

Slow Potentials in Taste Cells Induced by Frog Glossopharyngeal Nerve Stimulation

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Abstract

Intracellular recordings were made from the taste cells of atropinized bullfrogs while the glossopharyngeal (GP) nerve fibres were electrically stimulated. Two types of slow potential, slow hyperpolarizing potentials (HPs) and slow depolarizing potentials (DPs), were induced in the taste cells. The slow HPs appeared when the lingual capillary blood flow was kept above 0.7 mm/s, whereas the slow DPs appeared when the blood flow was slowed down below 0.7 mm/s. The membrane resistance of a taste cell increased during the generation of a slow HP, but decreased during the generation of a slow DP. The reversal potentials for the slow HPs and the slow DPs were recorded at the same membrane potential (–11 to –13 mV). Activation of non-selective cation channels possibly induced the slow DP and inactivation of those channels possibly induced the slow HP in the taste cell membrane. Electrical stimulation of the GP nerve activated a population of C fibres in the nerve and possibly released neurotransmitters from the nerve terminals. Released neurotransmitters might cause modulation of the membrane conductance in taste cells that leads to generation of the slow potentials. The present data suggest that slow HPs and slow DPs evoked in the taste cells of atropinized frogs by GP nerve stimulation are induced by putative neurotransmitters in the taste disc.

Introduction

A great number of fungiform papillae are distributed on the dorsal surface of the frog tongue. Each fungiform papilla has one taste disc at the top. Eight to 12 myelinated afferent fibres (Rapuzzi and Casella, 1965; Sato *et al.*, 1983) and many unmyelinated fibres (Graziadei and DeHan, 1971; Inoue and Kitada, 1991) innervate each taste disc. A taste disc is composed of several kinds of cells including taste cells. The physiological response properties of taste cells and myelinated gustatory nerve fibres in frogs to tastants have been studied extensively (Sato, 1976; Sato *et al.*, 1995).

Efferent nerve fibres innervating the cells control the sensitivity of hair cells in the auditory and vestibular organs (Furukawa, 1981). The existence of efferent synapses in taste cells has been suggested morphologically and physiologically (Brush and Halpern, 1970; Esakov and Bykov, 1971; DeHan and Graziadei, 1973; Yoshie *et al.*, 1996; Reutter *et al.*, 1997). However, postsynaptic potentials from the efferent synapses of taste cells have seldom been recorded. It has been reported that, when the glossopharyngeal (GP) nerve of the frog is stimulated, a slow potential similar to the postsynaptic potential appears in a taste cell (Esakov and Bykov, 1971; Kutyna and Bernard, 1977; Sato *et al.*, 2000, 2001a). However, this slow potential is not the synaptic potential but is instead due to a large liquid junction potential generating between the lingual

gland saliva, which is secreted by activation of parasympathetic nerve fibres and the lingual adapting solution (Sato *et al.*, 2000, 2001a). After the large physicochemical potential evoked in a frog taste cell is completely inhibited by blocking activity of the lingual gland cells with atropine, a small hyperpolarizing potential similar to the slow postsynaptic potential is elicited in taste cells by GP nerve stimulation (Sato *et al.*, 2001a). The purpose of the present experiments was to examine the properties of putative slow synaptic potentials induced in the taste cells of atropinized frogs. The preliminary report of the present experiments has been presented elsewhere (Sato *et al.*, 2001b).

Materials and methods

Preparation

Thirty-two bullfrogs (*Rana catesbeiana*) of 320–650 g were used during a period between June and November. The experiments were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University. The animals were deeply anaesthetized by intraperitoneally injecting a 50% urethane–Ringer solution at a dose of 3 g/kg body weight. The hypoglossal nerves and hyoglossal and geniohyoid muscles were bilaterally severed in order to remove spontaneous contractions of the tongue muscles.

The animals were placed in a supine position on a lucite plate and their tongues were pulled out as far as possible and pinned down on a silicone rubber plate. The tongues were always adapted to Ringer solution. Blood supply to the tongues through the lingual arteries and veins was maintained as long as possible. The whole GP nerve of either side was separated out from the surrounding tissues, cut centrally and immersed into mineral oil. All experiments were carried out at room temperature (24–27°C). At the end of the experiments the brains and spinal cords of the animals were completely destroyed.

Recordings and stimulations

Intracellular recordings were made from taste cells in the taste disc of the fungiform papillae on the tongue with a 3 M KCl-filled glass capillary microelectrode (30–65 M Ω). An indifferent electrode of chlorinated silver wire was inserted into the muscles of the forelimb.

The criteria for identifying intracellular penetration of single taste cells were as follows. The taste disc of bullfrogs is 200–300 μ m wide and 60–70 μ m thick and is divided into three layers: the superficial, intermediate and basal layers (Jaeger and Hillman, 1976). The cell bodies of taste cells are located in the middle and bottom parts of the intermediate layer. The cell bodies of wing cells (non-sensory cells) in the taste disc are also located in the upper part of the intermediate layer (Richter *et al.*, 1988; Witt, 1993; Osculati and Sbarbati, 1995). In order to avoid penetration of a microelectrode into a wing cell the microelectrode tip was vertically advanced within the intermediate layer between 30 and 50 μ m from the taste disc surface. As the cell bodies of basal cells in the basal layer are located at the periphery of the taste disc (Osculati and Sbarbati, 1995) the microelectrode tip was not penetrated into the peripheral portion of the taste disc. When the microelectrode was inserted into the taste cells, the usually negative potential shift of the resting potential showed a deflection of two or three steps because taste cell penetration occurred after passing through one or two taste disc cells having different resting potentials. Most of the penetrated taste cells responded to 0.5 M NaCl and 1 mM acetic acid with depolarizing or hyperpolarizing responses (Sato *et al.*, 1995).

The membrane potentials led off from the taste cell were amplified with a microelectrode amplifier (Nihon Kohden MEZ-8101, Tokyo, Japan) and recorded on a pen recorder. A bridge circuit housed in the amplifier was used for simultaneous current injection and membrane potential recording when measuring the input resistance and reversal potential for a slow potential in a taste cell. The bridge was balanced completely before the penetration of a taste cell in order to cancel the resistance of the microelectrode. In order to perform measurement of the membrane potential the potential difference between an intracellular microelectrode and an indifferent electrode was recorded. The potential

drop across a resistor was used for monitoring the current intensity injected through the microelectrode.

Anti-dromically conducting action potentials in afferent fibres evoked by stimulation of the GP nerve were recorded from a fungiform papilla in some experiments with a glass suction electrode of ~150 μ m internal diameter. The suction electrode was filled with a frog Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂ and 5 mM HEPES, pH 7.2). An indifferent chlorinated silver wire electrode was glued to the outside of the suction electrode. The action potentials were displayed on an oscilloscope.

Repetitive pulses with a pair of chlorinated silver wire electrodes suspending the nerve electrically stimulated the whole GP nerve, as the slow potential of a taste cell to a single stimulation was very small. The electrical pulses were 0.01 or 0.1 ms in duration and were changed from 0.1 to 30 V in strength. Usually repetitive pulses at a frequency of 30 Hz were given for 5–10 s in order to obtain the maximal slow potential.

The latency of slow potentials evoked in the taste cells by train stimulation was measured as the time between the start of the stimulation and the onset of a slow potential. The amplitude of slow potentials was measured at the peak.

Measurement of the velocity of capillary blood flow

The fungiform papillae were moved sidelong on the tongue surface by a small brush. The velocity of the blood flow in the capillary running along the stalk part of the fungiform papilla was measured by observing the movement of single red blood cells using an ocular micrometer and a stopwatch. It was difficult to pursue the movement of single red blood cells exactly when the velocity of the blood flow was very rapid during the 3 h after the start of the experiments because the density of red blood cells in the capillaries was very high. In order to correct the more rapid velocity of the blood flow measured during the intracellular recordings, approximately half of the entire blood volume in two frogs was exchanged with an equivalent volume of frog Ringer solution, which consisted of 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 5 mM HEPES, 20 mM glucose and 5% (w/v) dextran (Pharmacia A B, Uppsala, Sweden) (pH 7.2). The velocity of the blood flow was then measured in the capillary containing a reduced number of red blood cells.

Experimental procedure

When the GP nerve was strongly stimulated at 30 Hz, large non-synaptic slow potentials appeared on the tongue surface and in the taste cells of the fungiform papillae (Sato *et al.*, 2000, 2001a). These potentials were due to a liquid junction potential generating between saliva secreted from the lingual glands and lingual adapting solution (Sato *et al.*, 2000, 2001a). In order to remove these large non-synaptic potentials, which masked a questioned slow potential appearing in the putative efferent synapses of the taste cell, 1 mg/kg atropine sulphate (Tanabe Seiyaku, Osaka, Japan)

was injected intravenously at the beginning of each experiment. Disappearance of the slow liquid junction potentials lasted more than 9 h after the drug injection (Sato *et al.*, 2000). Intracellular recordings from the taste cells were started 30 min after the drug injection. It was confirmed every hour after the start of intracellular recordings from the taste cells that no slow extracellular potentials were elicited on the tongue surface by GP nerve stimulation in each atropinized frog.

Statistics

All data were expressed as mean \pm SEM values. The level of significance was set at $P < 0.05$ using Student's *t*-test.

Results

Relationship between capillary blood flow and the two types of slow potential

Since the animals' tongues were pulled out as far as possible and fixed on a plate, the velocity of the lingual blood circulation was gradually reduced in various time courses. Figure 1 shows the time course of the reduction in the velocity of the capillary blood flow in the fungiform papillae (Figure 1A) and the type and number of slow potentials recorded from the taste cells during the experiments in response to GP nerve stimulation (Figure 1B). The potentials recorded were either slow hyperpolarizing potentials (slow HPs) (Figure 2A) or slow depolarizing potentials (slow DPs) (Figure 2B). We were able to record slow potentials repeatedly every 30 min or 1 h for up to 8 h. The resting potential in the taste cells ranged from -20 to -50 mV. The time course of the capillary blood flow illustrated in Figure 1A is a typical example. The velocity of the blood flow was reduced to half of the initial velocity 4 h after the start of the recordings. During the first 4 h the slow potentials were induced in a hyperpolarizing direction, but after 4 h slow DPs appeared and gradually dominated the slow potentials (Figure 1B). When the capillary blood flow in Figure 1A was reduced to less than 20% of the initial level after 4.5 h, the amplitude of the resting potentials was decreased by only 15% (Figure 1C). The receptor potentials induced in the taste cells by 0.5 M NaCl for 2 h between 4.5 and 6.5 h after the start of the recordings were decreased in amplitude by only 10% of the initial value (Figure 1C). After a complete arrest of the blood flow no slow potentials were recorded any more. A great reduction in the lingual blood flow unusually occurred shortly after the beginning of the experiment and all the slow potentials in the taste cells at that time were slow DPs.

Properties of the slow potentials

When the GP nerve was stimulated at various frequencies with pulses of 0.1 ms duration and 15 V intensity, the slow HP recorded from a taste cell gradually increased in amplitude with increasing frequency of stimulation and reached a

maximum at 30–40 Hz (Figure 3A). This increment was due to summation of single slow potentials as seen in the inset of Figure 3A. The summation occurred above a frequency of 0.8 Hz. The same summation occurred in the slow DP. In the slow HPs of Figure 3A the latency between the start of the first stimulus and the onset of slow potentials was almost the same at approximately 0.8 s on single and train stimulation. When the GP nerve was stimulated by 30 Hz pulses of 0.1 ms duration with increasing stimulus intensity, the amplitude of the slow HP in a taste cell increased in a step-like manner (Figure 3B). The changes in the amplitude of the slow HPs in 10 taste cells examined occurred in one step (five cells), two steps (three cells) or three steps (two cells). This suggests that the slow HP was induced by a group of fibres with different thresholds. GP nerve stimulation with increasing intensities caused the amplitude of slow DPs to change in a step-like manner: of five taste cells examined three cells showed one step and two cells two steps.

Intracellular recordings were obtained from 1039 taste cells in 27 preparations in which both types of slow potentials appeared in response to GP nerve stimulation at 30 Hz for 5–10 s. Out of those cells 45% ($n = 466$) showed slow HPs and 20% ($n = 208$) slow DPs, whereas 35% ($n = 365$) did not respond to GP nerve stimulation.

The latency, duration and amplitude of the slow potentials were measured using data obtained for 2 h after the appearance of slow HPs or slow DPs. The mean latency was 1.4 ± 0.5 s ($n = 148$) for slow HPs and 1.6 ± 0.8 s ($n = 54$) for slow DPs. No difference was found between them ($P > 0.1$). The mean duration was 14.9 ± 0.8 s ($n = 54$) for slow HPs and 15.8 ± 0.8 s ($n = 27$) for slow DPs. No difference existed between them ($P > 0.1$). The mean amplitude was -2.1 ± 0.2 mV ($n = 153$) for slow HPs and 2.0 ± 0.8 mV ($n = 57$) for slow DPs.

Resistance change

The input resistance of single taste cells adapted to a Ringer solution was in the range of 20–80 M Ω (Sato and Beidler, 1975). The input resistance of the taste cell gradually increased during the generation of a slow HP and reached a maximum at the peak of the slow HP. As the maximum amplitude of the slow HP was gradually decreased, the membrane resistance gradually recovered (Figure 4A). In contrast, the membrane resistance of a taste cell gradually decreased during the development of a slow DP by GP nerve stimulation, reached a minimum at the peak and then gradually recovered to the control level (Figure 4B). The relationships between the amplitudes of the slow potentials and the changes in the taste cell resistances are shown in Figure 5. The mean increase in the resistance during slow HPs was $\sim 220\%$ at a 5 mV hyperpolarization (Figure 5A) and the mean decrease in the resistance during slow DPs was 55% at a 5 mV depolarization (Figure 5B). Slow potentials were always accompanied by changes in the membrane

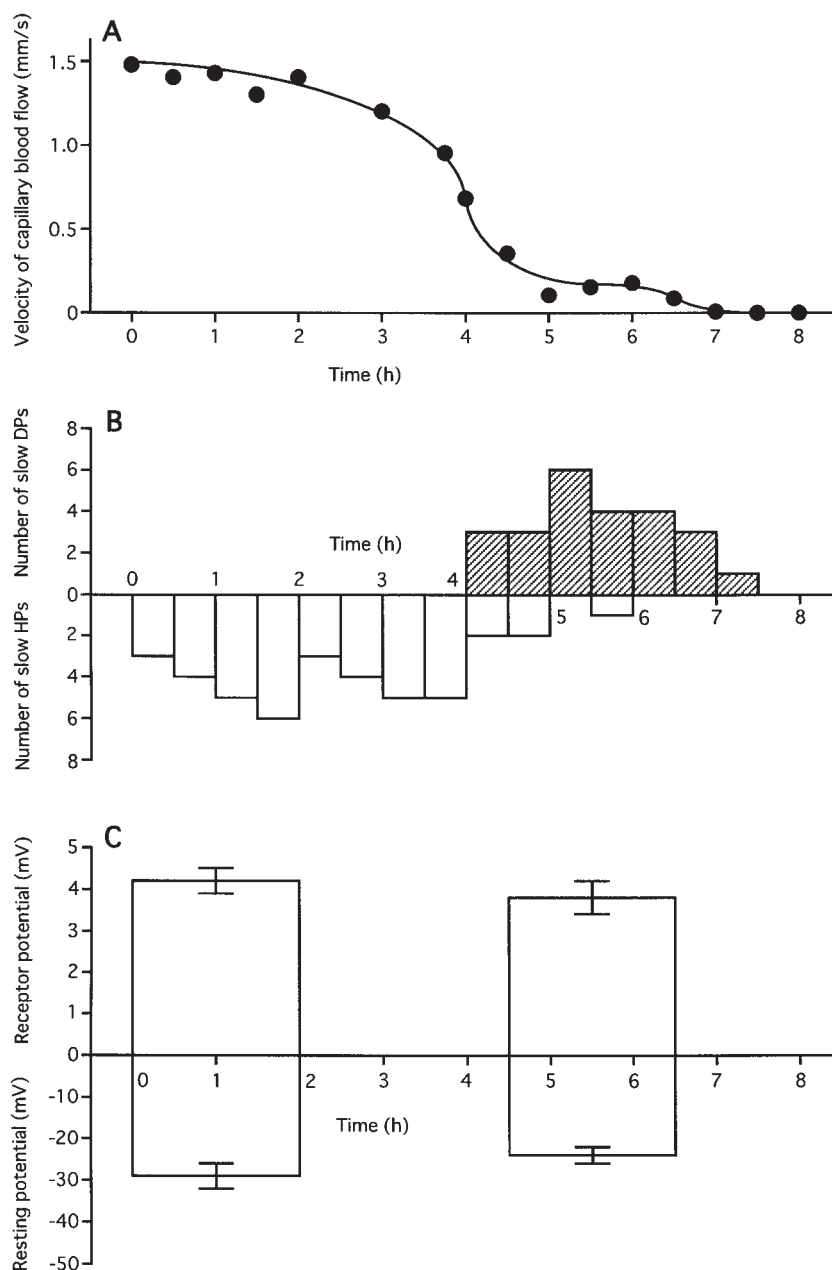


Figure 1 Relationship between the velocity of capillary blood flow, the occurrence of two types of slow potential induced by GP nerve stimulation and receptor potentials induced by 0.5 M NaCl in frog taste cells after the start of intracellular recordings. **(A)** Time course of the change in the velocity of the capillary blood flow in the fungiform papillae of a frog tongue after the beginning of the experiment. Each point is the mean from two to four measurements. **(B)** Number of slow HPs and slow DPs sampled from taste cells of the same tongue in successive times after the beginning of the experiment. **(C)** Receptor potentials induced by 0.5 M NaCl and resting potentials measured during 0–2 h and 4.5–6.5 h after the start of the experiment. The taste cells sampled were four cells for the first 2 h and five cells for the next 2 h. The GP nerve was stimulated at 30 Hz for 5–10 s with pulses of 0.1 ms duration and 15 V strength. All data were obtained from one frog.

resistance. This suggests that slow potentials are not induced by DC potentials of exogenous origin, but induced as intrinsic potentials, possibly by a transmitter released from the nerve terminals.

Reversal potential

The relationship between the membrane potential level and

the amplitude of the slow potentials evoked by GP nerve stimulation was measured. Figure 6A shows the changes in GP nerve-induced slow potentials by applied current in two taste cells generating a slow HP or a slow DP. Figure 6B shows the magnitudes of two slow HPs and two slow DPs plotted against the membrane potential level. The slow HP increased in amplitude as the membrane potential was

shifted in a hyperpolarizing direction (Figure 6B, solid triangles and stars). However, the amplitude of the slow HP decreased, reached a null value and finally changed into positive values as the membrane potential was shifted in a depolarizing direction. The reversal potentials for the slow HPs were obtained by extrapolating experimental points and the mean value was -13 ± 2 mV ($n = 6$ and range -9 to -20 mV). The behaviour of slow DP during change in the membrane potential was the opposite of that of the slow HPs (Figure 6B, solid circles and squares). The amplitudes of the slow DPs induced by GP nerve stimulation increased with cell hyperpolarization. There was a mean reversal point of the slow DPs of -11 ± 2 mV ($n = 7$ and range -10 to -14 mV). There was no difference between the reversal potentials for the slow HPs and slow DPs ($P > 0.1$). This suggests that the same ionic channels are involved in generating two types of slow potentials.

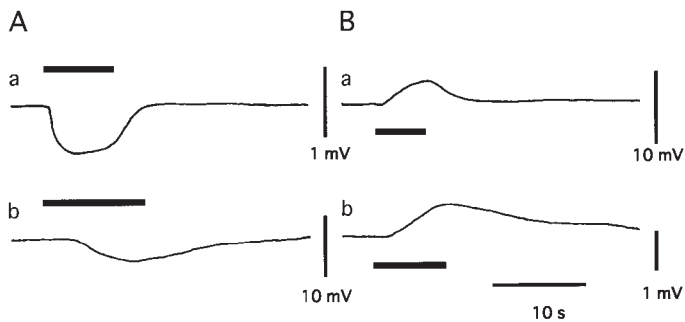


Figure 2 Intracellular recordings of slow potentials from frog taste cells. **(A)** Slow HPs. **(B)** Slow DPs. (a) An example of a relatively fast time course. (b) An example of slower time course. The horizontal bar above or underneath each response is the period of repetitive stimulation of the GP nerve at 30 Hz with pulses of 0.1 ms duration and 15 V strength. Four responses were obtained from four different taste cells.

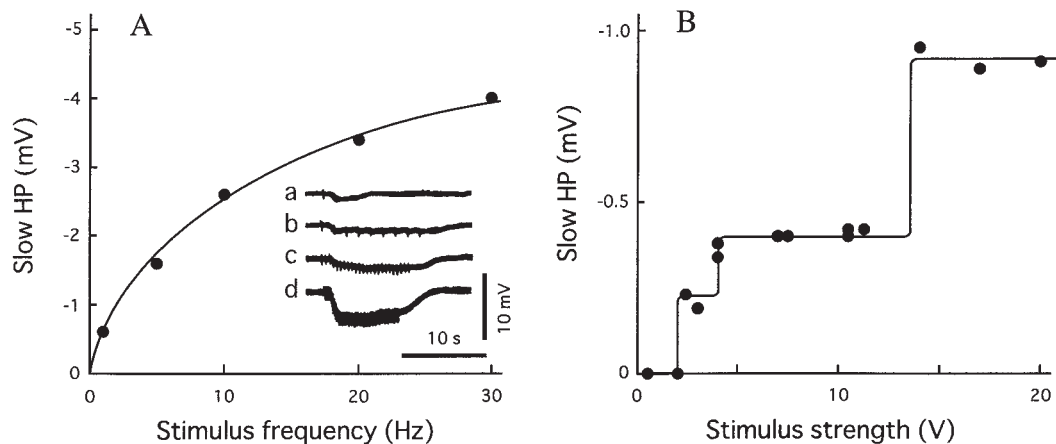


Figure 3 Stimulus frequency-dependent and stimulus strength-dependent slow HPs in frog taste cells. **(A)** Relationship between the amplitude of slow HPs and the stimulus frequency. The pulses for GP nerve stimulation were of 0.1 ms duration and 15 V strength. The inset shows slow HPs in a taste cell in response to (a) a single stimulus and (b) 0.8, (c) 5.0 and (d) 20 Hz stimulations. Stimulus artefacts are superimposed on the slow potentials. **(B)** Relationship between the amplitude of slow HPs and the stimulus strength. Pulses of 0.1 ms duration were applied to the GP nerve at 30 Hz. The data for (A) and (B) were obtained from two different taste cells.

Efferent fibre type innervating the taste cells

The taste disc is innervated by eight to 12 myelinated afferent fibres, which respond to mechanical or gustatory stimuli (Sato, 1976; Sato *et al.*, 1983) and many unmyelinated fibres, the origin and function of which are still unclear (Jaeger and Hillman, 1976). In order to determine the type of putative efferent fibres innervating taste cells, the electrical threshold of the efferent fibres was compared with that of the myelinated afferent fibres. The electrical thresholds of myelinated afferent fibres innervating the taste disc were measured by recording anti-dromic action potentials from a sucked fungiform papilla while stimulating the GP nerve with single pulses of 0.1 ms duration (Sato *et al.*, 2000). The mean threshold of the myelinated afferent fibres was 0.20 ± 0.01 V (range 0.06–0.50 V for 99 afferent fibres from 10 papillae) and the mean conduction velocity was 14.0 ± 0.7 m/s (range 4.2–23.0 m/s for 99 afferent fibres). The afferent fibres showing these velocities belong to the fibre type of A β –A δ . No slow potentials were obtained from the taste cells ($n = 28$) by stimulating the GP nerve with 30 Hz pulses of 0.06–0.50 V.

The electrical thresholds of possible efferent fibres generating slow potentials in the taste cells were measured by recording slow potentials (four slow HPs and three slow DPs) while stimulating the GP nerve with 30 Hz pulses of 0.1 ms duration. The threshold voltage of the GP efferent fibres was 4.9 ± 1.2 V (range 1.5–15.0 V for seven taste cells of seven different taste discs). The electrical threshold of efferent fibres eliciting the slow potentials in the taste cells was significantly much higher than that for eliciting action potentials of the myelinated afferent fibres of type A β –A δ ($P < 0.01$). This indicates that the efferent fibres innervating the taste cells are of a C type (Sato *et al.*, 2000).

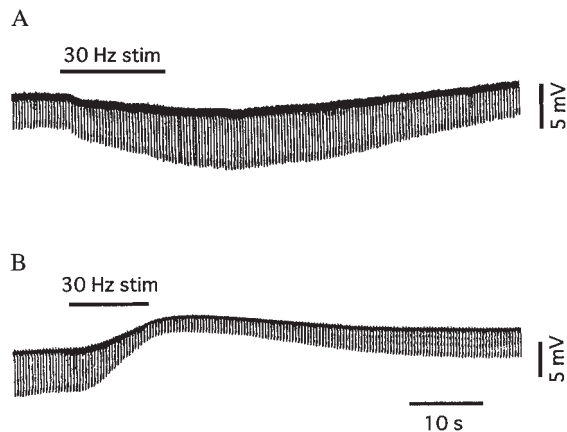


Figure 4 Change in the input resistance of a taste cell during generation of slow HPs and slow DPs. **(A)** Slow HP. **(B)** Slow DP. The GP nerve was stimulated at 30 Hz with pulses of 0.1 ms duration and 15 V strength (horizontal bar above the records). The resistance changes were measured by applying hyperpolarizing pulses. The data for (A) and (B) were obtained from different cells.

Discussion

In the present experiments, a microelectrode tip was vertically advanced into the central portions of the intermediate layer of a taste disc between 30 and 50 μm from the taste disc surface. The microelectrode tip probably penetrated the cell body of two kinds of taste cell, one with a rod-like process and one with a microvilli-bearing process (Richter *et al.*, 1988; Witt, 1993; Osculati and Sbarbati, 1995) or the cell body of a sustentacular cell (Jaeger and Hillman, 1976; Osculati and Sbarbati, 1995).

No slow potentials were recorded from 35% of 1039 cells examined in the intermediate layer of the taste discs. Those cells probably included sustentacular cells and a group of taste cells that were devoid of efferent innervation. Since sustentacular cells in the intermediate layer of taste discs have been reported to have a small diameter (Jaeger and Hillman, 1976; Osculati and Sbarbati, 1995), we presumed that the microelectrode mostly impaled taste cells having a larger diameter.

It is well known that there are non-selective cation channels in various kinds of cell (Siemen, 1993). Since the equilibrium potentials of Na^+ and K^+ in the frog taste cell have been estimated as 63 and -101 mV respectively (Sato *et al.*, 1984; Miyamoto *et al.*, 1993), the reversal potentials of -11 mV for GP nerve-induced slow DPs in the frog taste cells lie between the equilibrium potentials of K^+ and Na^+ . In a frog taste cell non-selective cation channels are spread throughout the whole membrane including the apical receptive surface and the proximal processes (Fujiyama *et al.*, 1993; Okada *et al.*, 1994). A permeability ratio of $P_{\text{K}}/P_{\text{Na}}$ of approximately 1.5 has been measured in the frog taste cell membrane (Fujiyama *et al.*, 1993; Okada *et al.*, 1994; Sato *et al.*, 1995). By using this permeability ratio, estimated intracellular concentrations of K^+ and Na^+ and

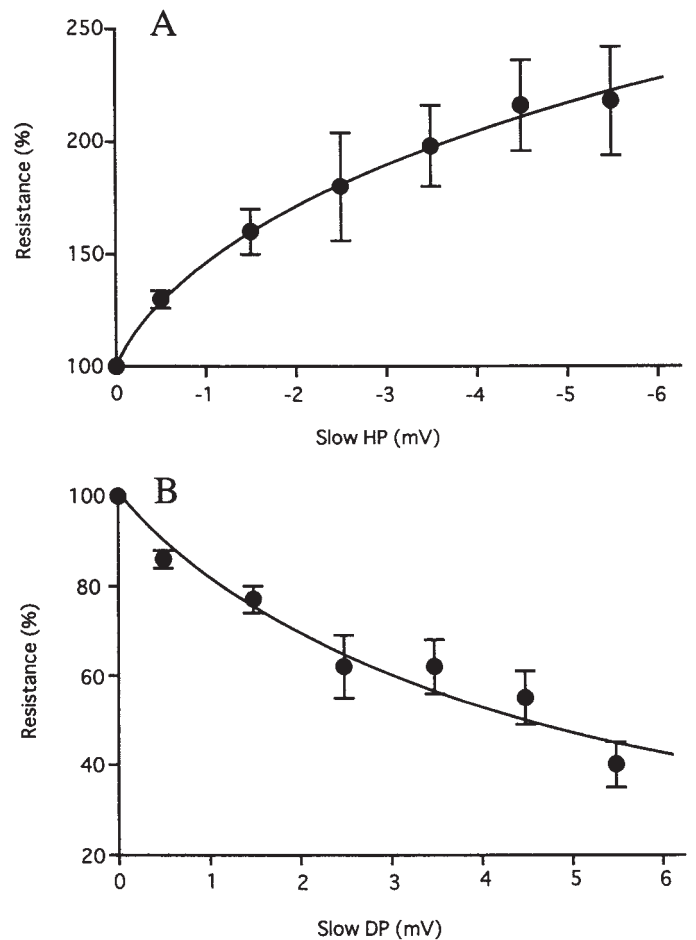


Figure 5 Relationship between the amplitudes of slow potentials and the amplitudes of membrane resistance in taste cells. **(A)** Slow HP. **(B)** Slow DP. The membrane resistance on the ordinate is presented as a percentage of the control input resistance of a taste cell without stimulation of the GP nerve. The resistances were measured at the peaks of slow potentials and indicated as the mean values from nine to 50 taste cells in slow HPs and from eight to 27 cells in slow DPs. The GP nerve was stimulated at 30 Hz with pulses of 0.1 ms duration and 15 V strength.

an ionic composition of Ringer solution, the reversal point for slow DPs in frog taste cells has been calculated as -12 mV from the Goldman-Hodgkin-Katz equation (Hille, 1984), which is close to our measured value. The slow DPs in taste cells are likely to be elicited by a diffusion of Na^+ and K^+ through the non-selective cation channels.

The slow HP in the taste cells that was induced by GP nerve stimulation increased in amplitude with cell hyperpolarization and reversed at -13 mV. Therefore, the slow HP could be explained by a mechanism involving a reduction in the membrane non-selective ionic conductance through which both Na^+ and K^+ are primarily permeated. In the frog, a taste cell membrane region underneath putative efferent nerve terminals would result in a large decrease in the non-selective cation conductance through which an outward current would flow. The same mechanism has been

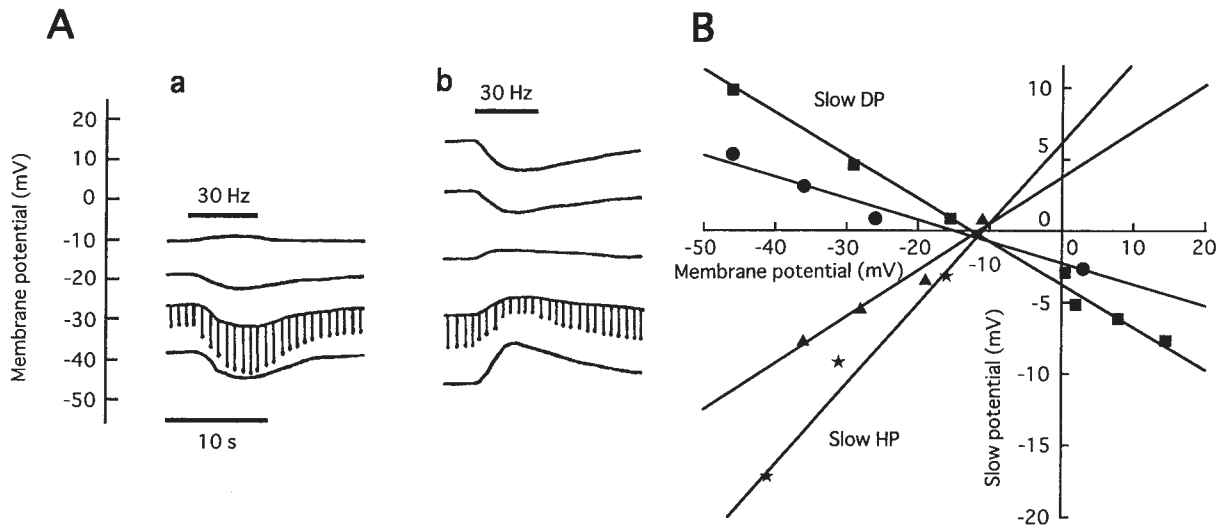


Figure 6 Relationship between the membrane potential level and the amplitude of slow potentials in taste cells. **(A)** Changes in the amplitudes of (a) slow HPs in a cell and (b) slow DPs in another cell by alteration of the membrane potential with applied currents. The upper bar denotes 30 Hz stimulation of the GP nerve with pulses of 0.1 ms duration and 15 V strength. Change in the membrane resistance was measured using hyperpolarizing pulses only in slow potentials induced at resting potentials of (a) -28 mV and (b) -29 mV. **(B)** The amplitudes of slow potentials plotted against membrane potentials. The data were obtained from two taste cells showing slow HPs (solid triangles and stars) and two other cells showing slow DPs (solid circles and squares). The data for the solid triangles and squares are shown in (a) and (b) of (A) respectively.

found in molluscan neurons (Gerschenfeld and Paupardin-Tritsch, 1974).

Large slow potentials with electropositivity are evoked on the tongue surface in non-atropinized frogs by stimulation of GP parasympathetic fibres (Sato *et al.*, 2000). In addition, non-synaptic slow DPs are evoked in taste cells by an outward current from the extrinsic electric source on the tongue, which is generated between saliva secreted from the lingual glands and lingual fluid (Sato *et al.*, 2000, 2001a). In the present experiments, these extrinsic slow potentials were completely inhibited by blocking muscarinic acetylcholine receptors in the lingual glands with atropine. Therefore, no slow potentials, as observed previously (Sato *et al.*, 2000), were evoked between the top and bottom of the fungiform papillae by GP nerve stimulation. The lingual gland-derived slow depolarizing potential in the taste cells is not accompanied by membrane conductance change and does not have reversal potentials (Kutyna and Bernard, 1977), whereas the slow HPs and slow DPs in this study were accompanied by a conductance change and had a reversal potential of -11 to ~ -13 mV. The time course of the lingual gland-derived slow potentials in the taste cells was approximately 20% shorter than that of the slow HPs and slow DPs. Under normal blood circulation the lingual gland-derived slow potentials are depolarizing, but after block of lingual gland activity the slow potentials are hyperpolarizing. The mean amplitude of the former is 4.4 mV (Sato *et al.*, 2000), but that of the latter is -2.1 mV. Therefore, the characteristics of lingual gland-derived slow potentials in the taste cell are quite different from those of the intrinsic slow potentials in the taste cells that were induced in this study.

In order to clarify that slow potentials recorded from taste cells in atropinized frogs are postsynaptic, the following basic criteria must be checked: (i) disappearance of the slow potential by application of Ca^{2+} -free solution to the pre-synaptic terminals, (ii) appearance of the slow potential by application of a transmitter candidate to the synaptic region and (iii) disappearance of the slow potential by application of antagonists to the synaptic region. These criteria have not yet been tested because of experimental limitation and non-detection of transmitter candidates and receptor antagonists. Although our findings are circumstantial, they suggest that the slow HPs and slow DPs induced by stimulating unmyelinated efferent fibres in the GP nerve are generated in the taste cells by neurotransmitters released from the efferent fibre terminals. Another possibility is that slow HPs and slow DPs could be evoked by neuromodulators released from remote regions.

The long latencies and slow time courses of postsynaptic potentials are well known in various synapses in the autonomic nervous system. Successive activation of metabotropic receptors, G-proteins, second messengers, protein kinases and ion channels are thought to mediate slow potentials (Kuno, 1995). Therefore, the long latencies and slow time courses of the HPs and DPs evoked in taste cells are also presumed to be due to the successive activation of receptors, G-protein, intracellular messengers, functional protein and ion channels as mentioned above.

Efferent innervation of sensory cells is known in hair cells in the hearing organ and the vestibular organ (Furukawa, 1981) and intrafusal fibres in the muscle spindle (Ganong, 1999). Myelinated efferent fibres innervate outer hair cells,

whereas unmyelinated efferent fibres innervate inner hair cells (Nakajima and Wang, 1974) and gamma efferent fibres innervate the muscle spindle. In the frog taste cells the putative efferent fibres are unmyelinated C fibres.

Efferent fibres usually reduce the sensitivity of a hair cell's response to sound (Furukawa, 1981), whereas the sensitivity of muscle spindles in response to stretch is elevated by gamma efferents (Ganong, 1999). In frog taste cells, where both slow DPs and slow HPs were induced by GP nerve stimulation, gustatory sensitivity may be modulated by these slow potentials. Since slow DPs appeared under a slower blood flow, it is supposed that the gustatory sensitivity of frog taste cells is elevated by slow DPs when the temperature in the living environment becomes lower. On the other hand, since slow HPs appeared under a higher blood flow, gustatory sensitivity is likely to be reduced by slow HPs when the environmental temperature becomes higher.

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