Chem. Senses 29: 651–657, 2004 doi:10.1093/chemse/bjh072

Analysis of Slow Hyperpolarizing Potentials in Frog Taste Cells Induced by Glossopharyngeal Nerve Stimulation

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Abstract

Electrical stimulation of the frog glossopharyngeal (GP) nerve evoked slow hyperpolarizing potentials (HPs) in taste cells. This study aimed to clarify whether slow HPs were postsynaptically induced in taste cells. The slow HPs were recorded intracellularly with a microelectrode. When Ca^{2+} concentration in the blood plasma was decreased to ~0.5 mM, the amplitude of slow HPs reduced and their latency lengthened. When the Ca^{2+} concentration was increased to ~20 mM, the amplitude of slow HPs increased and their latency shortened. Addition of Cd^{2+} to the plasma greatly reduced the amplitude of slow HPs and lengthened their latency. These data suggest that the slow HPs are dependent on presynaptic activities in the GP nerve terminals in the taste disk. Of various antagonists injected intravenously for blocking receptors of neurotransmitter biogenic amines and peptides, only antagonists for substance P blocked the slow HPs at 2–4 mg/kg body wt. Application of substance P of 2 mg/kg to the plasma induced hyperpolarizing responses in taste cells, whose amplitude was the same as that of the slow HPs induced by GP nerve stimulation. Application of a nonselective cation channel antagonist, flufenamic acid, to the plasma blocked the slow HPs. These results suggest that the slow HPs are generated by closing the nonselective cation channels in the postsynaptic membrane of taste cells following possible release of substance P from the GP nerve terminals in the taste disk.

Key words: flufenamic acid, frog taste cell, gustatory efferent synapse, slow hyperpolarizing potential, substance P

Introduction

Sensory hair cells in the auditory organ (Nakajima and Wang, 1974), the vestibular organ (Hillman, 1969) and the lateral-line organ (Hama, 1965) are innervated by both afferent and efferent nerve fibers. The former convey sensory information from the sensory cells and the latter control the sensitivity of the cells (Fex, 1962; Llinás and Precht, 1969; Furukawa, 1981). Afferent innervation of gustatory receptor cells has extensively been studied morphologically and physiologically (Murray, 1973; Lindemann, 1996). Efferent innervation of the gustatory receptor cells has been suggested by electron-microscopical studies (Nomura *et al.*, 1975; Yoshie *et al.*, 1996; Reutter *et al.*, 1997). Electrophysiological studies have suggested that receptor potentials in the frog taste cells are modulated by the glossopharyngeal (GP) nerve stimulation (Sato *et al.*, 2001). Our previous study (Sato *et al.*, 2002) showed that slow hyperpolarizing potentials (HPs) and slow depolarizing potentials (DPs) are elicited in frog taste cells by GP nerve stimulation. The former are evoked under normal blood circulation, but the latter are observed only when the blood circulation is lowered. Either slow potentials are likely to be postsynaptic responses since they are accompanied with a change of conductances in the taste cell membrane (Sato *et al.*, 2002). Slow types of postsynaptic potentials have been found in many neurons of autonomic and central nervous systems (Nicholls, 1994; Shepherd, 1994; Ganong, 2003). These potentials play important roles in modulating various functions such as cardiac output, hormone release, thirst, etc. (Pocock and Richards, 1999).

The objective of this study was to clarify whether slow HPs in frog taste cells evoked by the GP nerve stimulation are postsynaptic. We examined effects of (1) Ca^{2+} and Cd^{2+} and (2) neurotransmitter antagonists and agonists on GP nerve-induced slow HPs. The results suggest that the slow HPs are postsynaptically evoked in taste cells by substance P possibly released from the GP nerve terminals in the taste disk.

Materials and methods

Preparation

Fifty-four bullfrogs (*Rana catesbeiana*) of 330–720 g were used in the experiments. All the experiments were performed

in accordance with the Guidelines for Animal Experimentation of Nagasaki University. The animals were deeply anaesthetized by i.p. injection of a 50% urethane solution at a dose of 2–3 g/kg body wt. The hypoglossal nerves were bilaterally cut to remove lingual muscle contractions. The tongue of the animal placed in a supine position was pulled out from the mouth and pinned on a silicone rubber plate. Blood supply to the tongue through the lingual arteries and veins was carefully maintained. The whole GP nerves on both sides were separated out from connective tissues, cut centrally and immersed into mineral oil. All experiments were carried out at room temperature of 23–27°C.

Electrical recordings and stimulations

Intracellular recordings were made from taste cells in the taste disk of the fungiform papillae with a 3 M KCl-filled microelectrode (35–75 MΩ). An indifferent electrode of chlorided silver wire was inserted into the forelimb muscles. The criteria for identifying successful intracellular recordings were the same as described previously (Sato *et al.*, 2002).

The membrane potentials of the taste cells were amplified with a microelectrode amplifier (MEZ-8101; Nihon Kohden, Tokyo, Japan) and recorded on a pen recorder. The input resistance of single taste cells was always measured with a bridge circuit housed in the amplifier. When a slow HP was generated by GP nerve stimulation, the input resistance of an impaled taste cell increased (Sato *et al.*, 2002). When the input resistance did not increase, the cell was regarded as of no innervation with the GP nerve fibers. The distal side of the GP nerve transected was electrically stimulated with repetitive pulses of 30 Hz for 5–10 s, which produced the maximal amplitude of summated slow potentials in the taste cells (Sato *et al.*, 2002). The electrical pulses were 0.1 ms in duration and 15–30 V in strength for stimulating C-fibers.

Solutions and drugs

The tongue surface was adapted to a frog Ringer solution, which consisted of (mM) 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$ and 5 HEPES (pH 7.2). The synaptic region of taste cells in the taste disks was perfused via capillary vessels by intravenously injecting a Ringer solution containing pharmacological drugs to modulate synaptic transmission. Capillary vessels were abundant underneath the taste disks of the fungiform papillae (Jaeger and Hillman, 1976). Amount of injected Ringer solution was 2 ml/kg body wt. Applied agents were EGTA, $CaCl_2$ and $CdCl_2$. Ca^{2+} concentration in the blood plasma was decreased by injecting EGTA at a dose of 23.9 mg/kg and increased by injecting $CaCl_2$ at a dose of 116 mg/kg. Estimated low and high Ca^{2+} concentrations in the plasma were ~0.5 and ~20 mM, respectively, which were calculated from an even distribution of injected EGTA and $CaCl_2$ in the frog plasma volume (4% of the body wt; Thorson, 1964). Normal Ca^{2+} concentration in the frog plasma is ~2 mM (Wilson, 1979). $CdCl_2$ was intravenously injected at doses of 0.001–1 mg/kg. Estimated Cd^{2+} concentrations in the plasma were in the range of $\sim 6 \times 10^{-8}$–$\sim 6 \times 10^{-5}$ M.

To search for neurotransmitters which are released from the axon terminals in the taste disks, 10 receptor antagonists were used: DL-propranolol hydrochloride, prazosin hydrocloride, metergoline, tropine 3,5-dichlorobenzoate, spiperone, (±)-SKF-83566 hydrochloride, 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 and oxalate salt. Substance P acetate salt was used as an agonist (NK_1 agonist). All drugs were purchased from Sigma-Aldrich Co. (St Louis, MO). Stock solutions from prazosin hydrochloride, metergoline, spiperone, SKF-83566 hydrochloride and oxalate salt were prepared with ethanol. Stock solutions from tropine 3,5-dichlorobenzoate and CGRP fragment 8–37 were prepared with DMSO and 0.1% acetic acid, respectively. These solutions were kept at –20°C. Aliquots of stock solutions were added into the frog Ringer solution to obtain desired final concentrations when used for experiments. VIP fragment 6–28, [D-Arg^1, D-$Trp^{7,9}$, Leu^{11}] substance P acetate salt, substance P acetate salt and propranolol hydrochloride were directly dissolved in the Ringer solution. To remove the junction potential generated between the fluid secreted from lingual salivary glands following GP nerve stimulation and the lingual surface fluid, atropine sulfate (Sigma-Aldrich) dissolved in the Ringer solution was applied i.v. To block nonselective cation channels in taste cell membranes, flufenamic acid (Sigma-Aldrich) stocked in ethanol was injected i.v. after diluting with the Ringer solution. Ringer solutions containing Cd^{2+}, EGTA and excess Ca^{2+} were intravenously injected at a slow rate of 0.1 ml/min so as not to perturb the heart rate, but Ringer solutions containing the other drugs were injected at a rate of 0.1 ml/s.

Experimental procedure

Strong repetitive electrical stimulations of GP nerve produce a large slow potential on the lingual surface and in taste cells, which is derived from the physicochemical junction potential between a secreted saliva and a lingual surface solution (Sato *et al.*, 2000). This junction potential disturbs an analysis of slow HP responses elicited in taste cells. Therefore, before the start of intracellular recordings from taste cells, atropine sulfate was injected intravenously at a dose of 1 mg/kg to completely block the slow physicochemical junction potential. The effect of this injection lasted for >7 h.

The electrical activities of frog taste disks show a variation from individual to individual and from season to season. Comparison between control and test responses was performed using the slow HPs obtained from the same individual. Control response data were collected from 10–30 taste cells for 90 min before a drug injection and test

response data were collected from 10–30 taste cells for 90 min after a 30 min circulation of an injected drug.

Statistics

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

Results

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

Release of neurotransmitters from the presynaptic axon terminal is triggerd by Ca^{2+} influx at the terminal and modulated by changing Ca^{2+} concentrations at the terminal (Katz and Miledi, 1967). If slow HPs are postsynaptically evoked in taste cells by GP nerve stimulation, they may be modulated by changing Ca^{2+} concentration in presynaptic axon terminals of taste disks. To test this, Ca^{2+} concentration in the taste disks was altered by changing the amount of Ca^{2+} in the blood plasma. The slow HPs in taste cells induced by GP nerve stimulation at 30 Hz were significantly lower in amplitude and longer in latency at a low Ca^{2+} concentration (~0.5 mM) than at the control (~2 mM) ($P < 0.05$, $n = 12–20$; Figure 1A, Ba, Ca). On the other hand, the slow HPs were higher in amplitude and shorter in latency at a high Ca^{2+} concentration (~20 mM) than at the control ($P < 0.05$, $n = 15–20$; Figure 1A, Bb, Cb).

The amplitude and latency of postsynaptic potentials fluctuate during repetitive synaptic transmission (Katz and Miledi, 1965). To observe such a phenomenon, slow HPs in single taste cells were induced by stimulating GP nerve with train pulses (30 Hz for 5 s) at every minute for 7–16 min. Figure 2 shows fluctuation of amplitudes (A and B) and latencies (A and C) of the slow HPs when Ca^{2+} concentrations in the plasma were kept at the control and the low level. Either the amplitude or the latency distributed broadly at the low Ca^{2+} concentration (data at the high Ca^{2+} concentration not shown). To statistically compare the distribution of amplitudes and latencies of the slow HPs obtained under the low (~0.5 mM), the control (~2 mM) and the high (~20 mM Ca^{2+} concentrations in the plasma, the mean amplitude and mean latency of the slow HPs in each taste cell were respectively normalized as 100. Resultantly, means and SEMs of standard deviations in amplitude of the slow HPs were 46 ± 3 ($n = 8$) in the low , 21 ± 1 ($n = 6$) in the control and 10 ± 1 ($n = 8$) in the high Ca^{2+} concentration. On the other hand, means and SEMs of standard deviations in latency of the slow HPs were 49 ± 2 ($n = 7$) in the low, 18 ± 1 ($n = 6$) in the control and 11 ± 1 ($n = 9$) in the high Ca^{2+} concentration There were significant differences between any pairs of the three standard deviations in either amplitude or latency of the slow HPs ($P < 0.05$, $n = 6–9$). Both the amplitude and the latency of the slow HPs in taste cells more fluctuated as Ca^{2+} concentration decreased in GP nerve terminals of the taste disk.

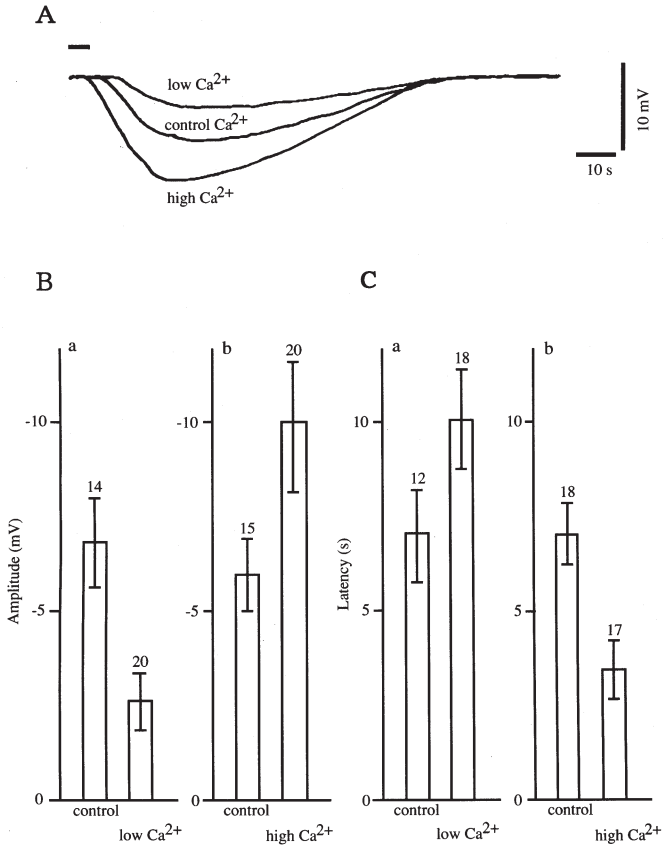

Figure 1 Change in amplitude and latency of slow HPs in frog taste cells by altering Ca^{2+} levels in blood plasma. **(A)** Slow HPs induced by GP nerve stimulation at low, control and high Ca^{2+} concentration in the plasma. The three superimposed traces were obtained from different taste cells. A horizontal bar above the traces denotes electrical stimulation of GP nerve at 30 Hz. **(B)** Amplitudes of GP nerve-induced slow HPs at control Ca^{2+} (a, b), low Ca^{2+} (a) and high Ca^{2+} (b) concentrations in the plasma. **(C)** Latencies of GP nerve-induced slow HPs at control Ca^{2+} (a, b), low Ca^{2+} (a) and high Ca^{2+} (b) concentrations in the plasma. Control Ca^{2+} concentration in the frog plasma is ~2 mM. Low and high Ca^{2+} concentrations in the plasma were obtained by injecting EGTA of 23.9 mg/kg body wt and $CaCl_2$ of 116 mg/kg body wt, respectively. Estimated low and high Ca^{2+} concentrations in the plasma are ~0.5 mM and ~20 mM. Vertical bars are SEMs and numerals above the bars are number of taste cells tested in this and the other figures.

Effect of Cd^{2+} on slow HPs

Cd^{2+} is a nonselective voltage-gated Ca^{2+} channel blocker (Randall, 1998). We tested effect of intravenously injected Cd^{2+} on the slow HPs in taste cells. The amplitude of slow HPs was dose-dependently decreased by Cd^{2+} (Figure 3). When $CdCl_2$ was injected at a dose of 1 mg/kg, the amplitude became 18% of that without Cd^{2+} ($P < 0.01$, $n = 18–21$). At a dose of 1 mg/kg, the concentration of Cd^{2+} in the plasma was estimated to be 60 μM. The IC_{50} of $CdCl_2$ for the slow HPs was at 0.013 mg/kg (~0.8 μM Cd^{2+}). The latency of slow HPs significantly increased from 5.0 ± 1.2 s ($n = 15$) in the controls to 10.8 ± 1.2 s ($P < 0.05$, $n = 18$) when $CdCl_2$ was injected at 1 mg/kg.

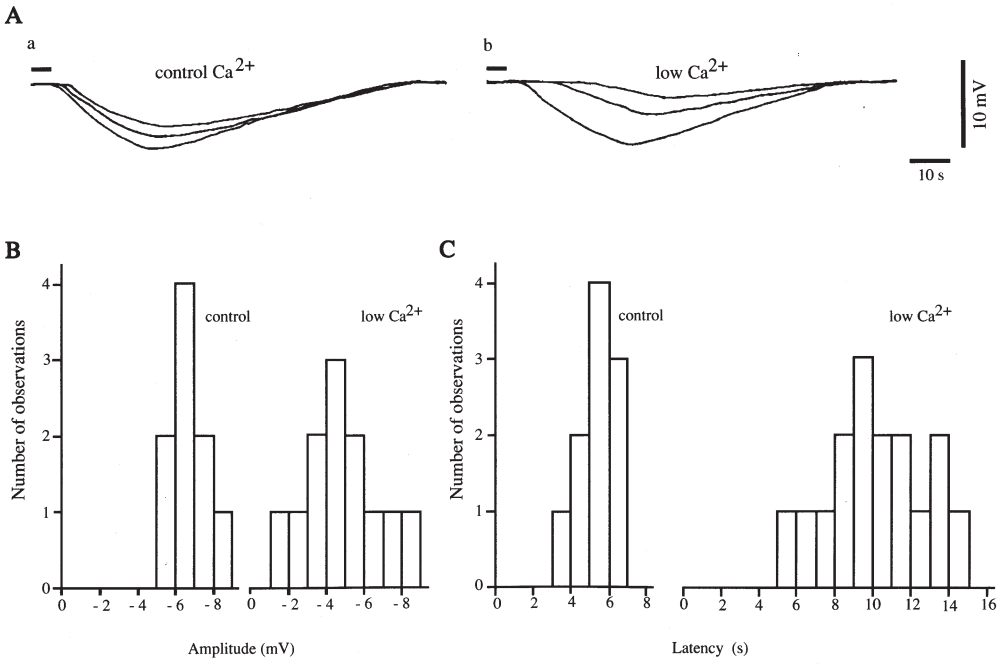

Figure 2 Fluctuation of amplitudes and latencies of slow HPs in single taste cells at control and low Ca^{2+} concentrations in the blood plasma. **(A)** Change in amplitudes and latencies of slow HPs in a taste cell induced by GP nerve stimulation at control Ca^{2+} (a) and low Ca^{2+} (b) concentrations in the plasma. Each record, in which three traces were superimposed, was taken from a taste cell. A horizontal bar above the traces denotes GP nerve stimulation at 30 Hz. **(B)** Distribution of amplitudes of GP nerve-induced slow HPs at control Ca^{2+} and low Ca^{2+} concentrations in the plasma. Each histogram was obtained from a taste cell. **(C)** Distribution of latencies of GP nerve-induced slow HPs at control Ca^{2+} and low Ca^{2+} concentrations in the plasma. Each histogram was obtained from different cells. Ca^{2+} concentration is ~2 mM in the control plasma and is ~0.5 mM in the low Ca^{2+} plasma.

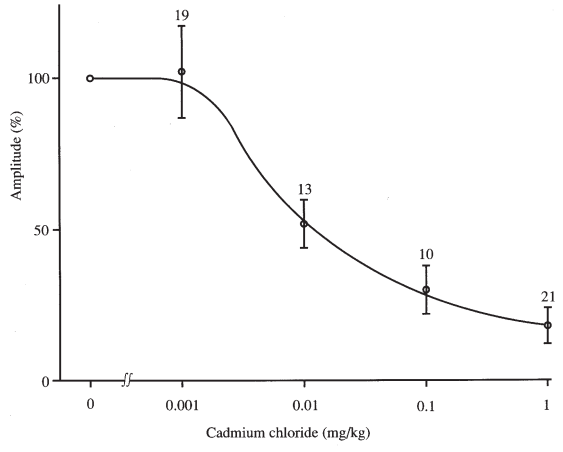

Figure 3 Relationships between amplitudes of GP nerve-induced slow HPs in taste cells and amounts of $CdCl_2$ injected i.v. The control amplitude of slow HPs in ordinate was taken as 100%, which was the mean value obtained from 18–22 taste cells before the drug injection. Test values were relative to the control. In abscissa injected amounts of $CdCl_2$ are shown in mg/kg body wt.

Effect of neurotransmitter receptor antagonists on slow HPs

First, effects of receptor antagonists for biogenic amines on the slow HPs were tested. Prazosin (a noradrenaline α_1 blocker), propranolol (a noradrenaline β blocker), metergoline (a blocker of serotonin $5HT_1$ and $5HT_2$ receptors), tropine 3,5-dichlorobenzoate (a selective blocker of serotonin $5HT_3$ receptor), SKF-83566 (a selective blocker of dopamine D_1 receptor) and spiperone (a selective blocker of dopamine D_2 receptor) were injected intravenously at 1 mg/kg body wt with no effects on the amplitude of slow HPs ($P > 0.1$, $n = 19–23$; Figure 4A).

Kuramoto (1988) and Kusakabe *et al.* (1996) found nerve fibers containing VIP, CGRP and substance P in the frog taste disks. Therefore, we tested receptor antagonists for these peptides. Injected drugs were VIP 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 acetate salt (a substance P receptor NK_1 blocker) and oxalate salt (a substance P receptor NK_1 blocker). Both VIP and CGRP receptor antagonists did not affect the slow HPs ($P > 0.1$, $n = 16–20$). Two types of substance P receptor antagonist reduced the slow HPs (Figure 4B). When oxalate salt was injected at doses of 0.2–4 mg/kg, the drug dose-dependently reduced the slow HPs. Oxalate salt of 4 mg/kg reduced the slow HPs by 99 ± 1% ($P < 0.01$, $n = 17–21$) and [D-Arg[1], D-Trp[7,9], Leu[11]] substance P acetate salt of 2 mg/kg reduced the slow HPs by 98 ± 2% ($P < 0.01$, $n = 18–22$). Therefore, it is suggested that a neurotransmitter candidate in generating the slow HPs in taste cells is substance P.

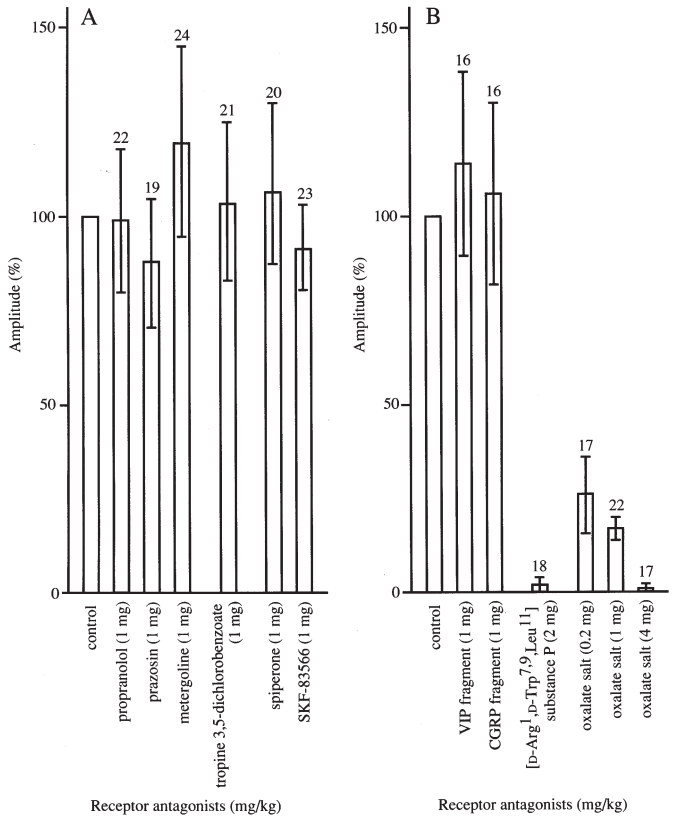

Figure 4 Effects of various antagonists of neurotransmitter receptors on amplitudes of GP nerve-induced slow HPs in taste cells. **(A)** Effect of antagonists of biogenic amine receptors on the slow HPs. **(B)** Effect of antagonists of neuropeptide receptors on the slow HPs. All antagonists were injected i.v. Injected amounts of the drugs are shown at mg/kg body wt. The control amplitude of slow HPs was taken as 100%, which was obtained from 18–25 taste cells before the injection of each drug.

Hyperpolarizing responses induced by substance P

Whether substance P elicits the slow HPs in taste cells was examined by i.v. injection at a dose of 2 mg/kg. Membrane potentials were recorded from many taste cells of a tongue before and after injection of substance P. A summary of recordings is illustrated in Figure 5. The resting membrane potential of taste cells was –32 ± 2 mV (n = 14) before the drug injection, but the membrane potential was increased to –43 ± 4 mV (n = 6) 30 min after the drug injection. The increased membrane potential, which was maintained for 25 min, resumed the resting membrane potential of –33 ± 2 mV (n = 10) 20 min after a substance P antagonist, oxalate salt, was injected at a dose of 2 mg/kg. The amplitude of hyperpolarizing responses in taste cells evoked by substance P of 2 mg/kg was –11 ± 1 mV (n = 31).

On the other hand, the amplitude of slow HPs evoked by electrical stimulation of GP nerve was –12 ± 3 mV (n = 8) when the resting potential was in a range from –28 to –36 mV (–32 ± 2 mV, n = 8). There was no difference in amplitude between substance P-induced hyperpolarization and GP

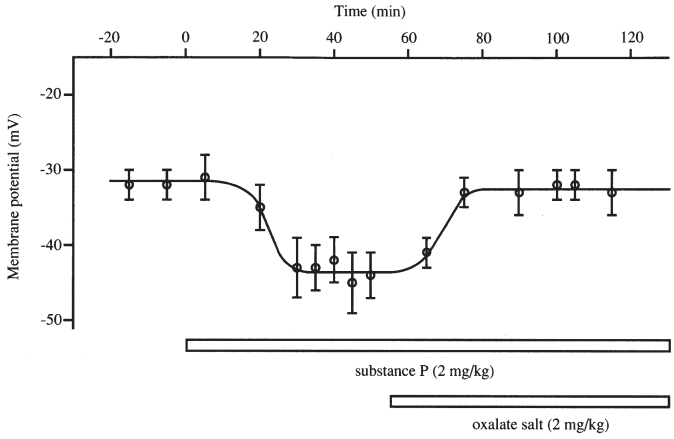

Figure 5 Hyperpolarizing responses in taste cells induced by i.v. injection of substance P. Membrane potentials were measured from different taste cells before and after injecting the drug at 2 mg/kg body wt. At time zero, substance P of 2 mg/kg was injected and a substance P antagonist, oxalate salt, of 2 mg/kg was injected 55 min after the substance P injection. Each point is the mean membrane potential from 4–10 taste cells tested for 5 min. All data were obtained from one individual.

nerve-induced slow HPs (P > 0.1, n = 8–31). The hyperpolarizing responses induced during application of substance P at 2 mg/kg were observed in 50 of 64 taste cells (78%) tested. The slow HPs induced by GP nerve stimulation were observed in 452 of 525 taste cells (86%).

Effect of flufenamic acid on slow HPs

Our previous study (Sato *et al.*, 2002) showed that the reversal potential for slow HPs in taste cells evoked by GP nerve stimulation is –13 mV and that the mechanism generating the slow HPs is an inactivation of nonselective cation channels which are most permeable to K^+ and Na^+. Flufenamic acid is a potent antagonist for the nonselective cation channels (Hescheler and Schultz, 1993). The slow HPs in taste cells were dose-dependently reduced by flufenamic acid (Figure 6). Flufenamic acid of 5 mg/kg completely inhibited the slow HPs (P < 0.01, n = 25–26), indicating that they are generated by flufenamic-acid-sensitive nonselective cation channels at the taste cell membrane.

Discussion

Sequential events of chemical synaptic transmission are as follows: (i) activation of voltage-gated Ca^{2+} channels at presynaptic axon terminal by a depolarization; (ii) release of a neurotransmitter from the presynaptic terminal; (iii) binding of the neurotransmitter to receptors at postsynaptic cell; and (iv) generation of a postsynaptic potential at the postsynaptic cell by direct or indirect opening or closing of ion channels (Shepherd, 1994).

The present study suggested that the amplitude and latency of the slow HPs in frog taste cells induced by the GP nerve stimulation were modulated by Ca^{2+} and Cd^{2+} in the nerve terminals of taste disk (Figures 1 and 3) and that the

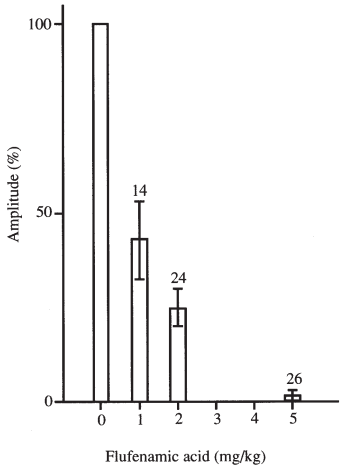

Figure 6 Relationships between amplitudes of GP nerve-induced slow HPs in taste cells and amounts of flufenamic acid injected i.v. The control amplitude of slow HPs was taken as 100%, which was obtained from 22–28 taste cells before the drug injection. Amounts of injected drug are denoted at mg/kg body wt.

fluctuations of the amplitudes and latencies of the slow HPs in taste cells were dependent on Ca^{2+} concentration in the nerve terminals of taste disk (Figure 2). Proton-gated nonselective cation channels of the apical receptive membrane in frog taste cells cause acid responses (Miyamoto *et al.*, 1988; Okada *et al.*, 1994; Sato *et al.*, 1995). Sensitivity of these nonselective cation channels to Cd^{2+} is low and the IC_{50} of Cd^{2+} for the acid responses in frog taste cells is estimated to be ~0.5 mM (Miyamoto *et al.*, 1988). On the other hand, the IC_{50} of Cd^{2+} for the slow HPs in frog taste cells was as low as 0.8 μM. Cd^{2+} is a potent blocker of voltage-gated Ca^{2+} channels in various tissues and the IC_{50} for these channels is ~1 μM (Randall, 1998), which is almost the same as the IC_{50} for the slow HPs in frog taste cells. In general, sensitivity to Cd^{2+} is much lower in nonselective cation channels than in voltage-gated Ca^{2+} channels (Wallnöfer *et al.*, 1989). Therefore, Cd^{2+}-dependent inhibition of slow HPs in taste cells may be due to an inactivation of voltage-gated Ca^{2+} channels at presynaptic axon terminals. Also, Ca^{2+}-dependence of the amplitude and latency of slow HPs may be due to a modulation of Ca^{2+} influxes through voltage-gated Ca^{2+} channels at the presynaptic axon terminals.

Of antagonists tested for receptors of biogenic amines and peptides only antagonists for substance P receptor completely blocked slow HPs in taste cells induced by GP nerve stimulation (Figure 4). The presence of substance P has been shown immunohistochemically at the nerve terminals of frog taste disk (Kuramoto, 1988; Kusakabe *et al.*, 1996). Intravenous application of substance P at 2 mg/kg, whose concentration in the plasma is estimated to be 28 μM, produced hyperpolarizing responses in taste cells. These results suggest that a candidate of neurotransmitter released from the presynaptic axon terminals in frog taste disk is substance P.

Our previous study (Sato *et al.*, 2002) indicated that GP nerve-induced slow HPs may be due to an inactivation of nonselective cation channels permeable to K^+ and Na^+ in the frog taste cells. Nonselective cation channels of 30 pS permeable to K^+ and Na^+ have been found in the basolateral membranes of frog taste cells (Fujiyama *et al.*, 1993). The present work showed that flufenamic acid as a potent blocker of the nonselective cation channel in the postsynaptic membrane of the taste cell suppressed the generation of slow HPs in taste cells (Figure 6). Since flufenamic acid is relatively insensitive to voltage-gated Ca^{2+} channels present in the presynaptic axon terminals (Hescheler and Schultz, 1993), the slow HPs is thought to be suppressed by binding of flufenamic acid to the nonselective cation channels in the taste cell membrane.

Substance P receptor, NK_1, is coupled to G-protein, and the second messengers of the intracellular transduction pathways following binding of substance P to NK_1 are IP_3 and DAG (Otsuka and Yoshioka, 1993; Bloom, 1996; Ganong, 2003). Since the membrane conductance decreases while slow HPs are generated in frog taste cells by GP nerve stimulation (Sato *et al.*, 2002), the slow HPs are probably generated by closing the nonselective cation channels through an intracellular transduction cascade after substance P binds to NH_1 in the postsynaptic membrane of the taste cells.

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Accepted 16 July 2004