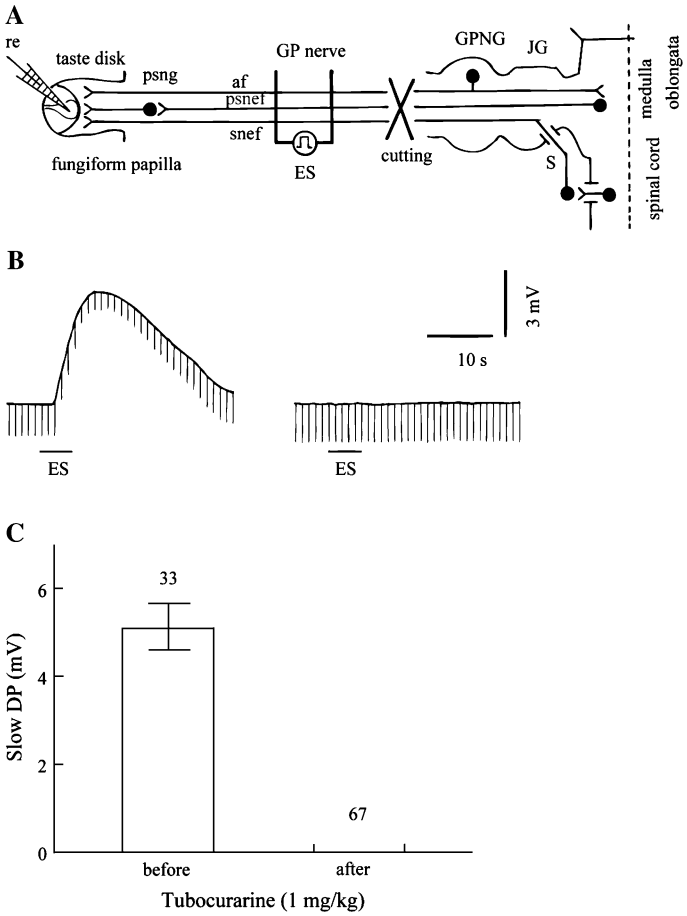

Figure 1 Effect of block of PSN ganglia in lingual branch of GP nerve on slow DPs. **(A)** Schematic diagram of afferent and efferent fibers in papillary nerve and lingual branch of GP nerve and positions of stimulating and recording electrodes. re, Recording microelectrode; psng, PSN ganglion; af, afferent fiber; psnef, PSN efferent fiber; snef, sympathetic nerve efferent fiber; GPNG, GP nerve ganglion; JG, jugular ganglion; S, sympathetic nerve trunk. GP nerve was transected in all experiments, and distal end of the transected nerve was stimulated. **(B)** Left, slow DP in taste cell induced by ES of GP nerve (30 Hz pulses of 0.1-ms duration and 15-V strength for 5 s in this and other figures) before i. v. injection of tubocurarine. Right, no response in taste cell by ES of GP nerve after i. v. injection of tubocurarine at 1 mg/kg b. w. To measure input resistance of a taste cell, HP pulses induced by constant hyperpolarizing currents were superimposed on membrane potential. Resting potential was −32 mV (left) and −29 mV (right). **(C)** Mean amplitude of slow DPs in taste cells induced by GP nerve stimulation before (left) and after (right) tubocurarine injection (1 mg/kg). Vertical bars are SEMs, and numerals near the bars are number of taste cells sampled in this and other figures. Resting potential was −31 ± 1 mV (n = 33) (before) and −32 ± 1 mV (n = 67) (after).

The amplitude and latency of postsynaptic potentials fluctuate while they are repeatedly generated (Katz and Miledi 1965). To confirm such a phenomenon, slow DPs were repeatedly elicited in single taste cells by stimulating the GP nerve at 1/min. Figure 3A shows examples of a fluctuation of slow DPs under 3 different Ca^{2+} concentrations. The amplitude and latency of slow DPs broadly distributed at a low Ca^{2+} level than at a normal Ca^{2+} level but narrowly

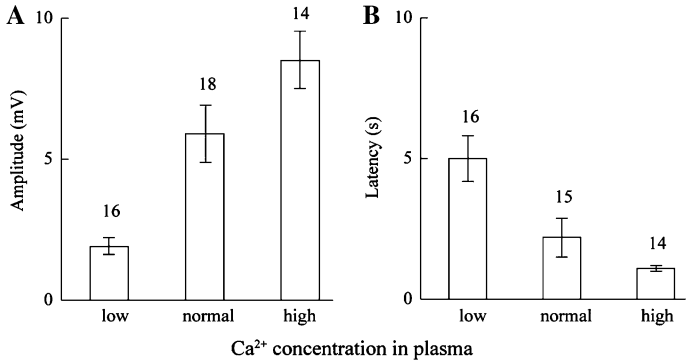

Figure 2 Effects of Ca^{2+} concentration in blood plasma on amplitude and latency of slow DPs in taste cells induced by PSN stimulation. **(A)** Amplitude of slow DPs in taste cells at low (~0.5 mM), normal (~2 mM), and high (~20 mM) Ca^{2+} concentrations. Resting potential was −31 ± 1 mV (n = 48). **(B)** Latency of slow DPs in taste cells at low, normal, and high Ca^{2+} concentrations. Resting potential was −30 ± 1 mV (n = 43).

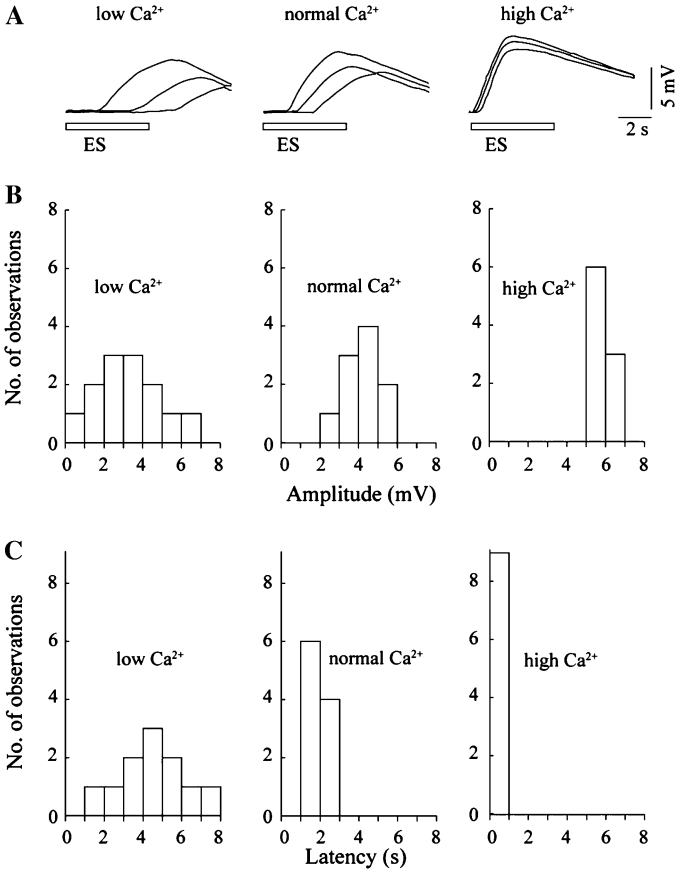

Figure 3 Fluctuation of amplitude and latency of slow DPs in single taste cells induced by PSN stimulation. **(A)** Records of slow DPs in 3 taste cells induced by ES of PSN at 1/min at low (~0.5 mM), normal (~2 mM), and high (~20 mM) Ca^{2+} concentrations. Resting potential was −29 to 2 mV. **(B)** Histogram of amplitudes of slow DPs at low, normal, and high Ca^{2+} concentrations. Each histogram was obtained from different taste cells. Resting potential was −30 to 32 mV. **(C)** Histogram of latencies of slow DPs at low, normal, and high Ca^{2+} concentrations. Each histogram was from different taste cells. Resting potential was −28 to 31 mV.

distributed at a high Ca^{2+} level than at a normal Ca^{2+} level (Figure 3B and C). To statistically examine the difference in fluctuation of amplitude and latency among slow DPs obtained at different Ca^{2+} levels, the mean amplitude and latency of slow DPs in each taste cell repeatedly stimulated were respectively normalized as 100, and the standard deviations (SDs) were compared as an index of fluctuation. SDs in amplitude of slow DPs were 53 ± 4 (SEM) (n = 10) at the low, 23 ± 2 (n = 6) at the normal, and 11 ± 2 (n = 5) at the high Ca^{2+} concentration. On the other hand, SDs in latency were 50 ± 5 (n = 7) at the low, 20 ± 2 (n = 6) at the normal, and 13 ± 2 (n = 5) at the high Ca^{2+} concentration. There were significant differences between any pairs of the 3 SDs in either amplitude or latency of the slow DPs ($P < 0.05$, n = 5–10). This indicates that the fluctuation in amplitude and latency of slow DPs becomes larger with decreasing Ca^{2+} concentration at the PSN axon terminals of the taste disks.

Effect of Cd^{2+} on slow DP

Cd^{2+} is a nonselective blocker of voltage-gated Ca^{2+} channels (Randall 1998). We tested the effect of i. v. injected Cd^{2+} on the slow DPs in taste cells induced by PSN stimulation. The amplitude of slow DPs gradually decreased with increasing concentrations of injected Cd^{2+} (Figure 4). The dose-response curve was obtained from a Hill plot. When cadmium chloride was injected at 1 mg/kg b. w., the concentration of Cd^{2+} in the blood plasma was estimated to be 130 μM from the amount of injected drug solution and 4% plasma volume of b. w. The IC_{50} (intermediate concentration for half an amplitude) of $CdCl_2$ for slow DPs was 0.01 mg/kg (~1.3 μM Cd^{2+}). The latency of slow DPs increased from 2.1 ± 0.3 s (n = 19) in the control to 6.3 ± 0.5 s ($P < 0.05$, n = 18) under $CdCl_2$ at 1 mg/kg.

Effect of neurotransmitter receptor antagonists on slow DP

First, effects of 6 antagonists for biogenic amine receptors on the PSN-induced slow DPs were tested. Prazosin (a noradrenaline α_1 blocker), propranolol (a noradrenaline β blocker), metergoline (a blocker of $5HT_1$ and $5HT_2$), tropine 3,5-dichlorobenzoate (a selective blocker of $5HT_3$), SKF-83566 (a selective D_1 blocker), and spiperone (a selective D_2 blocker) were all injected i. v. at 1 mg/kg b. w. No antagonists tested had any effects on slow DPs in taste cells ($P > 0.1$, n = 13–31) (Figure 5A).

Second, because the nerve fibers containing VIP, CGRP, and substance P have been found in frog taste disks (Hirata and Kanaseki 1987; Kuramoto 1988; Kusakabe et al. 1996), effects of antagonists of these peptides' receptors were tested on the slow DPs in taste cells. I. v. injected drugs were VIP

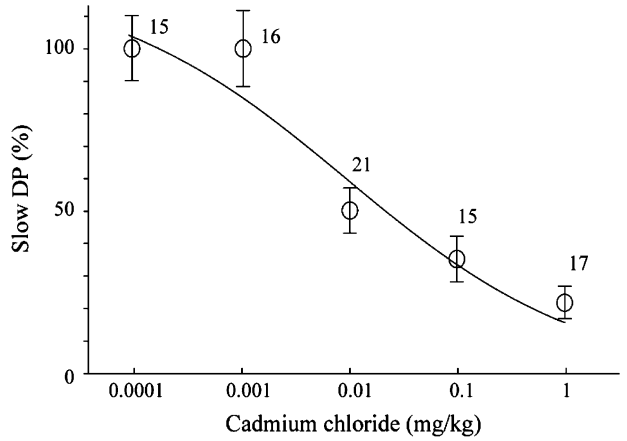

Figure 4 Effect of Cd^{2+} on amplitude of slow DPs in taste cells. $CdCl_2$ was injected i. v. at doses of 0.0001–1 mg/kg b. w. PSN efferent fibers in GP nerve were strongly stimulated at 30 Hz. Control amplitude of slow DPs before $CdCl_2$ injection (n = 20) was taken as 100%, and test amplitudes after the injection were relative to control. Resting potentials under injection at various doses of $CdCl_2$ were −31 ± 1 mV (n = 20) (control), −31 ± 1 mV (n = 15) (0.0001 mg/kg), −30 ± 2 mV (n = 16) (0.001 mg/kg), −32 ± 1 mV (n = 21) (0.01 mg/kg), −30 ± 1 mV (n = 15) (0.1 mg/kg), and −32 ± 1 mV (n = 17) (1 mg/kg).

fragment 6–28 (a potent VIP receptor blocker), CGRP fragment 8–37 (a selective CGRP receptor blocker), [D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P (a substance P neurokinin$_1$ [NK$_1$] receptor blocker), and L-703 606 (a substance P NK$_1$ receptor blocker). Neither VIP nor CGRP receptor antagonists affected the slow DPs in taste cells (P > 0.1, n = 15–20). Two types of substance P receptor antagonist reduced slow DPs (Figure 5B). Both L-703 606 at 3 mg/kg b. w. and [D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P at 2 mg/kg b. w. almost completely blocked the slow DPs (L-703 606: 1 ± 0%, P < 0.01, n = 15–17; [D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P: 0 ± 0%, P < 0.01, n = 18–20). These data suggest a neurotransmitter candidate for efferent synapses in frog taste cells is substance P.

DP induced by substance P

If substance P is released from the PSN axon terminals of the taste disk by excitation of the terminals, i. v. application of substance P will produce slow DPs in the taste cells. To test this possibility, changes in the membrane potential and the membrane resistance of taste cells were measured before and after the injection of substance P at 2 mg/kg b. w. (Figure 6).

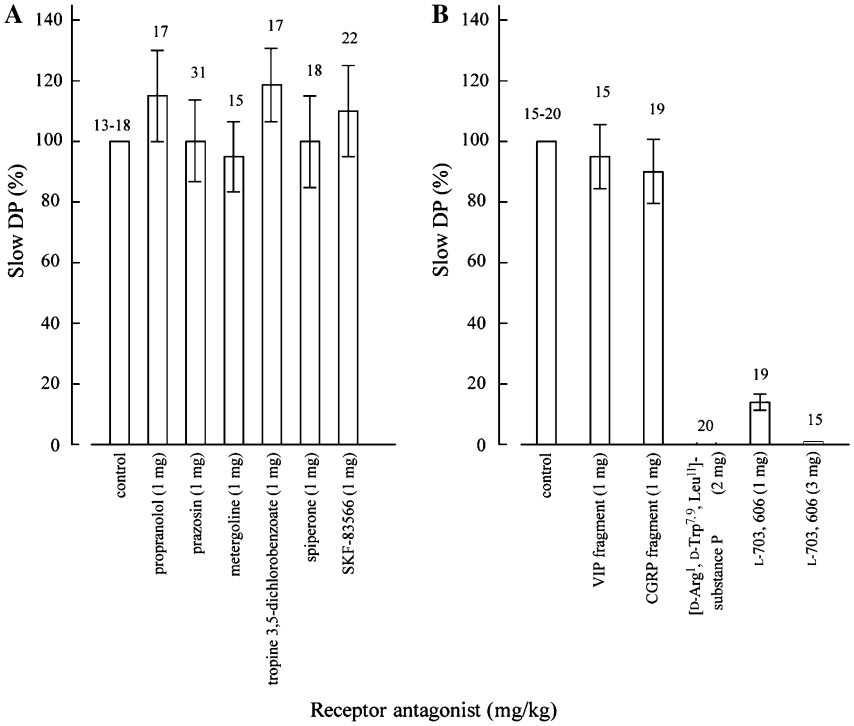

Figure 5 Effects of i. v. injection of various neurotransmitter receptor antagonists on slow DPs in taste cells. **(A)** Effects of i. v. injection of 6 biogenic amine receptor antagonists at 1 mg/kg b.w. Resting potentials were −30 ± 0 mV (n = 90) (control), −31 ± 1 mV (n = 17) (propranolol), −32 ± 1 mV (n = 31) (prazosin), −29 ± 2 mV (n = 15) (metergoline), −29 ± 2 mV (n = 17) (tropine 3,5-dichlorobenzoate), −32 ± 1 mV (n = 18) (spiperone), and −30 ± 1 mV (n = 22) (SKF-83566). **(B)** Effects of i. v. injection at 1–3 mg/kg b.w. of 3 peptide receptor antagonists. In both graphs, control amplitude before drug injection was taken as 100%. Resting potentials were −30 ± 1 mV (n = 108) (control), −30 ± 1 mV (n = 15) (VIP fragment), −30 ± 1 mV (n = 19) (CGRP fragment), −29 ± 1 mV (n = 20) ([D-Arg^1, D-Trp^{7,9}, Leu^{11}]-substance P), −32 ± 2 mV (n = 19) (L-703 606) and −30 ± 2 mV (n = 15) (L-703 606).

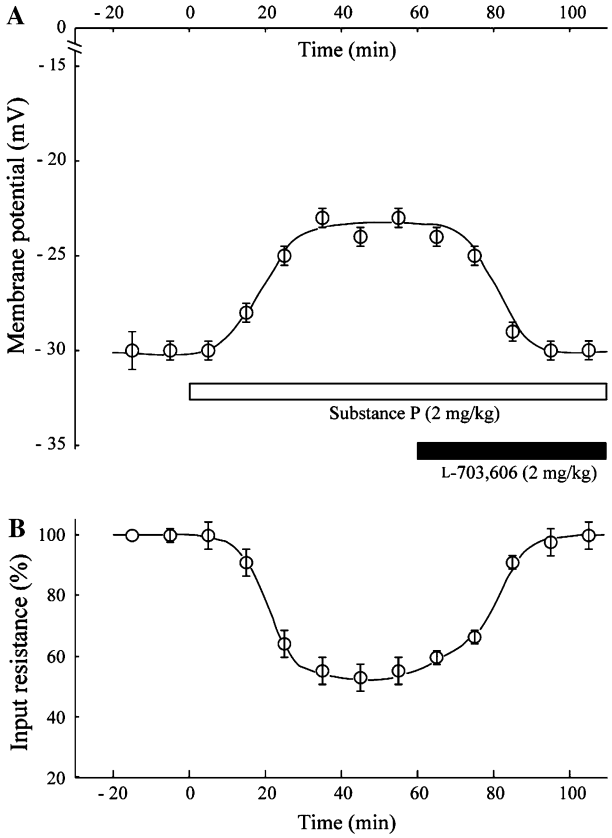

Figure 6 Change in membrane potential and input resistance of taste cells induced by i. v. injection of substance P. **(A)** Amplitude of DPs of taste cells by substance P (2 mg/kg b. w.) and the recovery by L-703 606 (2 mg/kg b. w.). Resting potential was −30 ± 1 mV (n = 30). **(B)** Decrease of input resistance of taste cells by substance P and the recovery by L-703 606. At time 0, substance P was injected i. v., and at time 60 min, L-703 606 was injected i. v. Intracellular recordings were obtained from 10–16 taste cells during each 10 min. Each experimental point is mean ± SEM from these cells. All data were from one frog.

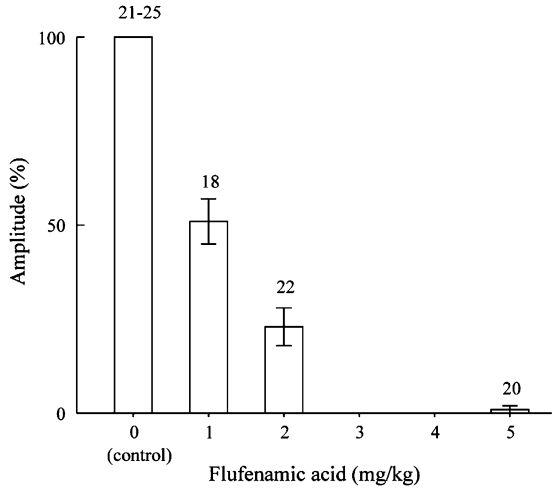

Figure 7 Effects of i. v. injection of flufenamic acid on slow DPs in taste cells. Control amplitude was taken as 100%, and test amplitudes were relative to control. Resting potentials under injection at various doses of flufenamic acid were −32 ± 1 mV (n = 46) (control), −30 ± 1 mV (n = 18) (1 mg/kg), −31 ± 2 mV (n = 22) (2 mg/kg), and −29 ± 1 mV (n = 20) (5 mg/kg).

Resting potentials of taste cells in this preparation were –30 ± 1 mV (n = 30) before the injection. The membrane potentials gradually decreased and the amplitude of DPs reached 7 ± 2 mV (n = 12) 30 min after the injection (Figure 6A). After an i. v. injection of L-703 606 at 2 mg/kg b. w. DPs gradually returned to the resting potential level of −30 ± 1 mV (n = 26). In parallel with the changes in the membrane potentials, the control input resistance taken as 100% (n = 15) gradually decreased and reached 52 ± 5% (n = 12) (Figure 6B). After the L-703 606 injection, the decreased membrane resistance gradually returned to the control input resistance. The maximal change in the membrane resistance of taste cells was 48% of the control during i. v. application of substance P.

Effect of flufenamic acid on slow DP

Our previous work (Sato et al. 2002) suggested that the reversal potential of GP efferent–induced slow DPs in frog taste cells is approximately –11 mV, and slow DPs are generated by opening nonselective cation channels permeable to Na^+ and K^+ on the proximal process of taste cells. We tested the effects of a potent blocker of nonselective cation channels on slow DPs in taste cells. Slow DPs were blocked dose dependently by i. v. injection of flufenamic acid (Figure 7). Flufenamic acid of 5 mg/kg b. w. completely blocked slow DPs ($P < 0.01$, n = 20–24). This indicates that the slow DPs in taste cells are elicited by flufenamic acid–sensitive nonselective cation channels on taste cell membrane.

Discussion

At chemical synaptic transmission, activation of voltage-gated Ca^{2+} channels at the presynaptic axon terminals releases neurotransmitters from the terminals. The neurotransmitters bind to receptors on the postsynaptic membrane and generate a postsynaptic potential by activating ion channels at the postsynaptic cell (Shepherd 1994). In the present experiments, changing Ca^{2+} concentration in the plasma and adding Cd^{2+} to the plasma greatly affected the amplitude and latency of slow DPs in frog taste cells. This suggests that Ca^{2+} channels at the presynaptic axon terminals of frog taste disks are activated in generating slow DPs in taste cells. The IC_{50} of Cd^{2+} for the slow DPs in frog taste cells was as low as 1.3 μM. Cd^{2+} is a potent blocker of voltage-gated Ca^{2+} channels in various tissues, and IC_{50} of Cd^{2+} for the channels is ~1 μM (Randall 1998), which is almost the same as that obtained in the present study. Because the sensitivity of Cd^{2+} to nonselective cation channels is very weak (Wallnöfer et al. 1989), Cd^{2+}-dependent inhibition of slow DPs in taste cells is likely to be derived from a block of voltage-gated Ca^{2+} channels at the presynaptic axon terminals of taste disk.

Of antagonists tested for blocking 9 receptors of biogenic amines and peptides, only 2 types of antagonist, [D-Arg^{1}, D-Trp^{7,9}, Leu^{11}]-substance P and L-703 606, for substance P NK_1 receptor completely blocked the slow DPs in taste cells induced by PSN stimulation. The i. v. injection of substance P also elicited DPs and increased the membrane conductance in taste cells. Substance P is immunohistochemically found in the presynaptic axon terminals within the taste disks of the frog fungiform papillae (Hirata and Kanaseki 1987; Kuramoto 1988, Kusakabe et al. 1996). These findings suggest that a neurotransmitter candidate released from the presynaptic terminals of PSN in the taste disks is substance P.

Our previous studies (Sato et al. 2002) suggested that GP nerve–induced slow DPs are due to the opening of nonselective cation channels permeable to Na^+ and K^+ on the frog taste cell membrane. Nonselective cation channels of 30 pS are present in the proximal processes of frog taste cells (Fujiyama et al. 1993; Sato et al. 1995). In the present work, a potent blocker, fulfenamic acid, of nonselective cation channels on the postsynaptic membrane of taste cells at the gustatory efferent synapse completely blocked slow DPs. Because flufenamic acid is relatively insensitive to the voltage-gated Ca^{2+} channels in the presynaptic axon terminals (Hescheler and Schultz 1993), the slow DPs induced in taste cells are very likely to be blocked by binding flufenamic acid to the nonselective cation channels on the postsynaptic membrane.

The PSN efferent fibers innervating frog taste cells generate either the slow HPs accompanied by membrane conductance decrease under normoxia or the slow DPs accompanied by membrane conductance increase under hypoxia (Sato et al. 2002). Our studies indicate that both slow HPs and slow DPs have the same reversal potential of approximately −12 mV, are activated by substance P as a neurotransmitter candidate, and are induced by closing or opening the nonselective cation channels on the proximal processes of taste cells (Sato et al. 2002, 2004). However, it is not clear whether the same taste cells in the frog taste disk elicit both slow HPs under normoxia and slow DPs under hypoxia because a microelectrode inserted into a taste cell cannot maintain the stable intracellular recording for a long time. Slow HPs are obtained from ~85% of the whole taste cells tested under normoxia (Sato et al. 2004), suggesting that the remaining ~15% in taste cells which do not respond to PSN stimulation may not be innervated by the PSN efferent fibers or premature cells. In the present experiments, slow DPs are also obtained from ~85% of tested taste cells. These data do not support the possibility that a subset of taste cells in the frog taste disk evokes slow HPs under normoxia and another subset of taste cells evokes slow DPs under hypoxia. Another possible explanation is that almost every taste cell can generate both slow HPs in normoxia (frog venous P_{O_2}: ~23 mmHg [Prosser and Brown 1965; Wilson 1979]) and slow DPs in hypoxia ($P_{O_2} < 4.5$ mmHg). What mechanism is involved in switching from slow HPs to slow DPs or vice versa? The exact mechanism is unknown. It is well known that when living organisms are encountered from normoxia to hypoxia, many types of O_2-sensitive ion channels in organs are inactivated in various degrees to adapt the activity of the living organisms to hypoxia (López-Barneo et al. 2001). When activities of the frog's whole body are adapted to hypoxic situation, switching from slow HPs to slow DPs in taste cells in response to GP nerve stimulation is reasonable for decreasing gustatory responses under a lowered lingual tissue respiration. At gustatory afferent synaptic transmission, slow DPs in a presynaptic taste cell induced by PSN may exert the presynaptic inhibition (Eccles 1973; Ganong 2003), resulting in a decrease in gustatory neural responses. This can keep gustatory activity lower during reduced formation of adenosine triphosphate in the mitocondoria. It is supposed that after G protein–coupled substance P NK_1 receptor (Otsuka and Yoshioka 1993; Bloom 1996) binds substance P under normoxia, nonselective cation channels on the taste cell are closed by phosphorylation via sequential events of cell-signal tansduction molecules (Sato et al. 2004). This may result in a generation of slow HPs in taste cells. Furthermore, one would suppose that the nonselective cation channels are opened by hypoxia-induced conformational changes in substance P NK_1 receptors, cell-signal transduction molecules, or cation channels, resulting in a generation of the slow DPs accompanied by an increase in the membrane conductance.

It is concluded that PSN-induced slow DPs in frog taste cells under hypoxia are generated by opening the nonselective cation channels on the taste cells following a possible release of substance P from the presynaptic PSN axon terminals in taste disk and that generation of slow DPs under hypoxia is necessary for adapting the gustatory system to hypoxia.

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