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The Origin of Slow Potentials on the Tongue Surface Induced by Frog Glossopharyngeal Efferent Fiber Stimulation

Toshihide Sato, Kazuo Toda[1], Takenori Miyamoto, Yukio Okada and Rie Fujiyama

Department of Physiology, Nagasaki University School of Dentistry, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan and [1]Section of Cognitive Neurobiology, Department of Maxillofacial Biology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, 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 (GP) nerve of the frog was stimulated electrically, electropositive slow potentials were recorded from the tongue surface and depolarizing slow potentials from taste cells in the fungiform papillae. The amplitude of the slow potentials was stimulus strength- and the frequency-dependent. Generation of the slow potentials was not related to antidromic activity of myelinated afferent fibers in the GP nerve, but to orthodromic activity of autonomic post-ganglionic C fibers in the GP nerve. Intravenous injection of atropine abolished the positive and depolarizing slow potentials evoked by GP nerve stimulation, suggesting that the slow potentials were induced by the activity of parasympathetic post-ganglionic fibers. The amplitude and polarity of the slow potentials depended on the concentration of adapting NaCl solutions applied to the tongue surface. These results suggest that the slow potentials recorded from the tongue surface and taste cells are due to the liquid junction potential generated between saliva secreted from the lingual glands by GP nerve stimulation and the adapting solution on the tongue surface.

Introduction

Most of the somatosensory and gustatory nerve fibers from the frog tongue pass through the glossopharyngeal (GP) nerve. On the other hand, most of the efferent fibers in the lingual branch of the GP nerve innervating the tongue region are secretory fibers supplying the lingual glands (Gaupp, 1904) and vasodilator and vasoconstrictor fibers supplying the arterioles in the tongue, which are all autonomic post-ganglionic C fibers (Krogh, 1920; Siggins and Weitsen, 1971; Inoue and Kitada, 1988, 1991; Inoue *et al.*, 1992, 1994). The presence of GP efferent fibers innervating taste cells has been suggested (Brush and Halpern, 1970; Esakov and Byzov, 1971; DeHan and Graziadei, 1973; Reutter *et al.*, 1997).

In single fungiform papillae of the frog there are ~10 myelinated fibers (Rapuzzi and Casella, 1965; Jaeger and Hillman, 1976; Sato *et al.*, 1983; Inoue and Kitada, 1991) and many unmyelinated fibers (Graziadei and DeHan, 1971; Inoue and Kitada, 1991). Most of the myelinated fibers in the fungiform papillae are gustatory and mechano-sensitive fibers (Kusano and Sato, 1957; Sato *et al.*, 1983).

Esakov and Byzov (Esakov and Byzov, 1971) recorded electropositive slow potentials from the tongue surface and hyperpolarizing slow potentials from taste cells in the papillary taste disk in response to electrical stimulation of the frog GP nerve. They speculated that the former are generated from the surface epithelium of the whole tongue independently of the activity of taste cells and the latter are generated from the taste cell as the post-synaptic response. On the other hand, Kutyna and Bernard (Kutyna and Bernard, 1977) mentioned that positive slow potentials were also recorded from the tongue surface and that depolarizing and hyperpolarizing slow potentials were recorded from supporting cells and taste cells in the taste disk, respectively, when the frog GP nerve was stimulated. Since all extracellularly and intracellulary recorded slow potentials exhibit latencies >1 s and intracellular slow potentials are not modified by alternation of the membrane potential by current injection, they assumed that the slow potentials were not generated by synaptic events but were due to peripheral interactions among antidromically activated gustatory nerve fibers. However, this conclusion is not enough to explain the mechanism underlying generation of slow potentials with a long latency.

In the human parotid gland a liquid junction potential of ~10 mV occurs between the secreted saliva and the body fluid (Inomata *et al.*, 1993, 1995). The time course of this potential is very similar to the secretory potential from the parotid (Inomata *et al.*, 1993, 1995). Large liquid junction

potentials usually appear between two solutions with different ionic compositions when an electrode or bath solution is exchanged during electrophysiological experiments. Such electrochemical potentials have to be corrected for to obtain true physiological values (Barry and Lynch, 1991). Therefore, the slow potential induced on the frog tongue surface following GP nerve stimulation may arise from the liquid junction potential between the tongue surface fluid and saliva secreted from the lingual glands, which are distributed abundantly beneath the dorsal surface of the tongue (Nalavade and Varute, 1971; Albanese Carmignani *et al.*, 1975, Albanese Carmignani and Zaccone, 1977). Electrical stimulation of the GP nerve induces secretion of mucous saliva from these glands (Gaupp, 1904).

In the present experiments, we have examined the origin and characteristics of slow potentials arising on the tongue surface and from taste cells when the frog GP nerve is stimulated.

Materials and methods

Eighteen bullfrogs (*Rana catesbeiana*) weighing 185–530 g were used in the experiments. The animals were deeply anesthetized with an i.p. injection of a 50% urethane–Ringer solution (3 g/kg body wt). The hypoglossal nerves on both sides were severed to avoid neurally driven contraction of the tongue muscles. An intact blood supply to the tongue through the lingual arteries and veins was maintained during the course of the experiments to obtain reproducible responses from the tongue surface. The whole tongue was pulled out from the mouth and pinned down on a cork plate. The whole GP nerve on either side was separated out from the surrounding connective tissues, cut centrally and immersed in mineral oil.

The whole nerve was electrically stimulated by single or repetitive pulses with a pair of Ag–AgCl wire electrodes lifting the nerve. The stimulatory electrical pulse was changed from 0.01 to 0.1 ms in duration and from 0.1 to 30 V in strength. Extracellular electrical recordings from the tongue surface and intracellular recordings from the taste cells in the taste disk of the fungiform papillae were performed with a glass microelectrode, filled with 3 M KCl and having a resistance of 30–60 MΩ. A reference Ag–AgCl wire electrode was positioned in the muscles of the forelimb. Electrical responses of the tongue surface and taste cells were amplified with a microelectrode DC amplifier (Nihon Kohden MZ 10, Tokyo) and recorded on a pen recorder. Criteria to identify intracellular responses of single taste cells were as described previously (Sato and Beidler, 1975).

In some experiments, electrically evoked impulses of the GP nerve fibers were recorded from single fungiform papillae with a glass suction electrode of ~150 μm internal diameter at the tip. The suction electrode was filled with frog Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.2). A reference chlorinated silver wire electrode was glued to the outside of the suction electrode. The impulses were displayed on an oscilloscope and photographed with a camera. C fiber activities of the fungiform papillae were detected by averaging impulses evoked by 100 stimulations of the GP nerve (Nihon Kohden ATAC750).

The Ringer and other adapting solutions were applied to the tongue surface at a flow rate of 0.13 ml/s with a semi-automatically controlled gustatory stimulator (Sato, 1972). In some experiments atropine sulfate (Tanabe Seiyaku, Osaka) was injected into the vein at a dose of 1 mg/kg. All experiments were carried out at a room temperature of 22–26°C.

Results

Characteristics of slow potentials

While the GP nerve was strongly stimulated repetitively, slow electrical activities were recorded either ipsilaterally or contralaterally from the whole dorsal tongue surface (Figure 1A). The GP nerve-induced slow potentials appeared when a recording microelectrode was positioned on the top of the fungiform and filiform papillae and between the papillae. The time course of the slow potentials did not differ when recorded ipsilaterally or contralaterally from the tongue surface. The slow potentials increased in magnitude with increasing stimulus strength and frequency (Figure 1A). The maximal response was obtained at 30 or 50 Hz. The latency and rise time of the slow potentials were longer when the stimulus frequency was lower. Of the slow potentials examined (n = 73), 93% were surface-positive, 3% were surface-negative and 4% were neutral. After the slow potential reached a peak, it gradually decreased and fell rapidly following a delay after the end of GP nerve stimulation. Table 1 shows the mean values of latency,

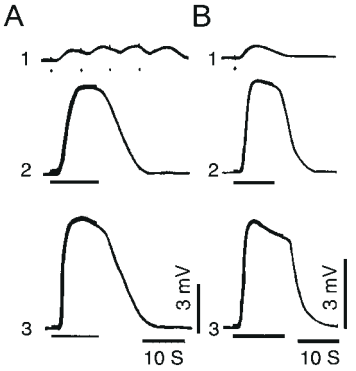

Figure 1 Slow potentials recorded from the tongue surface and a taste cell. **(A)** Extracellularly recorded slow potentials on the dorsal tongue surface. Stimulation of GP nerve, 1.4 Hz (1), 10 Hz (2) and 30 Hz (3) with pulses of 0.1 ms duration and 15 V. **(B)** Intracellularly recorded slow potentials in a taste cell of the taste disk. Stimulation of GP nerve, single stimulus (1), 10 Hz (2) and 30 Hz (3) with pulses of 0.1 ms duration and 15 V. The dot and bar underneath the slow potential show the period of electrical stimulation. The resting potential was –31 mV.

Table 1 Parameters of time course of slow potentials induced by GP nerve stimulation at 30 Hz

Slow potential	Latency (s)	Rise time (s)	Delay (s)	Fall time (s)	*n*
Positive slow potentials from tongue surface	1.4 ± 0.1	3.0 ± 0.2	1.9 ± 0.1	6.5 ± 0.5	44
Depolarizing slow potentials from taste cells	1.2 ± 0.1	3.5 ± 0.3	1.7 ± 0.2	6.3 ± 0.4	22

rise time, delay and fall time of positive slow potentials. The amplitude of electropositive slow potentials recorded from various portions of the tongue surface was 7.0 ± 0.5 mV (n = 68 recordings, a range of 2.1–20.6 mV) when the GP nerve was stimulated at 30 Hz. Negative slow potentials were observed from the tongue surface in two cases and the mean amplitude was –3.1 mV.

When the GP nerve was repetitively stimulated with 15 V pulses, blood circulation in the tongue surface was gradually increased with increasing stimulus frequency and the lingual arterioles were dilated. The maximal effect was observed with stimulation at 30 Hz.

When a recording microelectrode was inserted into the taste disk of the fungiform papillae from the top, intracellular slow potentials were obtained from supporting cells and taste cells (Figure 1B) on GP nerve stimulation. Intracellular slow potentials in taste cells also increased with increasing stimulus strength and frequency (Figure 1B). The mean values of latency, rise time, delay and fall time of the depolarizing slow potentials of taste cells are listed in Table 1. GP nerve-induced taste cell responses were depolarizing in 86% of the 71 cells examined, hyperpolarizing in 7% and neutral in 7%. The mean amplitudes of the depolarizing and hyperpolarizing slow potentials when the GP nerve was stimulated at 30 Hz were 4.4 ± 0.4 (n = 61) and –2.8 ± 0.5 mV (n = 5), respectively. Depolarizing slow potentials in the taste cells were significantly smaller than the positive slow potentials on the tongue surface ($P < 0.05$). The intracellular slow potentials recorded from eight supporting cells following GP nerve stimulation were all depolarizing and the mean amplitude was 6.4 ± 0.9 mV (n = 8).

A slow potential from the tongue surface in response to single electrical stimulation of the GP nerve is shown in Figure 2A. The mean amplitude of the slow potential was as small as 0.67 ± 0.19 mV (n = 8). The mean latency was 1.2 ± 0.2 s (n = 6) and the time constants in the rising and falling phases were 1.7 ± 0.2 s (n = 4) and 3.3 ± 0.4 s (n = 4), respectively. The relation between amplitude of the slow potential and stimulus pulse strength (Figure 2B) and the recovery of single pulse-induced slow potentials (Figure 2C) were similar to slow potentials elicited by repetitive stimulations.

Effect of microelectrode insertion into the fungiform papillae on slow potential

When a microelectrode was gradually inserted into the fungiform papilla from the top, extracellular slow potentials

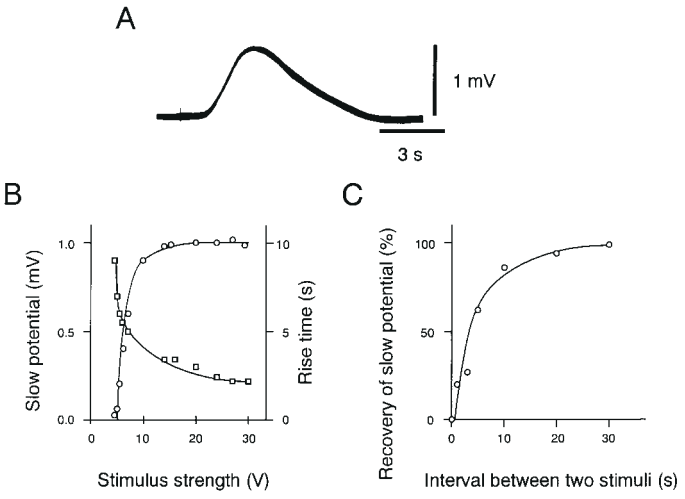

Figure 2 Slow potential from the dorsal tongue surface in response to single electrical pulses. **(A)** An example of the slow potential in response to a single electrical pulse (0.1 ms, 12 V). **(B)** Relationship between stimulus strength of single pulses and amplitude of slow potentials (○) and between the strength and rise time of slow potentials (□). **(C)** Relationship between recovery of slow potentials in response to second pulses and time interval of first and second pulses.

evoked by GP nerve stimulation reduced in amplitude (Figure 3A,B). This means that an increase in electrical resistance in the circuit for slow potential generation greatly reduces the electrical current.

Relation between firing of fibers in the GP nerve and occurrence of the slow potential

As shown in Figure 4A, a recording suction electrode was attached to a fungiform papilla and action potentials of the papillary nerve fibers were recorded. A microelectrode was placed on another fungiform papilla near the suction electrode to record the slow potential induced by repetitive stimulation at 30 Hz. Figure 4B shows the relation between the number of papillary fibers that fired and the strength of single stimulus pulses. When the stimulus duration was 0.01 ms, the threshold voltage was 0.6 V and nine A-type fibers included in the papilla were all fired at ~3.5 V (Figure 4B,D). The fiber type was determined by measuring the conduction velocities using conduction distance and impulse latencies. The calculated conduction velocities of the nine fibers in Figure 4D were in the range of 6.7–13.9 m/s. When all nine fibers of A type were fired by a 5 V pulse, no slow potentials were initiated from the tongue surface by 5 V

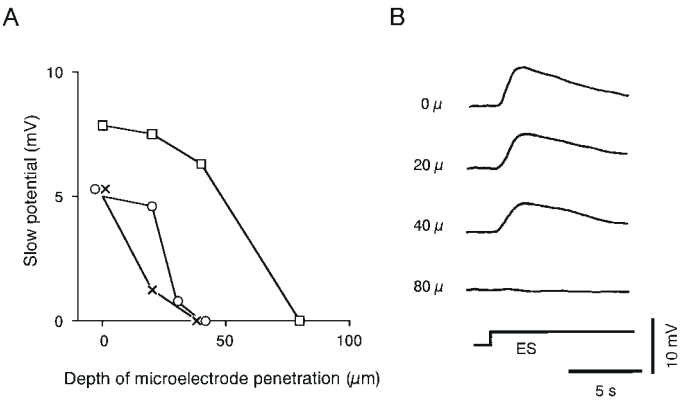

Figure 3 Relationship between depth of recording microelectrode insertion into the fungiform papilla and amplitude of extracellular slow potentials induced by GP nerve stimulation. **(A)** Relationship between depth of tip of microelectrode and slow potential. The electrode was placed on the top of a papilla at 0 μm and advanced vertically. Extracellular slow potentials were obtained from three fungiform papillae (□, ○, ×). **(B)** Examples of slow potentials from four different depths. The depth of the microelectrode tip was measured with a micrometer of manipulator. ES, electrical stimulation at 30 Hz with 12 V pulses of 0.1 ms duration.

pulses at 30 Hz. In this case, the slow potential appeared with 7 V pulses at 30 Hz and gradually increased with increasing strength of voltage pulses (Figure 4C). Similar results were obtained from the other 12 pairs of fungiform papillae.

In the neural recordings shown in Figure 4D, C fiber activities in the papilla were not seen, but C fiber impulses were detected by averaging impulses 100 times (Figure 4E). By simply dividing the conduction distance by impulse latencies obtained, conduction velocities of 0.66, 0.62, 0.58 and 0.53 m/s were obtained from three fungiform papillae. The slow potential was blocked by an injection of atropine [a blocker of muscarinic acetylcholine receptors (mAChR) in effectors innervated by parasympathetic post-ganglionic fibers] as described in detail later, so the slow potentials might be elicited by the activity of parasympathetic post-ganglionic fibers in the GP nerve. Averaged C fiber impulses were detected on electrical stimulation with >7 V pulses. Therefore, it is estimated that parasympathetic post-ganglionic fibers first fired with 7 V pulses of 30 Hz in the experiment shown in Figure 4.

Effect of adapting solutions on the slow potentials

If the GP nerve-induced slow potential derives from a liquid junction potential between saliva secreted from the lingual glands and lingual superficial fluid, the potential will change depending on the adapting solution on the tongue. This possibility was tested. When the tongue surface was adapted for 20 s to 0.001, 0.1 and 0.5 M NaCl and Ringer solution, the mean amplitudes of slow potentials on the tongue surface in response to GP nerve stimulation were 8.6 ± 0.4 mV (n = 5) for 0.001 M NaCl, 6.1 ± 1.1 mV (n = 5) for 0.1 M NaCl, 1.9 ± 1.0 mV (n = 9) for 0.5 M NaCl and 6.3 ± 1.4 mV (n = 5) for Ringer. The order of amplitudes was 0.001 M NaCl > 0.1 M NaCl = Ringer 0.5 M NaCl. When the adapting solution was 0.5 M NaCl, negative slow potentials were observed in two of nine recordings.

Effect of atropine on the slow potentials

The slow potential induced by GP nerve stimulation was compared before and after atropine (1 mg/kg) injection in a vein. When the lingual blood circulation was normal, the slow potential recorded from the tongue surface disappeared in the 2 min after atropine injection. When the lingual circulation was slower than normal, the slow potential disappeared in >2 min after injection (Figure 5). The slow potential recovered only when >5 h had elaspsed after injection. After the injection of atropine, depolarizing slow potentials in response to GP nerve stimulation were also barely recordable from the supporting and taste cells in the papillary disk. This atropine effect strongly suggests that the mechamism generating the slow potentials involves the activity of effectors innervated by parasympathetic post-ganglionic C fibers.

Discussion

Properties of slow potentials induced by GP nerve stimulation

Slow potentials on the frog tongue surface induced by GP nerve stimulation increased with increasing stimulus strength and stimulus frequency applied to the nerve (Figures 1 and 2) and adapting solutions applied to the tongue. The slow potentials were maximal in amplitude when the electrode was not inserted into the tongue but placed on the dorsal tongue surface adapted to Ringer solution. The amplitude of the slow potentials gradually reduced and finally disappeared as the microelectrode was inserted into the fungiform papilla (Figure 3). This suggests that the density of electric current flowing from a slow potential-generating source is gradually lowered inside the papillae.

In the present experiment, the mean amplitude of electropositive slow potentials recorded from the tongue surface was 7.0 mV and that of depolarizing slow potentials recorded intracellularly from the taste cells was 4.4 mV. Similar types of positive slow potentials have been recorded from the tongue surface of frogs (Esakov and Byzov, 1971; Kutyna and Bernard, 1977). Esakov and Byzov (Esakov and Byzov, 1971) recorded a hyperpolarizing slow potential from a frog taste cell in the taste disk following GP nerve stimulation. On the other hand, Kutyna and Bernard (Kutyna and Bernard, 1977) recorded depolarizing slow potentials from supporting cells but hyperpolarizing slow potentials from taste cells in the frog taste disk.

Relationship of the slow potential to neural fiber type

The relationship between firing of papillary nerve fibers and

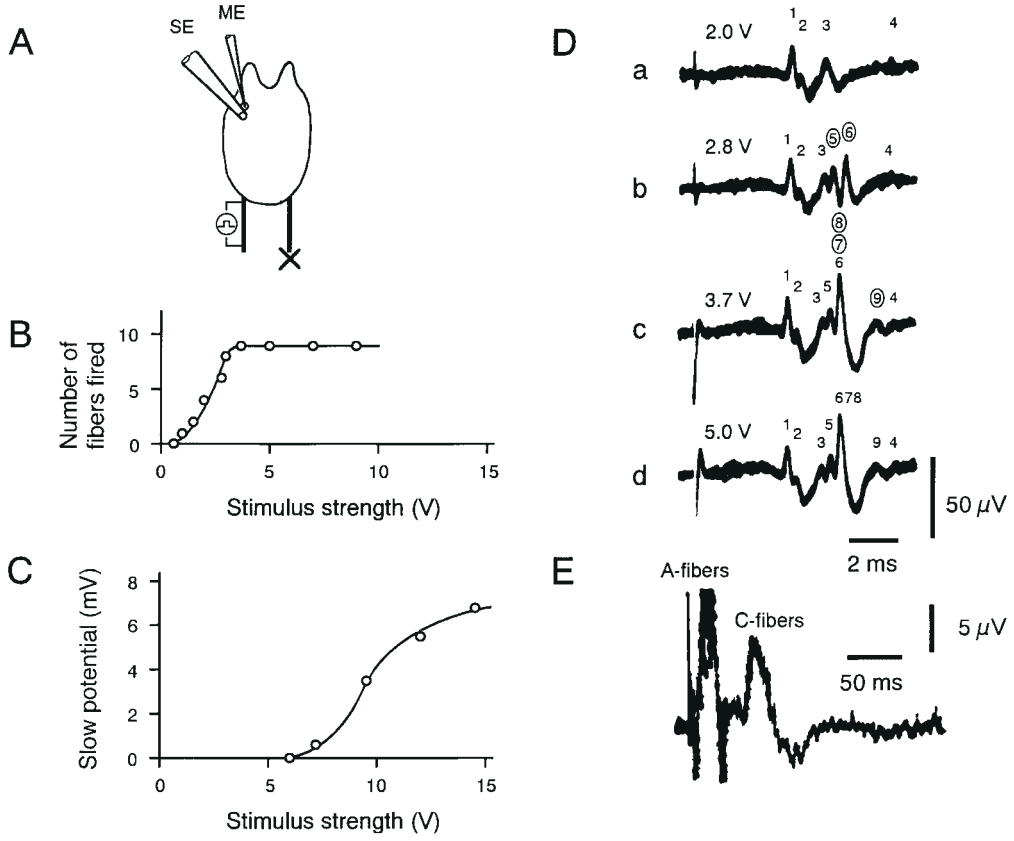

Figure 4 Relationship between generation of slow potential on the dorsal tongue surface and activity of nerve fiber types in a fungiform papilla. **(A)** Positions of recording electrodes on the tongue surface. ME, microelectrode on top of a fungiform papilla; SE, suction electrode attached to another fungiform papilla. Electrical stimuli were given to the right GP nerve. **(B)** Relationship between stimulus strength of single pulses (0.01 ms duration) and number of myelinated fibers that fired in the fungiform papilla. **(C)** Relationship between stimulus strength of 30 Hz pulses (0.01 ms duration) and amplitude of slow potentials recorded from the top of the fungiform papilla. **(D)** Examples of impulse firings in experiment B. Stimulus strength was increased from a to d. Numbers on impulses give the order of impulse firing. Impulses 6–8 formed a compound action potential. **(E)** C fiber impulses evoked by 100 stimulations of the GP nerve (0.01 ms, 30 V, 1 Hz). The upper and lower parts of A fiber impulses are truncated. The conduction velocity of the C fibers was 0.66 m/s.

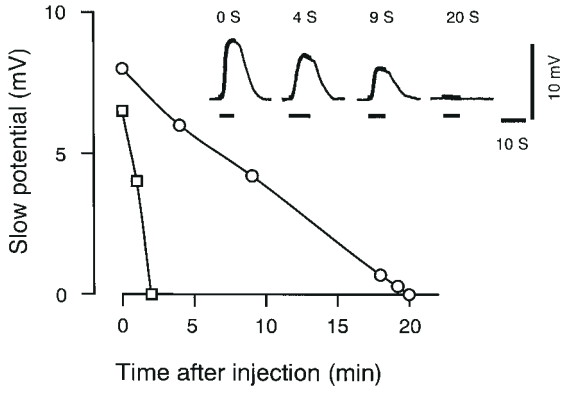

Figure 5 Reduction in amplitude of slow potentials on the dorsal tongue surface after i.v. injection of atropine. Atropine (1 mg/kg) was injected into two frogs whose lingual blood circulation was kept normal (○) or slowed (□). The GP nerve was stimulated at 30 Hz with pulses of 0.1 ms duration and 12 V. Insets show examples of slow potentials from the tongue surface before and after atropine injection into the frog with a slower lingual circulation (○).

occurrence of slow potentials on the tongue surface suggests that the activity of myelinated afferent fibers in the lingual branch of the GP nerve is not concerned with generation of the slow potentials (Figure 4). Since all gustatory nerve fibers supplying taste organs in the frog tongue are myelinated (Sato *et al.*, 1989), antidromic activity of these fibers is not responsible for the GP nerve stimulation-induced slow potential. A relationship between the slow potentials and antidromic activity of myelinated gustatory fibers was proposed by Kutyna and Bernard (Kutyna and Bernard, 1977). In the present study, the occurrence of the slow potential induced by GP nerve stimulation paralleled the activity of C fibers. Intravenous injection of atropine completely blocked the slow potentials induced by GP nerve stimulation (Figure 5). Since atropine is a blocker of mAChR, blockage of mAChR in the effectors innervated by parasympathetic post-ganglionic fibers of C type might abolish the slow potentials elicited by GP nerve stimulation. Parasympathetic post-ganglionic fibers in the frog GP nerve

are mainly composed of vasodilator fibers supplying lingual arterioles (Krogh, 1920), secretory fibers supplying lingual gland cells (Gaupp, 1904) and fibers supplying the taste disk cells (Inoue *et al.*, 1994). The activities of C fibers recorded in this study are concerned with activities of these parasympathetic post-ganglionic fibers.

Origin of slow potentials

Intravenous injection of atropine does not block excitation of parasympathetic post-ganglionic fiber terminals, but blocks chemical transmission between the fiber terminals and the effectors. After the blockage of chemical transmission by atropine, the slow potentials did not appear. Therefore, parasympathetic fiber terminal activity itself is not an electrical source for the generation of slow potentials.

The major candidates for the origin of slow potentials generated on the frog tongue surface may be the following effector activities: (i) smooth muscle fibers in the lingual arterioles; (ii) taste disk cells; (iii) lingual gland cells; (iv) a liquid junction potential between saliva secreted from the lingual glands and the tongue surface fluid.

The amplitude of action potentials recorded intracellularly from excitable cells is as large as 100 mV, but that recorded extracellularly from the cells is greatly reduced to <1 mV (Eccles, 1957). As shown in the present experiment (Figure 4), even when neural fiber activity in a fungiform papilla was recorded by separating it from surrounding tissues using a suction electrode, the amplitude of the action potentials was maximally 80 μV. If a suction electrode was not used, no detectable action potentials were recorded from the fungiform papillae. It is well known that electrocardiograms and electroretinograms show a largest extracellular recording value of ~1 mV (Wilson, 1979). In the present recordings, in which a microelectrode was positioned in Ringer solution flowing on the tongue surface, the amplitude of electropositive slow potentials ranged from 2.1 to 20.6 mV (mean 7.0 mV).

Of the candidates mentioned above as sources of GP nerve-evoked slow potentials, the extracellular activity of smooth muscle fibers in the lingual arterioles, taste disk cells and lingual gland cells might not produce as large a potential as the mean of 7 mV in the present recordings. Potential activities were probably not detected because electrical currents arising from these fibers and cells diffused in various directions in the Ringer solution covering the tongue surface. Therefore, the most acceptable candidate is the liquid junction potential generated between the saliva secreted from the many lingual glands underneath the tongue surface (Nalavade and Varute, 1971; Albanese Carmignani *et al.*, 1975; Albanese Carmignani and Zaccone, 1977) during GP nerve stimulation and the Ringer solution covering the tongue surface.

Slow potentials recorded from the tongue surface were altered in amplitude depending on the concentration of adapting NaCl solutions applied to the frog tongue surface. This supports the hypothesis that the slow potentials derive from the liquid junction potential between saliva secreted from the lingual glands and the adapting solution on the tongue because it changes depending on the concentration of NaCl solution covering the tongue. Atropine might block the activity of all effectors innervated by parasympathetic post-ganglionic fibers, but not those innervated by sympathetic post-ganglionic fibers, in the GP nerve (Inoue and Kitada, 1988). If the recording microelectrode, which was placed in the Ringer on the tongue, recorded the electrical activity of the effector cells, the effector cell activity induced by stimulation of sympathetic nerve fibers in the GP nerve would be picked up by the electrode even after atropine injection. Since this was not the case in the present study, electrical activity was not picked up from effector cells innervated by either sympathetic or parasympathetic post-ganglionic fibers. Therefore, the slow potentials evoked on the frog tongue surface following GP nerve stimulation are derived from the liquid junction potential between the saliva and Ringer on the tongue. Similar slow potentials have been recorded between human saliva and body fluid (Inomata *et al.*, 1993, 1995).

The depolarizing slow potentials of supporting and taste cells in the frog taste disk which were elicited by GP nerve stimulation might be generated by an outward current passing through these cells due to the liquid junction potential with the tongue surface positive. The hyperpolarizing slow potentials of frog taste disk cells might be initiated by an inward current through the cells due to the liquid junction potential with the tongue surface negative. Since the input resistance of frog taste cells measured by intracellular recording and whole-cell recording is 32–40 MΩ (Sato and Beidler, 1975) and 2 GΩ (Miyamoto *et al.*, 1991), respectively, and the resistance of the whole frog tongue is 710 Ω/cm^2 (Soeda and Sakudo, 1985), the mean depolarizing response of 4.4 mV in taste cells may be generated by the slow potentials of 7.0 ± 0.5 mV on the lingual surface.

Synaptic potential

Esakov and Byzov (Esakov and Byzov, 1971) suggested that intracellularly recorded hyperpolarizing responses in frog taste cells following GP nerve stimulation may be an inhibitory post-synaptic potential (IPSP). In our experiments, negative slow potentials were rarely recorded from taste cells. Even if an IPSP is induced in taste cells by GP nerve stimulation, it would be masked by the positive slow potentials that we recorded. The positive slow potentials were eliminated by blocking mAChR in lingual gland cells with atropine. If under atropine treatment any synaptic event occurs between taste cells and efferent nerve fibers, this must be mediated by transmitters that involve synaptic receptors other than mAChR.

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