Chem. Senses 32: 817–823, 2007

doi:10.1093/chemse/bjm049
Advance Access publication July 25, 2007

# Characteristics of Biphasic Slow Depolarizing and Slow Hyperpolarizing Potential in Frog Taste Cell Induced by Parasympathetic Efferent Stimulation

**Toshihide Sato[1], Kazuhisa Nishishita[2], Yukio Okada[1] and Kazuo Toda[1]**

Divisions of [1]Integrated Sensory Physiology and [2]Oral Pathopharmacology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan

*Correspondence to be sent to: Toshihide Sato, Division of Integrated Sensory Physiology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan. e-mail: toshi@net.nagasaki-u.ac.jp*

## Abstract

When the velocity of capillary blood flow in the frog tongue declined to an intermediate range of 0.2–0.7 mm/s, the glossopharyngeal nerve stimulation induced a biphasic slow depolarizing and slow hyperpolarizing potential (HP) in taste cells. The objective of this work was to examine the generative mechanisms of the biphasic slow potentials. The biphasic slow response was always preceded by a slow depolarizing potential (DP) component and followed by a slow HP component. Intravenous injection of tubocurarine completely blocked the biphasic slow responses, suggesting that both components of the biphasic slow potentials are evoked by the parasympathetic nerve (PSN) fibers. Membrane conductance of taste cells increased during slow DPs and decreased during slow HPs. The reversal potential of either component of a biphasic slow response was the almost same value of −12 mV. An antagonist, L-703,606, for neurotransmitter substance P neurokinin$_1$ receptor completely blocked both components of the biphasic slow responses. An antagonist, flufenamic acid, for nonselective cation channels on the taste cell membrane completely blocked the biphasic slow responses. These results suggest that PSN-induced biphasic slow responses are postsynaptically elicited in taste cells by releasing substance P at the PSN axon terminals. It is concluded that the slow DP component may be generated by opening one type of nonselective cation channel on taste cells and that the slow HP component may be generated by closing the other type of nonselective cation channel. We discussed that a second messenger inositol 1,4,5-trisphosphate might be related to a slow DP component and another second messenger diacylglycerol might be related to a slow HP component.

**Key words:** efferent synapse, frog taste cell, hypoxia, second messenger, slow depolarizing and slow hyperpolarizing potential

## Introduction

Frog taste cells are innervated by afferent and efferent nerve fibers (Sato 1976; Sato et al. 2002, 2004, 2005). The former are mostly gustatory nerve fibers, and the latter are parasympathetic nerve (PSN) efferent fibers (Inoue et al. 1992; Sato et al. 2005). Tastant-induced taste cell responses are modulated by PSN (Sato et al. 2005).

In our studies, tissue blood flow has been estimated from the velocity of red blood cells in the lingual capillaries and assumed to be an indicator of oxygen delivery. Normoxia is assumed under conditions where the normal velocity of blood flow is observed at the earlier stage of experiments. Hypoxia is assumed under conditions where the velocity declines spontaneously during the course of experiments as a result of a decline of cardiovascular function. When the velocity of capillary blood flow in the tongue is at an almost normal range of 0.7–1.5 mm/s, PSN efferent stimulation elicits monophasic slow hyperpolarizing potentials (HPs) alone in frog taste cells (Sato et al. 2002, 2004, 2005). These are equivalent to slow inhibitory postsynaptic potentials in neurons (Sato et al. 2006). During a generation of slow HPs, the membrane conductance of taste cells is decreased (Sato et al. 2002; Sato, Nishishita, Mineda, et al. 2007). Because gustatory efferent synapses are formed on taste cells as presynaptic cells, presynaptic facilitation (Hodge 1972; Ganong 2003) will occur in the cells during slow HPs and gustatory afferent transmission will be enhanced (Sato et al. 2006).

The decline of the velocity of lingual capillary blood flow to <0.2 mm/s causes monophasic slow depolarizing potentials (DPs) alone in taste cells by the glossopharyngeal nerve (GPN) stimulation (Sato et al. 2002; Sato, Nishishita, Okada, Toda 2007). In this case, the membrane conductance of taste cells is increased (Sato et al. 2002). It is suggested that presynaptic inhibition (Eccles 1973; Ganong 2003) will occur in taste cells during the generation of slow DPs and gustatory afferent transmission will be inhibited (Sato,

Nishishita, Okada, Toda 2007). In an intermediate range (0.2–0.7 mm/s) of the velocity of capillary blood flow in the tongue, either monophasic slow HP or monophasic slow DP has been observed in different taste cells by GPN stimulation (Sato et al. 2002).

There is the possibility that single taste cells in frogs are capable of generating a biphasic slow response composed of a slow DP and a slow HP at a lesser degree of hypoxia when the velocity of lingual capillary blood flow is at 0.2–0.7 mm/s. Therefore, we attempted to intracellularly record GPN-induced biphasic slow responses in frog taste cells. The purpose of the present study is to elucidate the physiological properties and the generative mechanisms of GPN-induced biphasic slow responses in the taste cells.

## Materials and methods

### Preparation, electrical recording, and stimulation

All experiments were carried out under Nagasaki University Guideline for Animal Experimentation with adult bullfrogs (*Rana catesbeiana*) weighing 380–650 g. The animals were anesthetized by intraperitoneally injecting a 50% urethane–Ringer solution at a moderate dose of 1.5–3.5 g/kg body weight (b.w.). All experiments were performed at a room temperature of 23–26 °C. The methods for preparation, electrical recording from taste cells, and electrical stimulation (ES) of GPN were the same as described previously (Sato et al. 2002, 2004). Briefly, intracellular recordings from taste cells were made with a capillary microelectrode filled with 3 M KCl and having 30–70 MΩ. The tip of the microelectrode was inserted into the lower portion of the intermediate layer of the taste disks in the fungiform papillae and penetrated into a receptor cell of type III or II (Sato, Nishishita, Mineda, et al. 2007). In order to avoid penetrating basal cells, the microelectrode was advanced into the central portion of taste disks. The criteria for a penetration of taste receptor cells were an appearance of 3-step potential changes in the membrane potential of taste disk cells (Sato, Nishishita, Mineda, et al. 2007). Both GPNs separated free from the connective tissues were cut centrally. The distal portion of GPNs was stimulated at 30 Hz with pulses of 0.1-ms duration and 15-V strength to excite the PSN efferent fibers and to obtain the maximal slow potential from taste cells (Sato et al. 2002, 2004).

### Drugs and solutions

Tubocurarine chloride, atropine sulfate salt, flufenamic acid, and L-703,606 oxalate salt were used. All drugs were purchased from Sigma-Aldrich Co. (St Louis, MO). Stock solutions from the first 2 drugs were prepared with a frog Ringer solution, and stock solutions from the last 2 drugs were prepared with ethanol and methanol, respectively. All stock solutions were diluted to various concentrations with frog Ringer solution just before use. The frog Ringer solution was composed of 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES [4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid], and the pH was adjusted to 7.2 with a Tris [tris(hydroxymethyl)aminomethane] buffer.

### Experimental procedure

Strong ES at 30 Hz of GPN produces a large physicochemical potential of 2–21 mV on the lingual surface and in taste disk cells. This comes from the junction potential generated between saliva secreted from the lingual glands and lingual superficial solution (Sato et al. 2000). The junction potential disturbed the analysis of physiological slow potentials in taste cells induced by ES of the GPN, so that atropine sulfate was injected intravenously (i.v.) at a dose of 1 mg/kg to block the junction potential. The atropine effect lasted >7 h.

Because the biphasic responses composed of a slow DP and a slow HP were usually induced for ~1 h after 4 h anesthesia, it was difficult to obtain both control and test responses before and after intravenous injection of a drug from the same animal. Therefore, the control and test biphasic slow responses were obtained from taste cells of different animals. Usually, the control responses were sampled from taste cells within 1 h after the first observation of biphasic slow response, and the test responses were obtained within 40 min after 20 min circulation of a drug injected i.v. at the first initiation of the biphasic response. The right precaval vein and the postcaval vein were used for drug injections.

### Measurement of velocity of capillary blood flow

First, a 4% methylene blue solution was injected i.v. at a dose of 0.4 ml/kg b.w., and red blood cells were partially stained at varying densities. The velocity of capillary blood flows was measured by observing the movement of the most densely stained red cells flowing in the capillaries of the fungiform papillae. The observation was carried out with an ocular micrometer and a stopwatch.

### Statistics

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

## Results

### Time course of GPN-induced slow potential type in taste cell

After the animal was anesthetized with urethane, the velocity of lingual capillary blood flow was gradually decreased because the heart rate and the contractility of cardiac muscles declined. The time course of changes in slow potential types in taste cells induced by GPN stimulation was dependent on individuals. A typical example of the time course is illustrated in Figure 1. Although the velocity of capillary blood

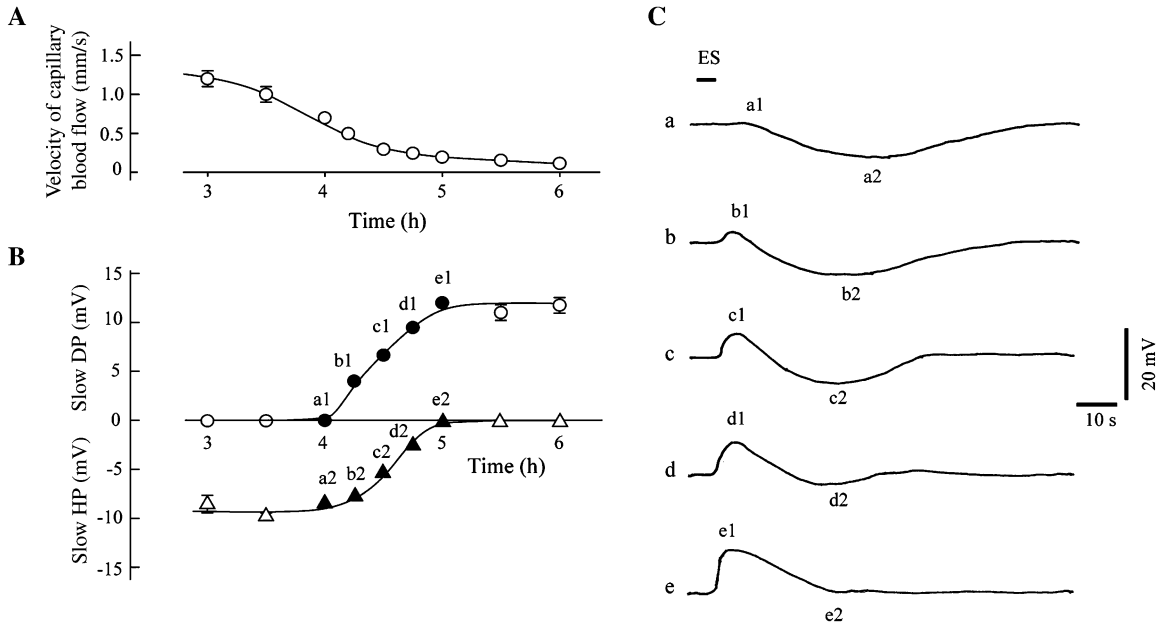


**Figure 1** Time course of change in slow potential types in frog taste cells induced by GPN stimulation. **(A)** Time course of velocity of capillary blood flow in tongue after start of anesthesia. Each point is mean of 4–7 measurements from fungiform papillae. **(B)** Time course of changes in amplitude of slow potentials in taste cells induced by GPN stimulation after anesthesia. Three types of slow potential: 1) monophasic slow HP, 2) biphasic slow potential composed of slow DP and slow HP components, and 3) monophasic slow HP appeared. Points marked (a1, a2) to (e1, e2) were obtained from (C). Each of the other points is a mean from 7 to 11 taste cells. Resting potential was −31 ± 1 mV ($n$ = 40). **(C)** Records of slow potentials from 2 taste cells: a monophasic slow HP (a), biphasic slow potentials (b) to (d), and a monophasic slow DP (e). Records (b) to (e) were from one taste cell having resting potential of −32 mV, and record (a) was from another taste cell having resting potential of −29 mV. ES: electrical stimulation of GPN at 30 Hz.

flow declined from 1.5 mm/s (Sato et al. 2002) to 0.7 mm/s (Figure 1A) within 4 h after anesthesia, the slow potential type induced by GPN stimulation was mostly monophasic slow HPs alone. The mean amplitude of the slow HPs was stable and approximately −9 mV (Figure 1B). When the velocity of lingual capillary blood flow further spontaneously declined from 0.7 to 0.2 mm/s within 1 h after 4 h anesthesia, a biphasic slow depolarizing and slow hyperpolarizing response was newly evoked in single taste cells by GPN efferent stimulation (Figure 1B,C). The slow DP component always preceded the slow HP component. The traces (b) to (e) in Figure 1C were recorded from a single taste cell, and the trace (a) was recorded from another taste cell. The amplitudes of slow DP components (a1–e1) and slow HP components (a2–e2) in Figure 1C are plotted in Figure 1B (closed triangle, closed circle). The amplitude of slow DP components gradually increased with time, but the amplitude of slow HP components decreased with time. In the traces (b) to (e), the resting potential was initially −32 mV and drifted in the range of ±2 mV for 45 min. Approximately 5 h after start of anesthesia, the slow HP phase disappeared and the slow DP phase alone was evoked by GPN stimulation with further decreasing velocity of lingual blood flow.

When the GPN was stimulated at 30 Hz for 5 s, the latency, peak time, and fall time of monophasic slow DPs were 2 ± 0 s ($n$ = 13), 5 ± 1 s ($n$ = 13), and 33 ± 2 s ($n$ = 13), respectively. On the other hand, the latency, peak time, and fall time of monophasic slow HPs were 8 ± 0 s ($n$ = 70), 21 ± 1 s ($n$ = 70), and 74 ± 4 s ($n$ = 58), respectively (Sato, Nishishita, Mineda, et al. 2007). The 3 values are all significantly smaller at slow DPs than at slow HPs ($P < 0.05$, $n$ = 13–70).

### Distribution of slow potential type

As shown in Figure 1, the types of GPN-induced slow potentials in frog taste cells are composed of 1) a monophasic slow HP, 2) a biphasic slow depolarizing and hyperpolarizing potential, and 3) a monophasic slow DP. Figure 3 illustrates a sequential distribution of the 3 types of slow potential after the animal was anesthetized. The data are the means from 5 animals. At 3.5 h after the anesthesia, all responses were monophasic slow HPs, but at ~3.75 h, a biphasic slow potential was observed in 10% of tested taste cells and then the percentage of biphasic slow potential type gradually increased. At 4.5 h after anesthesia, all responses observed were biphasic. Then, the percentage of biphasic responses reduced, and within 45 min after 4.5 h anesthesia both biphasic slow response and monophasic slow DP were observed. Then all responses became monophasic slow DPs. The resting potentials were −31 ± 1 mV ($n$ = 110), −30 ± 1 mV ($n$ = 120), and −31 ± 1 mV ($n$ = 95) for each 1 h after 3, 4, and 5 h anesthesia. No difference was found between them ($P > 0.05$). The mean

15% (a range of 5–25%, 5 animals) of all impaled taste cells (325 cells) did not respond to GPN stimulation. These cells were regarded as receptor cells that might not be morphologically or functionally innervated by GPN efferent fibers. These cells were omitted in Figure 3.

### Effect of tubocurarine on biphasic response

It has been established that monophasic slow HPs and slow DPs are induced by an excitation of PSN efferent fibers (Sato et al. 2005; Sato, Nishishita, Okada, Toda 2007). We examined whether slow DP and slow HP components of biphasic responses in taste cells are similarly elicited by PSN. To block the nicotinic acetylcholine receptors on the parasympathetic ganglia, tubocurarine was injected i.v. at a dose of 0.2–1 mg/kg b.w. The amplitude of control biphasic responses before the drug injection was 6.5 ± 0.9 mV ($n$ = 21) in slow DP component and −5.3 ± 0.8 mV ($n$ = 21) in slow HP component. Each value was taken as 100%, and test tubocurarine effects are shown in Figure 3. Both components of the biphasic slow potentials decreased dose dependently and were completely blocked at 1 mg/kg b.w., indicating that the biphasic slow responses in taste cells are induced by PSN activities.

### Membrane conductance change

Changes in the membrane conductance of a taste cell during a biphasic slow response were measured from a change in the HP pulses induced by constant hyperpolarizing current pulses injected into the taste cell (Figure 4A). It is seen that the membrane conductance was increased during a slow DP component and decreased during a slow HP component. The relative membrane conductance was expressed as (input resistance at rest)/(input resistance at the biphasic response). The relative membrane conductance in taste cells was linearly increased with increasing amplitude of slow DPs but linearly decreased with increasing amplitude of slow HPs (Figure 4B,C). This result suggests that nonselective cation channels on taste cell membrane are opened during slow DPs and closed during slow HPs.

### Reversal potential for biphasic slow response

Our previous work indicated that the reversal potential for monophasic slow HPs and slow DPs in taste cells induced by PSN stimulation is −12 to 14 mV (Sato et al. 2002). We measured the reversal potentials of slow DP and slow HP components in a biphasic slow response. The membrane potential level was altered by injecting constant depolarizing and hyperpolarizing currents into an impaled taste cell. As shown in Figure 5A, the amplitudes of slow DP and slow HP components in the resting potential level were together increased with increasing membrane potential and decreased together with decreasing membrane potential. The amplitude of either slow DP or slow HP component became null at the membrane potential of approximately −12 mV in the taste cell. Then, the amplitude of both components of the biphasic response reversed in polarity. This relation is plotted in Figure 5B, where the reversal point of both components of a biphasic response was the same as that of −12 mV. The reversal potentials in biphasic slow responses determined from 11 taste cells were −12 ± 2 mV ($n$ = 11) for slow DP components and −11 ± 2 mV ($n$ = 11) for slow HP components. No significant difference was found between the 2 values ($P > 0.05$, $n$ = 11).

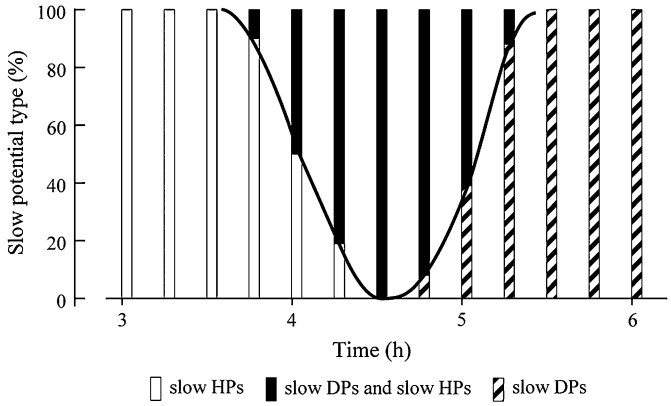


**Figure 2** Time course of distribution of 3 types of slow potential in taste cells after anesthesia. Data were sampled at 15-min intervals, and each column was obtained from 14 to 34 taste cells in 5 animals. Resting potential was −30 ± 1 mV ($n$ = 325).

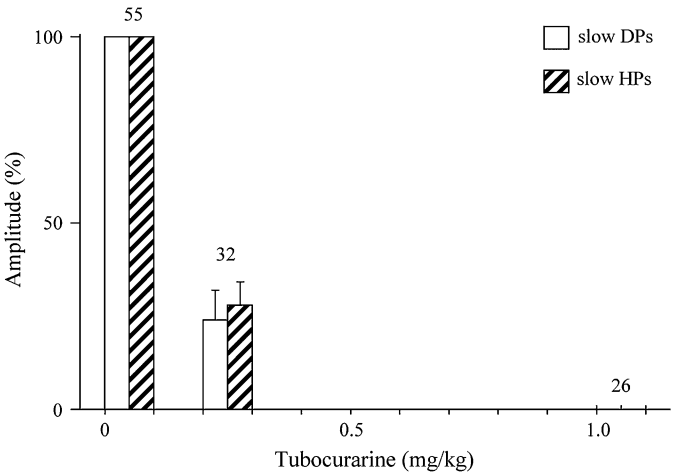


**Figure 3** Effect of tubocurarine on biphasic slow potentials in taste cells induced by GPN stimulation. Tubocurarine was injected i.v. Each control amplitude of biphasic slow DP and slow HP components was taken as 100%, and test amplitudes of biphasic responses were relative to control. Numerals above columns are number of taste cells tested and vertical bars are standard errors of means in this and other figures. Resting potential was −32 ± 1 mV ($n$ = 21; control), −32 ± 1 mV ($n$ = 18; 0.2 mg/kg), and −29 ± 2 mV ($n$ = 20; 1 mg/kg). Data from 2 animals.

### Effect of antagonist for substance P neurokinin$_1$ receptor on biphasic slow response

Intravenous application of a substance P neurokinin$_1$ ($NK_1$) receptor antagonist, L-703,606, at 4 mg/kg b.w. is known to

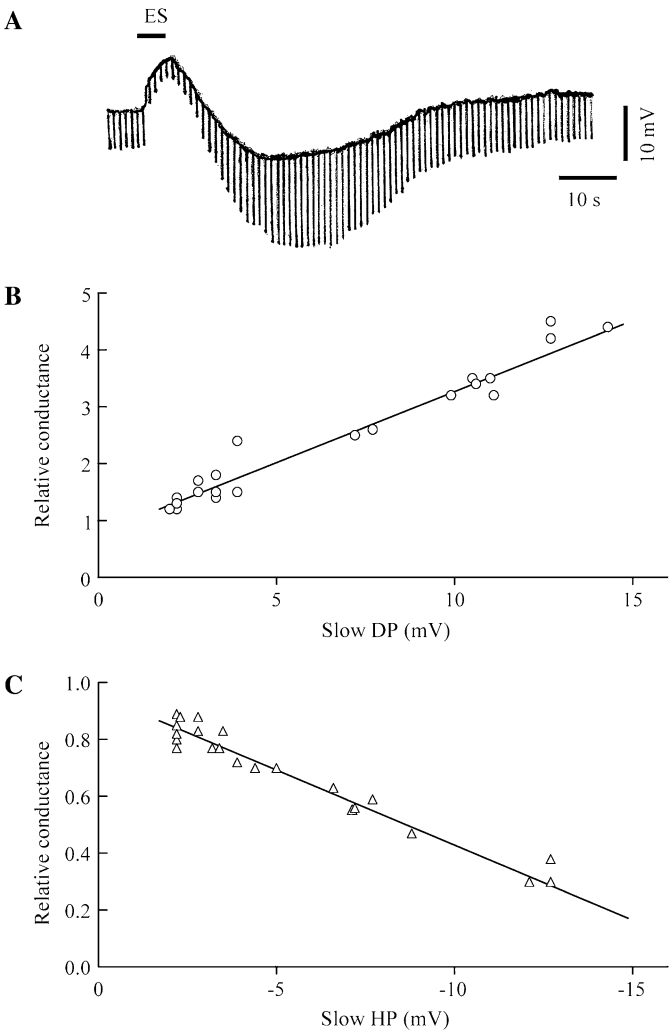


**Figure 4** Change in membrane conductance of taste cells during biphasic slow responses induced by PSN stimulation. **(A)** Record of biphasic slow potential in a taste cell on which HP pulse trains induced by constant hyperpolarizing current pulse trains were superimposed to measure membrane conductance. ES: electrical stimulation of PSN at 30 Hz. Resting potential was −33 mV. **(B)** Relationship between amplitudes of slow DP components and amplitudes of membrane conductances (open circle). **(C)** Relationship between amplitudes of slow HP components and amplitudes of membrane conductances (open triangle). Relative membrane conductance in ordinate was expressed as (input resistance at rest)/(input resistance at biphasic slow response) in taste cells. Data of (B) and (C) were obtained from biphasic slow responses of 21 taste cells having resting potential of −30 ± 1 mV ($n$ = 21).

completely block monophasic slow DPs and slow HPs induced by PSN stimulation (Sato et al. 2004; Sato, Nishishita, Okada, Toda 2007). We elucidated how the slow DP and slow HP components in a PSN-induced biphasic response are blocked by L-703,606. As shown in Figure 6, both components of the biphasic slow response were dose dependently blocked by L-703,606, suggesting that neurotransmitter substance P $NK_1$ receptors on the taste cell membrane mediate the biphasic responses in taste cells.

### Effect of nonselective cation channel blocker on biphasic slow response

It is known that monophasic slow HPs and slow DPs in taste cells are blocked by flufenamic acid of a nonselective cation channel blocker (Sato et al. 2004; Sato, Nishishita, Okada, Toda 2007). We measured how a nonselective cation channel blocker, flufenamic acid, affects each component of biphasic slow responses in taste cells when the velocity of capillary blood flow was at 0.2–0.7 mm/s. As shown in Figure 7, the amplitudes of slow DP and slow HP components in biphasic responses were dose dependently decreased and completely blocked at 5 mg/kg injected i.v. This result suggests that the slow DP and slow HP components are produced by opening and closing nonselective cation channels on taste cells, respectively. The resting potential of taste cells was −30 ± 2 mV ($n$ = 55) before an intravenous injection of flufenamic acid but became −34 ± 2 mV ($n$ = 32) and −35 ± 2 mV ($n$ = 26) after the injection at 2 and 5 mg/kg b.w., respectively. These hyperpolarizations following flufenamic acid treatment indicate that nonselective channels on the taste cell membranes were open at rest.

## Discussion

The present studies indicate that both components of a biphasic slow response in frog taste cells induced by PSN stimulation are completely blocked by L-703,606 (a blocker of neurotransmitter substance P $NK_1$ receptor) and by flufenamic acid (a strong blocker of nonselective cation channel). The reversal potential for both components of a biphasic slow response is the almost same value of −11 to 12 mV, and the membrane conductance of taste cells is increased during a slow DP and decreased during a slow HP. These results are the same as those obtained from monophasic slow HPs and slow DPs in frog taste cells induced by PSN stimulation (Sato et al. 2002, 2004; Sato, Nishishita, Okada, Toda 2007). Our previous studies suggested that the slow HPs are initiated by closing nonselective cation channels on the proximal processes of taste cells after a release of substance P at the presynaptic PSN axon terminals (Sato et al. 2004, 2005). Similar serotonin-induced slow HPs, which accompany a decrease in membrane conductance and induce an enhancement of receptor potentials, have been found in *Necturus* taste cells (Ewald and Roper 1994). On the other hand, the slow DPs are initiated by opening nonselective cation channels after the release of substance P (Sato et al. 2002; Sato, Nishishita, Okada, Toda 2007).

Most of the single taste cells are capable of eliciting a biphasic response consisting of a slow DP and a slow HP at an intermediate velocity of 0.2–0.7 mm/s in the lingual capillary blood flow. Anytime, a biphasic slow response is an initial slow DP followed by a slow HP. When the PSN is stimulated at 30 Hz for 5 s, the mean time course of monophasic slow DPs is composed of a latency of 2 s, a peak time of 5 s, and a fall time of 33 s, resulting in the whole duration of 38 s.

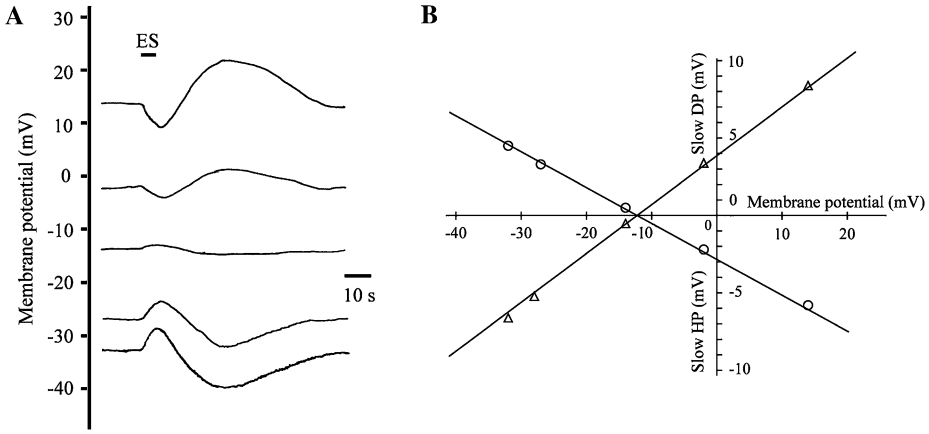


**Figure 5** Reversal potential of biphasic slow potential in a taste cell induced by PSN stimulation. **(A)** Records of biphasic slow potentials under different membrane potentials of a taste cell induced by PSN stimulation at 30 Hz for 5 s (horizontal bar lettered ES). Resting potential was −27 mV. **(B)** Amplitudes of biphasic slow potentials in a taste cell in (A) are plotted against membrane potentials.

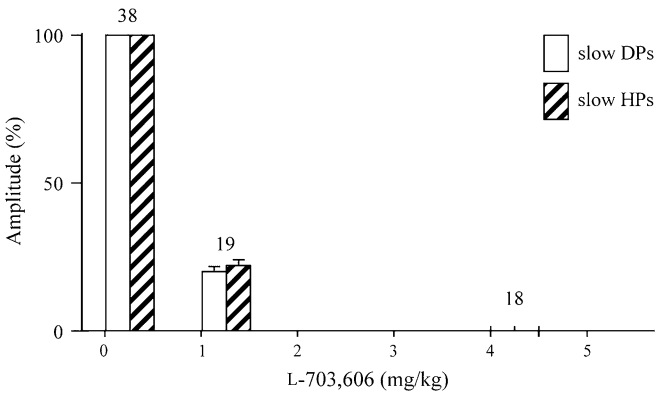


**Figure 6** Effect of L-703,606 on biphasic slow potentials in taste cells induced by PSN stimulation. L-703,606 was injected i.v. Each control amplitude of biphasic slow DP and slow HP responses was taken as 100%, and test amplitudes of biphasic slow responses were relative to control. Resting potential was −31 ± 1 mV ($n$ = 38; control), −28 ± 2 mV ($n$ = 19; 1 mg/kg), and −32 ± 2 mV ($n$ = 18; 4 mg/kg). Data from 2 animals.

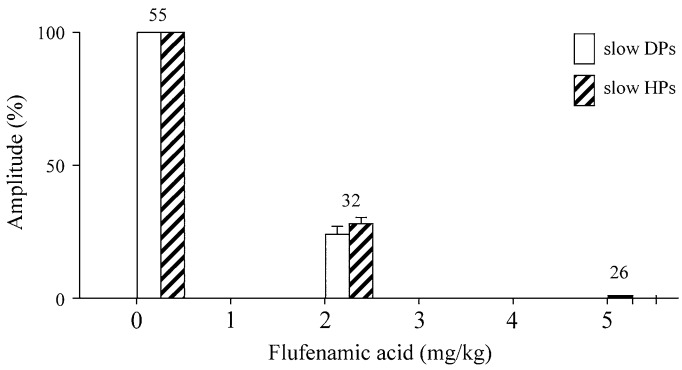


**Figure 7** Effect of flufenamic acid on biphasic slow potentials in taste cells induced by PSN stimulation. Flufenamic acid was injected i.v. Each control amplitude of biphasic slow DP and slow HP responses was taken as 100%, and test amplitudes of biphasic slow responses were relative to control. Resting potential was −30 ± 2 mV ($n$ = 55; control), −34 ± 2 mV ($n$ = 32; 2 mg/kg), and −36 ± 2 mV ($n$ = 26; 5 mg/kg). Data from 4 animals.

On the other hand, when PSN is stimulated at the same condition, the mean time course of single monophasic slow HPs induced by PSN shows a latency of 8 s, a peak time of 21 s, and a fall time of 74 s, resulting in the duration of 74 s (Sato, Nishishita, Mineda, et al. 2007). The latency of the monophasic slow HPs is 4 times longer than that of the monophasic slow DPs. The peak time and fall time of the monophasic slow HPs are, respectively, 4 and 2.5 times longer than those of the slow DPs. The total duration of the monophasic slow HPs is 2.5 times longer than that of the monophasic slow DPs.

The present results clearly show that when the velocity of lingual capillary blood flow is almost normal at 0.7–1.5 mm/s, PSN stimulation induces monophasic slow HPs alone, but when the velocity of the capillary blood flow greatly declines to <0.2 mm/s, PSN stimulation induces monophasic slow DPs alone. At an intermediate velocity of 0.2–0.7 mm/s in the capillary blood flow, a biphasic slow depolarizing and slow hyperpolarizing response appears in single taste cells. The important facts are that either slow DP or slow HP component is generated in the same taste cell, that the response polarity is reversal, and that the time course of single slow DP components is much faster than that of single slow HP components. The resting potential of frog taste cells is −31 ± 2 mV ($n$ = 85) at the normal velocity of lingual capillary blood flow (1.2–1.5 mm/s) for 3 h after anesthesia (Sato et al. 2006). The resting potential is also maintained to the same value during the next 3 h after 3 h anesthesia as shown in this study (Figure 2). No differences exist between resting potentials under normoxia and hypoxia. The resting potentials of frog taste cells are composed of ionic and metabolic components. The metabolic component is maintained by $Na^+$–$K^+$ exchange pump. Because the metabolic component

in the resting potential of frog taste cells is relatively resistive to hypoxia (Sato et al. 1983, 1995; Okada et al. 1986), the amplitude of resting potentials in the taste cells is kept stable for >6 h.

Neurotransmitter substance P $NK_1$ receptor is coupled to G protein, and the second messenger in cell signal transduction is inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG) (Otsuka and Yoshioka 1993; Bloom 1996; Ganong 2003). Substance P is known to activate various nonselective cation channels in neurons (Inoue et al. 1995). The difference in the time course and membrane conductance change between slow HPs and slow DPs in frog taste cells suggests that the DAG and $IP_3$ of the 2 second messengers exert different effects on a generation of slow HPs and slow DPs. One would speculate that one second messenger DAG predominantly acts on a generation of slow HPs. At an almost normal velocity of 0.7–1.5 mm/s in the lingual capillary blood flow, activation of DAG might elicit monophasic slow HPs alone by closing one type of nonselective cation channels on taste cells. This closing may be due to a phosphorylation of the cation channels by activating protein kinase C (PKC) via DAG pathway. PKC must also be activated by cytoplasmic $Ca^{2+}$ released by the other second messenger $IP_3$ (Nicholls et al. 2001). As the velocity of capillary blood flow declines to <0.7 mm/s, adenosine triphosphate is probably lacking because of hypoxia, so that the closing of the phosphorylation-dependent nonselective cation channel is gradually inhibited, resulting in a reduction of the amplitude of a slow HP component of a biphasic slow response in taste cell. Inversely, $Ca^{2+}$ released by $IP_3$ is gradually used to open the other type of intracellular $Ca^{2+}$-dependent nonselective cation channels, resulting in appearance of the slow DP component in a biphasic slow potential. A slow DP component is elicited with a rapid time course of 6 s before the onset of a slow HP component because the phosphorylation may not be necessary for opening the intracellular $Ca^{2+}$-dependent cation channels. In this process, the DAG pathway gradually begins not to function. Therefore, it is supposed that the amplitude of slow HP component is greatly inhibited, but the amplitude of slow DP component is growing. If cation influx inducing a slow DP component occurs through the inactivated phosphorylation-dependent nonselective cation channels, the slow DP component will be evoked during a slow HP component. This was not the case. Therefore, the slow DP component may be induced by phosphorylation-independent and $Ca^{2+}$-dependent nonselective cation channels. A great variety of nonselective cation channels have been found in varying tissues (Siemen 1993).

In conclusion, PSN-mediated monophasic slow HP in frog taste cells under normoxia may be elicited by DAG pathway in cell signal transduction, and the monophasic slow DP under a more severe hypoxia may be elicited by $IP_3$ pathway. At a lesser degree of hypoxia, a biphasic slow response consisting of a slow DP and a slow HP component may be generated by an interaction of DAG and $IP_3$ pathway in taste cells.

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*Accepted June 26, 2007*