Chem. Senses 26: 79–84, 2001

Non-synaptic Transformation of Gustatory Receptor Potential by Stimulation of the Parasympathetic Fiber of the Frog Glossopharyngeal Nerve

Toshihide Sato, Takenori Miyamoto, Yukio Okada and Rie Fujiyama

Department of Physiology, Nagasaki University School of Dentistry, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan

Correspondence to be sent to: Toshihide Sato, Department of Physiology, Nagasaki University School of Dentistry, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan. e-mail: toshi@net.nagasaki-u.ac.jp

Abstract

When the glossopharyngeal nerve (GP) in the frog was strongly stimulated electrically, slow potentials were elicited from the tongue surface and taste cells in the fungiform papillae. Injection of atropine completely blocked these slow potentials. The present and previous data indicate that the slow potentials induced in the tongue surface and taste cells are due to a liquid junction potential between saliva secreted from the lingual glands due to parasympathetic fiber activity and an adapting solution on the tongue surface. Intracellularly recorded depolarizing receptor potentials in taste cells induced by 0.5 M NaCl and 3 mM acetic acid were enhanced by depolarizing slow potentials induced by GP nerve stimulation, but were depressed by the hyperpolarizing slow potentials. On average, the receptor potential of taste cells for 0.5 M NaCl was increased by 25% by the GP nerve-induced slow potential, but the receptor potential of taste cells for 3 mM acetic acid was decreased by 1% by the slow potential. These transformations of receptor potentials in frog taste cells were not due to a synaptic event initiated between taste cells and the efferent nerve fiber, but due to a non-synaptic event, a lingual junction potential generated in the dorsal lingual epithelium by GP nerve stimulation.

Introduction

An extracellular slow potential is induced on the frog tongue surface by electrical stimulation of the glossopharyngeal (GP) nerve (Esakov and Byzov, 1971; Kutyna and Bernard, 1977; Sato *et al.*, 2000). Similar intracellular slow potentials are also recorded in the disk cells such as supporting and taste cells of the frog fungiform papillae following GP nerve stimulation (Esakov and Byzov, 1971; Kutyna and Bernard, 1977; Sato *et al.*, 2000). Recently we have proposed that the origin of the extracellular slow potential on the tongue surface and the intracellular slow potential of taste cells is due to a liquid junction potential between saliva secreted from the lingual glands (Nalavade and Varute, 1971; Albanese Carmignani *et al.*, 1975) due to stimulation of parasympathetic nerve fibers and an adapting solution on the tongue surface (Sato *et al.*, 2000). This conclusion arises from: (i) slow potentials in the lingual surface and taste cells are completely blocked by atropine; (ii) the amplitude and polarity of the slow potentials are changed by the kind of adapting solution on the tongue surface; (iii) electrical activities of effector cells innervated by parasympathetic nerve fibers are not recorded by a microelectrode positioned on the dorsal tongue surface. Therefore, the intracellular slow potential in taste cells is not initiated by a synaptic event occurring between the taste cell and an efferent fiber in the GP nerve, but by a passive electric current flowing from the slow potential generating source which exists underneath the dorsal lingual epithelium.

There are numerous lingual glands along the whole dorsal surface of the frog tongue (Albanese Carmignani *et al.*, 1975). The frog lingual glands are innervated by parasympathetic post-ganglionic fibers in the GP nerve (Gaupp, 1904) whose transmitter is acetylcholine. Therefore, when muscarinic acetylcholine receptors in lingual gland cells are completely blocked by atropine, GP nerve-induced slow potentials arising from the liquid junction potential disappear (Sato *et al.*, 2000). This situation may indicate that saliva is no longer secreted from the lingual glands.

The present experiment is aimed at determining if tastant-induced receptor potentials are non-synaptically altered by slow potentials induced by GP nerve stimulation.

Materials and methods

Sixteen bullfrogs (*Rana catesbeiana*) weighing 185–510 g were used in the experiments. The animals were anesthetized by i.p. injection of 50% urethane–Ringer solution (3 g/kg body wt). The hyperglossal nerves of either side were severed to avoid contraction of the tongue muscles. Normal blood circulation to the tongue through the lingual arteries and veins was maintained to obtain reproducible responses

from the tongue. The whole tongue was pulled out from the mouth and pinned to a cork plate in the experimental chamber. The whole GP nerve of either side was separated from the surrounding tissues, cut centrally and immersed in mineral oil.

The whole GP nerve was electrically stimulated with a pair of chlorinated silver wires lifting the nerve. Electrical stimulation of the GP nerve was usually with repetitive pulses at 30 Hz (0.1 ms duration and 12 V). Extracellular electrical recordings from the tongue surface and intracellular recordings from taste cells in the fungiform papillae were achieved with glass microelectrodes. The microelectrodes were filled with 3 M KCl and had a resistance of 30–60 M. An indifferent chlorinated silver wire electrode was positioned in the forelimb muscles. Electrical responses from the tongue surface and taste cells were amplified with a microelectrode DC amplifier (MZ 10; Nihon Kohden, Tokyo, Japan) and recorded with a pen recorder. Criteria for taste cell penetration were the same as those described previously (Sato and Beidler, 1975).

The tongue surface was usually adapted to normal Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.2) and rinsed with Ringer after taste stimulation. Taste and Ringer solutions were applied to the tongue surface at a rate of 0.13 ml/s with a semi-automatically controlled gustatory stimulator (Sato, 1972). Taste stimuli used were 0.5 M NaCl and 3 mM acetic acid, which were prepared with deionized water (reagent grade water; Millipore, Bedford, MA). All experiments were carried out at room temperature (22–26°C).

The tongues of three bullfrogs were used for histological sections. The tongues were fixed in Bouin solution, routinely embedded in paraffin, transversly cut into 10 μm serial sections and stained with hematoxylin and eosin.

Results

Distribution of lingual glands

In transverse sections of the tongue the tops of the corpus glandulae of the lingual glands existed at mean depths of 20 and 100 μm from the dorsal surface. The lingual glands were of simple tubular or ramificate tubular type. The length of the tubular glands was 121 ± 4 μm (mean ± SE, n = 49). The lingual glands were almost equally distributed across the whole dorsal surface of the tongue excepting the periphery. The mean number of the glands was 220/mm^2. Considering the mean area of the tongue surface (648 mm^2) and a 20% decrease in lingual glands in the periphery of the tongue, the total number of lingual glands was estimated to be 14 000 per tongue.

Properties of slow potentials from the tongue surface and taste cells

When parasympathetic fibers in the frog GP nerve were strongly stimulated at a frequency of 30 Hz, slow potentials were recorded from the whole dorsal tongue surface with an extracellular microelectrode which was positioned on the tops of the fungiform and filiform papillae and between the papillae (Sato *et al.*, 2000). The time course of the extracellularly recorded slow potentials was very similar to that of intracellularly recorded slow potentials from the taste cells, as shown in the inset of Figure 1. Extracellular slow potentials from the tongue surface following GP nerve stimulation showed surface positivity in 31 of 33 recordings (94%) using three frogs and surface negativity in two recordings (6%) when the tongue was adapted to normal Ringer solution. The mean amplitudes of the extracellular slow potentials measured from three different areas of the tongue surface were 7.7 ± 1.5 (mean ± SE) mV (n = 9) in the apical portion, 6.6 ± 0.8 mV (n = 15) in the middle portion and 7.0 ± 1.3 mV (n = 9) in the proximal portion. In a total of 31 extracellular recordings the mean amplitude of the slow potentials was 7.1 ± 0.7 mV, with a range of –2.1 to 18.8 mV. No significant differences were found among the amplitudes of slow potentials obtained from the three different portions ($P > 0.05$).

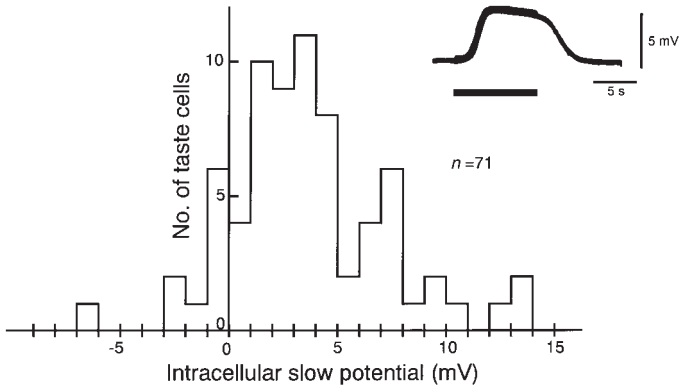

Figure 1 Distribution of intracellular slow potentials in 71 taste cells of the fungiform papillae induced by GP nerve stimulation. The tongue was adapted to normal Ringer. The inset shows an example of a depolarizing slow potential in a taste cell induced by GP nerve stimulation (horizontal bar, 30 Hz).

Of 71 taste cells examined with intracellular microelectrodes, depolarizing slow potentials in response to GP nerve stimulation were found in 61 cells (86%) and hyperpolarizing slow potentials in five cells (7%) when the tongue was adapted to Ringer (Table 1). Figure 1 illustrates the amplitude distribution of intracellularly recorded slow potentials of 71 taste cells in the fungiform papillae of five frogs when the GP nerve was stimulated at 30 Hz. The mean amplitudes of the intracellular slow potentials from taste cells were 3.4 ± 0.5 mV (n = 34) in the apical portion, 3.6 ± 0.6 mV (n = 24) in the middle portion and 3.7 ± 0.7 mV (n = 13) in the proximal portion. In a total of 71 taste cells the mean amplitude of the intracellular slow potentials was 3.6 ± 0.6 mV, with a range of –6.1 to 13.5 mV. No sig-

Table 1 Type of GP nerve-induced intracellular slow potentials in taste cells during application of Ringer, NaCl and acetic acid to the tongue

Adapting or stimulus solution	Positive change		Negative change		No change		Total	
	n	%	*n*	%	*n*	%	*n*	%
Ringer	61	86	5	7	5	7	71	100
0.5 M NaCl	20	63	8	25	4	12	32	100
3 mM Acetic acid	5	56	4	44	0	0	9	100

nificant differences existed among the intracellular slow potentials obtained from different tongue portions.

As listed in Table 1, during Ringer adaptation of the tongue surface hyperpolarizing slow potentials elicited by GP nerve stimulation were observed in five of 71 taste cells (7%). On the other hand, during gustatory stimulation of taste cells with 0.5 M NaCl and 3 mM acetic acid the percentage of hyperpolarizing slow potentials generated by GP nerve stimulation was increased to 25 and 44%, respectively. This is due to changes in the liquid junction potential elicited between saliva secreted by the lingual glands and the altered lingual surface fluid.

Under Ringer adaptation the latency and rise time of depolarizing and hyperpolarizing slow potentials in taste cells induced by GP nerve stimulation were 1.3 ± 0.1 (n = 66) and 3.6 ± 0.2 s (n = 66), respectively. On the other hand, under gustatory stimulation of the taste cells with 0.5 M NaCl the latency and rise time of GP nerve-induced depolarizing and hyperpolarizing slow potentials were 1.3 ± 0.1 (n = 28) and 2.9 ± 0.3 s (n = 28), respectively, and under stimulation with 3 mM acetic acid the latency and rise time of these potentials were 1.4 ± 0.1 (n = 9) and 2.7 ± 0.5 s (n = 9), respectively. The peak time of GP nerve-induced slow potential under stimulation with NaCl and acetic acid was significantly shorter than that under Ringer. The latencies of the hyperpolarizing slow potentials elicited by GP nerve stimulation were 1.8 ± 0.3 s (n = 5) under Ringer, 1.2 ± 1.8 s (n = 8) under 0.5 M NaCl and 1.5 ± 0.3 s (n = 4) under 3 mM acetic acid. No differences were found among these values ($P > 0.05$).

Thirty minutes after atropine (1 mg/kg) was injected into three frogs, extracellular recordings were taken from 29 loci on the tongue surface. Neither positive nor negative slow potentials appeared at any loci on the tongue surface following GP nerve stimulation. When intracellular recordings were taken from 21 taste cells adapted to Ringer solution, neither depolarizing nor hyperpolarizing slow potentials were recorded from 19 cells, but small hyperpolarizing slow potentials of <1 mV (–0.7 and –0.8 mV) were observed in two cells. The latency of these slow potentials was ~0.6 s.

Interaction between the gustatory receptor potential and the GP nerve-induced slow potential in taste cells

Transformation of the gustatory receptor potential in taste cells by GP nerve stimulation was tested. Figure 2 illustrates examples of enhancement (Figure 2A) and depression (Figure 2B) of depolarizing receptor potentials for 0.5 M NaCl and 3 mM acetic acid when the GP nerve was stimulated at 30 Hz. Figure 3A and C illustrates the distribution of receptor potentials elicited by 0.5 M NaCl (Figure 3A) in 32 taste cells and 3 mM acetic acid in nine taste cells (Figure 3C). Figure 3B and D shows the distribution of GP nerve-induced intracellular slow potentials when the taste cells were stimulated with 0.5 M NaCl (Figure 3B) and 3 mM acetic acid (Figure 3D). The mean amplitudes of the gustatory receptor potentials and GP nerve-induced slow potentials are given in each graph. During gustatory stimulation of 32 taste cells with 0.5 M NaCl, GP nerve-induced enhancement of the receptor potentials was seen in 20 cells, depression in eight cells and no change in four cells. On

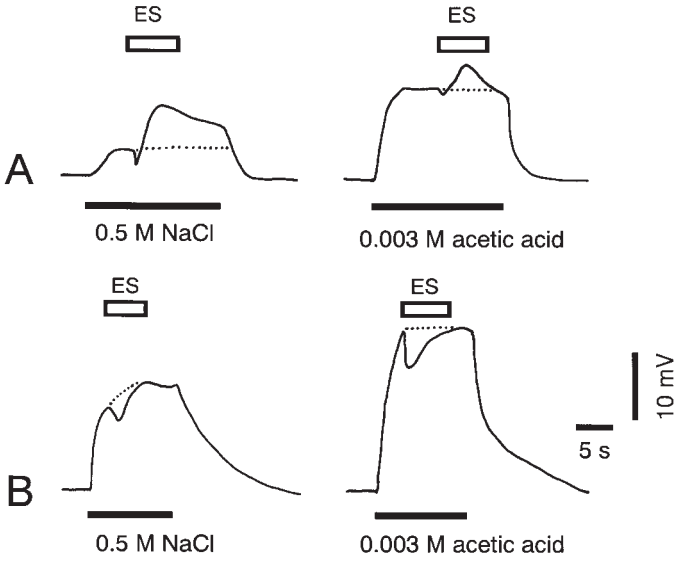

Figure 2 GP nerve stimulation-induced slow potentials superimposed on the depolarizing receptor potentials recorded intracellularly from taste cells. **(A)** Nerve-induced depolarizing slow potentials superimposed on receptor potentials elicited by 0.5 M NaCl and 3 mM acetic acid. **(B)** Nerve-induced hyperpolarizing slow potentials superimposed on receptor potentials elicited by the NaCl and acetic acid. Records (A) and (B) were obtained from different taste cells. Resting potential was –32 mV in (A) and –27 mV in (B). ES, electrical stimulation of the GP nerve with pulses of 0.1 ms, 12 V and 30 Hz. Dotted lines show the time course of receptor potentials without electrical stimulation of the GP nerve.

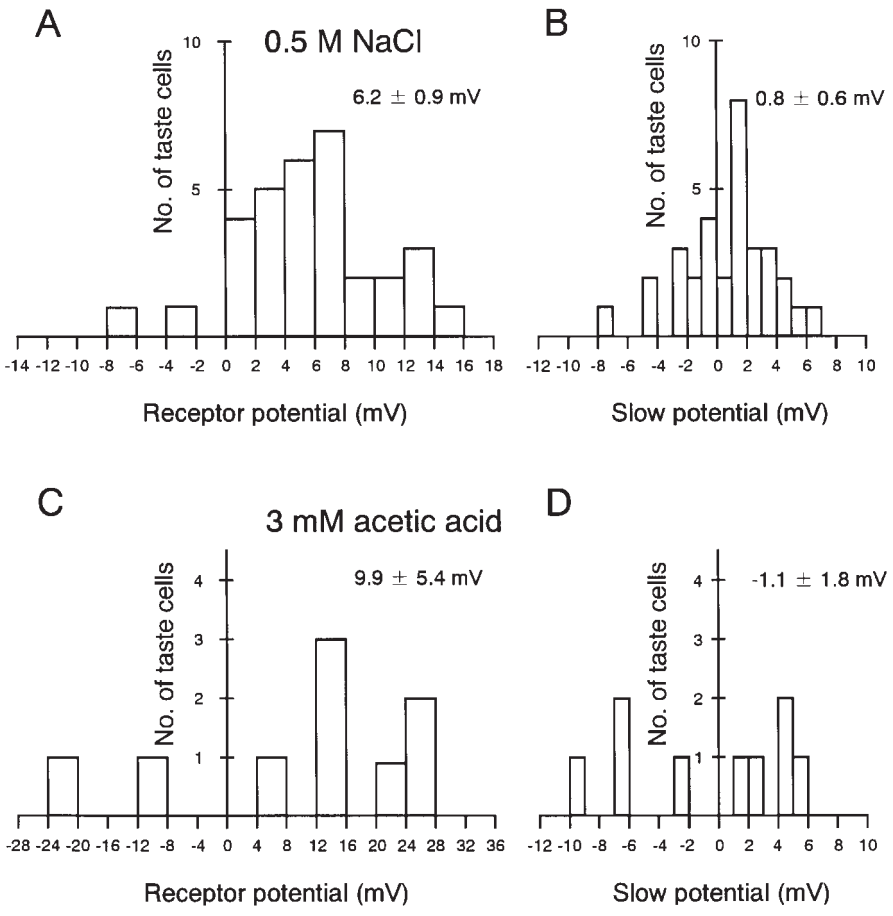

Figure 3 Histogram of intracellular receptor potentials in taste cells induced by tastant and intracellular slow potentials induced by GP nerve stimulation. **(A)** Distribution of 0.5 M NaCl-induced receptor potentials (n = 32 taste cells). **(B)** Distribution of GP nerve-induced slow potentials under 0.5 M NaCl stimulation (n = 32 taste cells). **(C)** Distribution of 3 mM acetic acid-induced receptor responses (n = 9 taste cells). **(D)** Distribution of the nerve-induced slow potentials under 3 mM acetic acid stimulation (n = 9 taste cells). In (B) and (D) the GP nerve was stimulated with 30 Hz pulses of 0.1 ms duration and 10–15 V, 3–10 s after application of the tastant.

average, the amplitude of 0.5 M NaCl-induced receptor potentials was enhanced by 25 ± 11% of the controls (n = 32, range –68 to 200%). During 3 mM acetic acid stimulation of nine taste cells, GP nerve-induced enhancement of receptor potential was observed in three cells and depression in six cells. The amplitude of the 3 mM acetic acid-induced receptor potentials was depressed by –1 ± 15% of the controls (n = 9, range –42 to 100%) by GP nerve stimulation. There were negative correlations between the amplitude of tastant-induced depolarizing receptor potentials and percentage change in the depolarizing receptor potentials induced by GP nerve stimulation when taste cells were stimulated with either 0.5 M NaCl ($P < 0.05$, n = 30, r = –0.394) or 3 mM acetic acid ($P < 0.03$, n = 7, r = –0.804).

Discussion

Mostly electropositive slow potentials are initiated on the frog tongue surface by stimulation of parasympathetic fibers in the GP nerve. These slow potentials are caused by a liquid junction potential generated between saliva secreted from the lingual glands and the tongue surface fluid and are completely abolished by blocking the liquid junction potential with an i.v. injection of atropine into the frog (Sato *et al.*, 2000). Depolarizing slow potentials are also induced in frog taste disk cells, such as supporting and taste cells, by GP nerve stimulation (Kutyna and Bernard, 1977; Sato *et al.*, 2000), which are completely abolished by atropine. Therefore, GP nerve-induced depolarizing slow potentials in frog taste cells are generated by a non-synaptic outward current through the disk cells due to the liquid junction potential between saliva from the lingual glands (Gaupp, 1904; Nalavade and Varute, 1971; Albanese Carmignani *et al.*, 1975; Albanese Carmignani and Zaccone, 1977) and the lingual surface fluid. An electric source for GP nerve-induced slow potentials does not exist inside the fungiform papillae, but beneath the lingual epithelium.

The input resistance of the frog taste cell is 32–40 MΩ as determined by an intracellular recording technique (Sato and Beidler, 1975) and 2 GΩ by a whole cell recording

technique (Miyamoto *et al.*, 1991). Although the measured value of input resistance depends on the recording method, a mean depolarizing potential of 4.4 mV may always appear in taste cells because a several millivolt difference exists between the top and bottom of the taste disk, due to the liquid junction potential.

The ionic composition of saliva secreted from the frog lingual glands is unknown. The liquid junction potential generated between the secreted saliva and the lingual fluid can be calculated from a theoretical equation for the potential (Wilson, 1979) using the composition of cations and anions in the saliva and lingual fluid. Important ions may be Na^+, K^+, Cl^-, HCO_3^- and mucous glycoprotein anions ($Prot^-$). When the adapting or stimulating fluid on the frog tongue surface is 0.001, 0.1 or 0.5 M NaCl or Ringer solution, the mean amplitudes of the slow potentials induced by GP nerve stimulation are in the order 0.001 M NaCl > 0.1 M NaCl = Ringer > 0.5 M NaCl (Sato *et al.*, 2000). This order reverses if inorganic ions alone, whose composition was estimated from mammalian saliva, are used in the equation for the liquid junction potential. Therefore, the concentrations of $Prot^-$ within the lingual glands and on the tongue surface are important in determining the liquid junction potential. $Prot^-$ with slower ionic mobilities control the movement of inorganic ions.

When the frog tongue surface was adapted to Ringer, 0.5 M NaCl or 3 mM acetic acid in the present study, hyperpolarizing slow potentials in taste cells occurred in a few cases with Ringer but frequently with NaCl and acetic acid (Table 1 and Figure 3). This may derive from the $Prot^-$ concentration in the lingual gland or on the lingual surface being elevated or lowered in a local region of the tongue. When stimulation of the GP nerve induces an electropositive or electronegative slow potential at recording loci on the frog tongue surface, depolarizing or hyperpolarizing slow potentials, respectively, are generated in taste cells at the same loci by an outward or inward current passing through the taste cell membrane.

If the concentrations of NaCl and KCl in the saliva of frog lingual glands are 20–30 mM, as in mammalian saliva (Bradley, 1995), taste cells adapted to 0.001 M NaCl or 3 mM acetic acid in the present experiments would be slightly depolarized by Na^+ and K^+ ions. As suggested by Ye *et al.* (1991), some cations and anions in saliva could pass through the tight junction connecting taste cells. K^+ from the frog lingual gland might pass through the tight junction because the K^+ concentration in saliva is higher than that in extracellular fluid, but Na^+ and Cl^- do not, because their concentrations in saliva are lower.

Parasympathetic vasodilator fibers in the GP nerve innervate arterioles in the frog tongue and excitation of these fibers enhances the lingual blood flow (Sato *et al.*, 2000). If the blood flow becomes slower, the activity of frog taste cells is gradually depressed by a decrease in Na^+–K^+ ATPase activity and the intracellular signal transmission mechanism.

Many researchers (Brush and Halpern, 1970; Esakov and Byzov, 1971; DeHan and Graziadei, 1973; Reutter *et al.*, 1997) have proposed that there may be synapses between taste cells and efferent fibers, but morphological and physiological evidence is still lacking. It is probable that efferent fibers in the frog GP nerve make close contacts on the taste cell and modulate the cell functions. The kind of efferent fiber is considered to be one of sympathetic post-ganglionic fibers, parasympathetic post-ganglionic fibers or A-type fibers in the GP nerve. In the present study a small hyperpolarizing slow potential of <1 mV was observed in two taste cells on stimulation of the GP nerve after a large intracellular slow potential, which was evoked by the liquid junction potential between saliva secreted from the lingual gland and the lingual surface fluid and was completely blocked by atropine. This slow potential had a shorter latency (~0.6 s) than the large intracellular slow potentials (~1.3 s). Since the activity of effector cells innervated by parasympathetic post-ganglionic nerve fibers (Inoue and Kitada, 1991) is completely blocked by atropine, the hyperpolarizing response elicited by GP nerve stimulation probably derives from the activity of post-synaptic taste cells innervated by either sympathetic post-ganglionic fibers (Inoue and Kitada, 1988) or A-type fibers. The amplitude of the hyperpolarizing taste cell responses (~0.8 mV) is five times smaller than non-synaptically evoked depolarizing taste cell responses. Therefore, the presumed small post-synaptic potential appearing in a taste cell might be masked by the large non-synaptic slow potential elicited by GP nerve stimulation, if atropine is not injected. A similar hyperpolarizing response evoked by GP nerve stimulation has been recorded in a frog taste cell and has been suggested to be an inhibitory post-synaptic potential (Esakov and Byzov, 1971). However, effects of atropine and other blockers on the hyperpolarizing response were not tested in their experiment. Post-synaptic properties of the GP nerve-induced small hyperpolarizing slow potential in frog taste cells after injection of atropine and other blockers will be investigated in a further study.

The amplitudes of 0.5 M NaCl-induced receptor potentials in frog taste cells were changed in the range 68% decrease to 200% increase by electrical stimulation of the GP nerve. The amplitudes of 3 mM acetic acid-induced receptor potentials were altered in the range 42% decrease to 100% increase by GP nerve stimulation. It should be noted that these large changes in the receptor potentials in taste cells were caused by non-synaptic outward and inward currents through the taste cell membrane from an electric source underneath the dorsal tongue epithelium (Sato *et al.*, 2000). The GP nerve-induced enhancement of frog receptor potentials might be concerned with an increase in transmitters released from the taste cells. On the other hand, GP

nerve-induced depression of the taste receptor potentials might be concerned with a decrease of the transmitters.

References

Albanese Carmignani, M.P. and **Zaccone, G.** (1977) *Histochemical studies on the tongue of anuran amphibians-II. Comparative morphochemical study of the taste buds and the lingual glands in* Bufo viridis *Laurenti and* Rana graeca *Boulenger with particular reference to the mucosaccharide histochemistry*. Cell. Mol. Biol., 22, 203–217.

Albanese Carmignani, M.P., Zaccone, G. and **Cannata, F.** (1975) *Histochemical studies on the tongue of anuran amphibian: I. Mucopolysaccharide histochemistry of the papillae and lingual glands in* Hyla arborea *L.,* Rana esculenta *L. and* Bufo vulgaris *Laur*. Ann. Histochim., 20, 47–65.

Bradley, R.M. (1995) Essentials of Oral Physiology. Mosby, St Louis, MO, pp. 161–186.

Brush, A.D. and **Halpern, B.P.** (1970) *Centrifugal control of gustatory responses*. Physiol. Behav., 5, 743–746.

DeHan, R.S. and **Graziadei, P.P.C.** (1973) *The innervation of frog's taste organ: 'a histochemical study'.* Life Sci., 13, 1435–1449.

Esakov, A.I. and **Byzov, A.L.** (1971) *Electrical response of the frog's gustatory cells to centrifugal stimulation.* Bull. Exp. Biol. Med. (Engl. Trans.), 72, 723–726.

Gaupp, E. (1904) A. Ecker's und R. Wiedersheim's Anatomie des Frosches, Vol. III. F. Vieweg und Sohn, Braunschweig, Germany.

Inoue, K. and **Kitada, Y.** (1988) *On the origin and course of sympathetic nerve fibers in the fungiform papillae of the frog's tongue*. Okajimas Folia Anat. Jpn., 65, 171–176.

Inoue, K. and **Kitada, Y.** (1991) *Parasympathetic postganglionic cells in the glossopharyngeal nerve trunk and their relationship to unmyelinated nerve fibers in the fungiform papillae of the frog*. Anat. Rec., 230, 131–135.

Kutyna, F.A. and **Bernard, R.A.** (1977) *Effects of antidromic activity in gustatory nerve fibers in taste disc cells of the frog tongue*. J. Comp. Physiol., 118, 291–306.

Miyamoto, T., Okada, Y. and **Sato, T.** (1991) *Voltage-gated membrane current of isolated bullfrog taste cells.* Zool. Sci., 8, 835–845.

Nalavade, M.N. and **Varute, A.T.** (1971) *Histochemical studies on the mucins of the vertebrate tongues. I. Histochemical analysis of mucosubstances in the amphibian tongue*. Histochemie, 27, 351–365.

Reutter, K., Witt, M. and **Valentincic, T.** (1997) *Ultrastructure of the taste buds of cave-dwelling amphibian* Proleus anguinus *(Caudata).* Chem. Senses, 22, 777–778.

Sato, T. (1972) *Adaptation of primary gustatory nerve responses in the frog*. Comp. Biochem. Physiol., 43A, 1–12.

Sato, T. and **Beidler, L.M.** (1975) *Membrane resistance change of the frog taste cells in response to water and NaCl.* J. Gen. Physiol., 66, 735–763.

Sato, T., Toda, K., Miyamoto, T., Okada, Y. and **Fujiyama, R**. (2000) *The origin of slow potential on the tongue surface induced by frog glossopharyngeal efferent fiber stimulation.* Chem. Senses, 25, 583–589.

Ye, Q., Heck, G.L. and **DeSimone, J.A.** (1991) *The anion paradox in sodium taste reception: resolution by voltage-clamp studies.* Science, 254, 724–726.

Wilson, J.A. (1979) Principles of Animal Physiology, 2nd Edn. Macmillan, New York.

Accepted August 10, 2000