

The Receptor Potential of Frog Taste Cells in Response to Cold and Warm Stimuli

Toshihide Sato¹, Kazuhisa Nishishita², Yukio Okada¹ and Kazuo Toda¹

¹Division of Integrative Sensory Physiology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan and ²Division of 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 Integrative 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

Accepted April 11, 2010

Abstract

Temperature sensitivity of frog taste cells was studied. The taste cell designated Type thermosensitive (TS) I cell was depolarized by warm stimulus at 30 °C and hyperpolarized by cold stimulus at 10 °C. The taste cell designated Type TS II cell was depolarized by the cold stimulus and hyperpolarized by the warm stimulus. Menthol solution at 20 °C, which selectively activates transient receptor potential (TRP) M8 channels sensitive to cold stimuli, depolarized Type TS II cells but not Types TS I cells. Thermal stimuli-induced receptor potentials were all blocked by a nonselective cation channel blocker flufenamic acid. The results indicate that Type TS I cells have warm sensor channels alone, Type TS II cells have cold sensor channels alone and both the channels are a nonselective cation channel. The candidate of cold sensor channel in Type TS II cells is a TRPM8 channel and that of warm sensor channel in Type TS I cells is likely to be a TRPM4-like channel from the published data. In a subset of taste cells, Types TS III and TS IV cells were found. The former was depolarized by both cold and warm stimuli, but the latter was hyperpolarized by both stimuli. Types TS III and TS IV cells might have both TRPM4-like and TRPM8 channels. It is supposed that depolarizations induced by both cold and warm stimuli were dominant in Type TS III cells and hyperpolarizations induced by both the thermal stimuli were dominant in Type TS IV cells.

Key words: frog taste cell, menthol, receptor potential, thermal stimuli, TRP channel

Introduction

Taste cells respond to various types of gustatory stimuli with depolarizing and hyperpolarizing receptor potentials (Ozeki and Sato 1972; Sato and Beidler 1975, 1983; Akaike et al. 1976). In studies on gustatory neural fiber responses of the rat and hamster, Ogawa et al. (1968) have found that significant correlations exist between thermal and gustatory responses induced by pairs of cold and HCl stimuli, cold and quinine stimuli, and warm and sucrose stimuli. This finding suggests that thermosensitive ion channels exist in taste cells and that there is an interaction between thermal and gustatory responses. Recent molecular biological studies have clarified molecular structures of gustatory receptors and transduction cascades for bitter, sweet, and umami tastes (Sato et al. 1994, 1995; Lindemann 1996; Zhang et al. 2003; Chandrashekar et al. 2006). Many types of transient receptor potential (TRP) channels responding to thermal

stimuli have been found in invertebrates and vertebrates (Papoutian et al. 2003; Damann et al. 2008). An interaction exists between gustatory transduction molecules and thermosensitive TRP channels (Zhang et al. 2003; Talavera et al. 2005). A warmed sweet solution stimulates both sweet receptors and TRPM5 channels in mammalian taste cells, resulting in a large sweet response (Pérez et al. 2002; Talavera et al. 2005; Damann et al. 2008). In humans, it has been clarified that the perceived intensity of 4 basic tastes is reduced by cooling the tongue or decreasing the temperature of gustatory solutions (Bartoshuk et al. 1982; Green and Frankmann 1987, 1988) and that strongly warming or cooling the tongue induces the weak sensation of sweetness, sourness, saltiness, or bitterness (Cruz and Green 2000).

The knowledge of receptor potential of taste cells to thermal stimuli is lacking. The purpose of the present study is to

investigate the electrical properties of receptor potentials of frog taste cells induced by cold and warm stimuli.

Materials and methods

Preparation

Bullfrogs (*Rana catesbeiana*) weighing 390–550 g were used. All the experiments were carried under the Guidance of Animal Experimentation of Nagasaki University. Both hypoglossal nerves were cut to remove spontaneous twitches, and both glossopharyngeal nerves were cut to remove the effects of parasympathetic nerve fibers on taste cell membrane potentials (Sato et al. 2005, 2006, 2007). Care was taken to keep the lingual circulation normal as long as possible. The tongue was pinned on a cork plate. The experiments were carried out at room temperature of 20–22 °C.

Electrical recording and chemical stimulation

Microelectrodes filled with 3 M KCl and having a resistance of 30–55 M Ω were used. A microelectrode was vertically inserted into the central area of taste disk of the fungiform papilla to penetrate a taste cell. Because the 3 cell body layers in the taste disk are horizontally arranged, the slow advancement of a microelectrode tip made an appearance of the resting potentials with 3 steps. This was the criteria for identifying a taste cell because the taste cell body layer was located in the deepest part (Sato et al. 2007). The change in the membrane potentials of taste cells induced by thermal and gustatory stimuli was amplified with a microelectrode amplifier (Nihon Kohden MEZ-8101) (Sato et al. 2002, 2004). The output of microelectrode amplifier was amplified with various sensitivities of the 4 amplifiers in a 4-channel pen recorder. When an adapting solution was flowed on the tongue surface, the spontaneous noisy shift of membrane potentials in taste cells was 0.1 ± 0.0 mV ($n = 30$) and its time course was 1.3 ± 0.1 s ($n = 85$). The frequency of the membrane noises was 3.4 ± 0.2 /min ($n = 32$). Because the receptor potentials were a sustained change during thermal and taste stimulation, the physiological responses were easily discriminated from the membrane noises. The membrane resistance of taste cells was measured by intracellularly injecting constant hyperpolarizing current pulses via an electric bridge housed in the microelectrode amplifier (Sato and Beidler 1975; Miyamoto et al. 1988).

Taste cells and fibers of frogs respond to water with large receptor potentials and massive neural impulses (Sato and Beidler 1975; Akaike and Sato 1976; Sato et al. 1983; Okada et al. 1993). These water responses are mostly inhibited by 10 mM NaCl but NaCl-sensitive taste cells and neural fibers slightly respond to 10 mM NaCl (Sato and Beidler 1975; Akaike and Sato 1976). Therefore, the tongue surface was always adapted to 10 mM NaCl at 20 °C to remove the water response in this study.

As thermal stimulation of taste cells, 10 mM NaCl solutions at 10 and at 30 °C were always used for cold and warm stimuli, respectively. When a thermal sensitivity range of taste cells was tested, 10 mM NaCl solutions of 5–35 °C were applied to the tongue. Four basic stimuli used were 100 mM NaCl, 10 mM quinine (Q)-HCl, 1 M sucrose, and 0.3 mM acetic acid. The last 3 substances were dissolved in 10 mM NaCl to inhibit the water response of taste cells. In some experiments, menthol solutions that specifically activate TRPM8 channels were used (Peier et al. 2002; Damann et al. 2008; Myers et al. 2009). Menthol was first dissolved in ethanol to make a stock solution and then the stock solution was diluted with 10 mM NaCl. Adapting solution of 10 mM NaCl at 20 °C that was stocked in a glass bottle was flowed on the tongue surface at a rate of 0.3 mL/s through a small-diameter tubing of a solution delivering device (Sato and Beidler 1975; Okada et al. 1993). In part of the tubing a small port was built to inject thermal and gustatory stimuli. As soon as a stimulus was injected, the adapting solution flow was stopped. Gustatory stimulus solutions and menthol solution were kept at 20 °C. Because a menthol solution induces an evaporative cooling, a temperature drop was measured during menthol stimulation of the tongue. The temperature drop for 30 s by menthol stimuli at 20 °C was 0.1 ± 0.1 °C ($n = 4$) for 0.01 mM, 0.2 ± 0.1 °C ($n = 8$) for 0.1 mM, and 0.5 ± 0.1 °C ($n = 5$) for 1 mM. A microelectrode was inserted into a fungiform papilla located near the outlet of gustatory stimulator. A probe of thermistor was located near the inserted papilla.

Statistics

Experimental data were expressed as means \pm standard error of means (SEMs). The level of significance was set at $P < 0.05$ with a Student's *t*-test.

Results

Response of taste cells for cold and warm stimuli

Microelectrodes were inserted into 81 taste cells all of which responded to 100 mM NaCl. The 75 (93%) of 81 taste cells tested responded to thermal stimuli. The resting potential of taste cells was -38.9 ± 1.1 mV ($n = 75$) under an adapting solution of 10 mM NaCl. From thermal response patterns for cold (10 mM NaCl at 10 °C) and warm (10 mM NaCl at 30 °C) stimuli, taste cells were classified into 4 types: Type TS I, Type TS II, Type TS III, and Type TS IV cells (Figure 1A). The taste cell designated Type TS I cell responded to warm stimulus with a depolarization and to cold stimulus with a hyperpolarization (Figure 1Aa). The taste cell designated Type TS II cell responded to warm stimulus with a hyperpolarization and to cold stimulus with a depolarization (Figure 1Ab). The taste cell designated Type TS III cell responded to either cold or warm stimuli with a depolarization (Figure 1Ac), and the taste cell designated Type TS IV cell responded to either cold or warm stimuli with

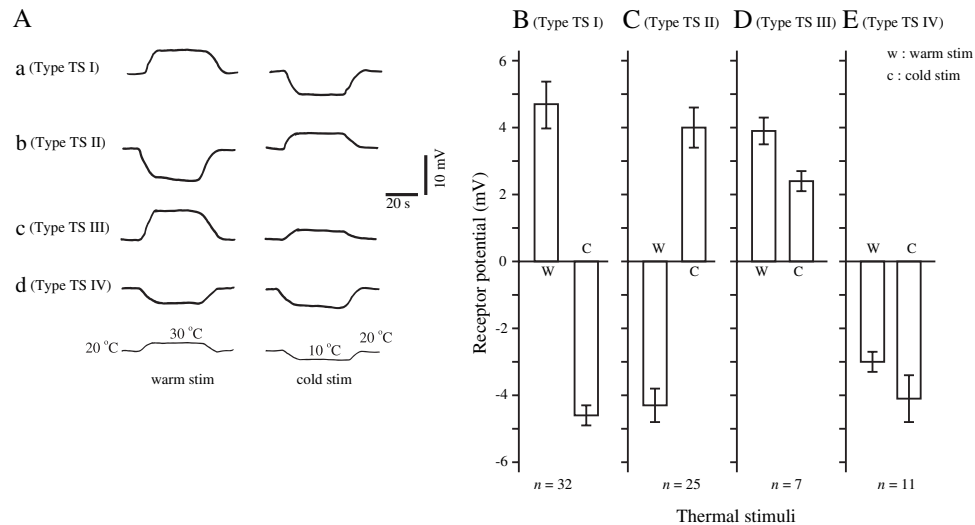


Figure 1 Receptor potentials of 4 types of taste cells for cold and warm stimuli. **(A)** Thermal responses of taste cells—**(a)** A Type TS I cell, **(b)** A Type TS II cell, **(c)** A Type TS III cell, and **(d)** A Type TS IV cell. stim, stimulus. **(B–E)** The mean amplitudes of depolarizing and hyperpolarizing responses for cold and warm stimuli in 4 types of cells. **(B)** Type TS I cell. **(C)** Type TS II cell. **(D)** Type TS III cell. **(E)** Type TS IV cell. The vertical bars are SEMs in all figures. In all experiments, adapting solution on the tongue surface was 10 mM NaCl solution at 20 °C, and cold and warm stimuli were 10 mM NaCl solutions at 10 and 30 °C.

a hyperpolarization (Figure 1Ad). Figure 1B–E shows the mean amplitudes of responses for cold and warm stimuli in 4 types of taste cells. There was no difference between depolarizing responses evoked by warm stimulus in Type TS I cells and by cold stimulus in Type TS II cells ($P > 0.05$, $n = 25$ –32) (Figure 1B,C). Also no differences were found between hyperpolarizing responses evoked by cold stimulus in Type TS I cells and by Type TS II cells ($P > 0.05$, $n = 25$ –32) (Figure 1B,C). In Type TS III cells, depolarizing responses for warm stimuli were larger than those for cold stimuli ($P < 0.05$, $n = 7$). In Type TS IV cells, hyperpolarizing responses for cold stimuli were larger than those for warm stimuli ($P < 0.05$, $n = 11$).

Temperature–response relation

The temperature of stimulus solutions used was a range of 5–35 °C. The temperature of adapting solution of 10 mM NaCl flowed on the tongue was at 20 °C. The relationships between the temperature ranges of thermal stimuli and the magnitudes of depolarizing and hyperpolarizing responses in taste cells were divided into 4 types (Figure 2A–D). 1) The Type TS I cell was depolarized by warm stimuli at a temperature >20 °C and hyperpolarized by cold stimuli at a temperature <20 °C (A). 2) The Type TS II cell was depolarized by cold stimuli at <20 °C and hyperpolarized by warm stimuli at >20 °C (B). 3) and 4) The Type TS III cell was depolarized by both cold stimuli at <20 °C and warm stimuli at >20 °C (C) and the Type TS IV cell was hyperpolarized by the same cold and warm stimuli (D).

Menthol stimulation

Menthol is a potent substance that selectively activates TRPM8 channels (Damann et al. 2008). Menthol solutions

of 20 °C depolarized Types TS II and TS III cells but hyperpolarized Types TS I and TS IV cells. Figure 3Aa,Ab shows examples of intracellular responses for various concentrations of menthol in a Type TS I and a Type TS II cell. The threshold for menthol was 0.1–1 μ M and the amplitudes of hyperpolarizing responses in Types TS I and TS IV cells (Figure 3B) and depolarizing responses in Types TS II and III cells (Figure 3C) for menthol were proportionate to the log of the molar concentration.

We compared responses induced by cold (10 °C) and 1 mM menthol (20 °C) stimuli. The Types TS I and TS IV cells were hyperpolarized by both cold and menthol solutions (Figure 4Aa,Ad). On the other hand, Types TS II and TS III cells were depolarized by both cold and menthol stimuli (Figure 4Ab,Ac). Figure 4B–E illustrates the mean amplitudes of receptor potentials for cold and 1 mM menthol stimuli in Types TS I–TS IV cells. In Types TS II and TS III cells, depolarizations for cold and menthol stimuli were the same in amplitude. The depolarizing response to 1 mM menthol was larger in Type TS II cell than in Type TS III cell ($P < 0.05$, $n = 3$ –12). In the Type TS I cell, 1 mM menthol-induced hyperpolarizing response was larger than cold stimuli-induced hyperpolarizing response (Figure 4B). After a Type TS I cell was adapted to the cool adapting solution (10 mM NaCl) of 10 °C, cold stimuli of 10 °C had no effect on the cell, but 1 mM menthol of 10 °C induced a hyperpolarizing response of -7.1 ± 0.5 mV ($n = 5$). This implies that menthol exerts an inhibitory action on the warm sensors in Type TS I cells.

Membrane resistance

The membrane resistance decreased during the depolarizing responses in Type TS I cell induced by warm stimuli (Figure 5A, left) and during those in Type TS II cell induced

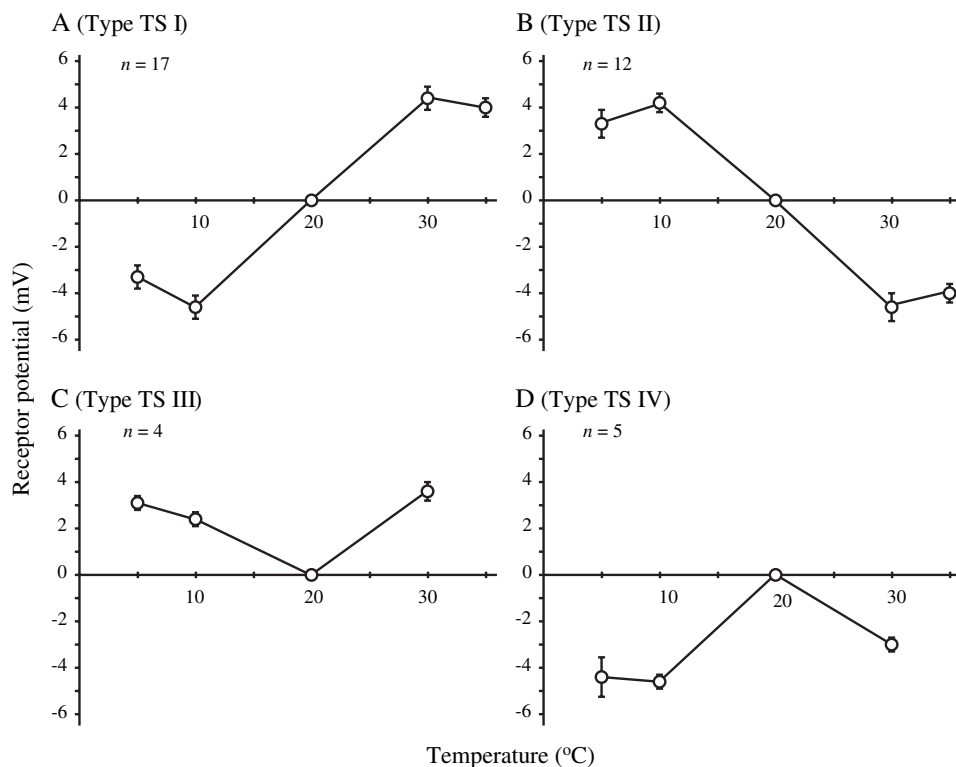


Figure 2 Temperature sensitivity of 4 taste cell types for cold and warm stimuli of 5–35 °C. **(A)** Type TS I cell. **(B)** Type TS II cell. **(C)** Type TS III cell. **(D)** Type TS IV cell. The ordinate denotes receptor potential and abscissa denotes temperature of thermal stimuli.

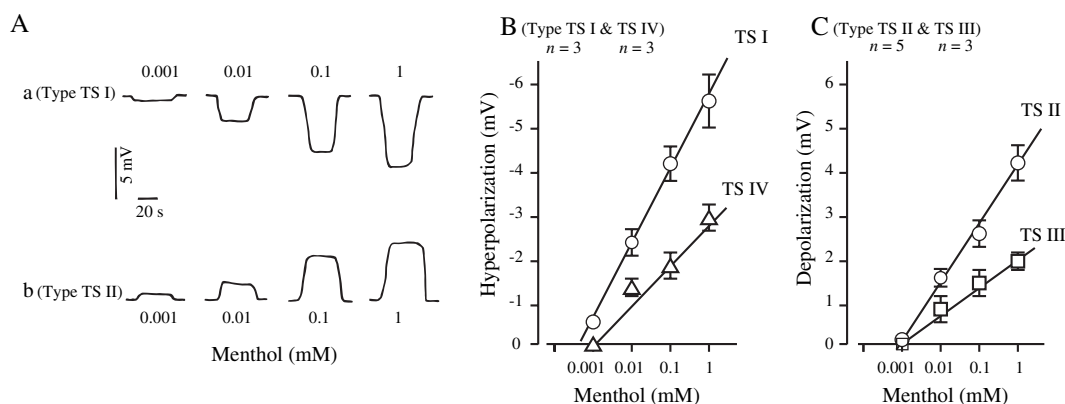


Figure 3 Receptor potentials of taste cells to menthol. **(A)** Hyperpolarizing and depolarizing responses induced by various concentrations of menthol at 20 °C—**(a)** A Type TS I cell and **(b)** A Type TS II cell. **(B)** Relation between the concentration of menthol and the amplitude of hyperpolarizations in Types TS I and TS IV cells. **(C)** Relation between the concentration of menthol and the amplitude of depolarizations in Types TS II and TS III cells.

by cold stimuli (Figure 5B, right). On the other hand, the membrane resistance increased during hyperpolarizations induced by cold stimuli in Type TS I cell (Figure 5A, right) and by warm stimuli in Type TS II cell (Figure 5B, left). In Type TS III cells, the membrane resistance decreased during depolarizations evoked by both cold and warm stimulations. The membrane resistance of Type TS IV cells increased during hyperpolarizations elicited by both stimulations. Also, the membrane resistance decreased during menthol-induced depolarizations in Types TS II and TS III cells and increased

during menthol-induced hyperpolarizations in Types TS I and TS IV cells.

The change in the membrane resistance depended on the amplitude of thermal stimuli-induced responses. Figure 6 shows the relationship between the amplitude of input resistances and the amplitude of thermal stimulus-induced depolarizations and hyperpolarizations in Types TS I–TS IV cells. The resistance change is expressed as percentage of the control in taste cells at rest. The mean amplitude of membrane resistances resulted in a 40% decrease at 10 mV

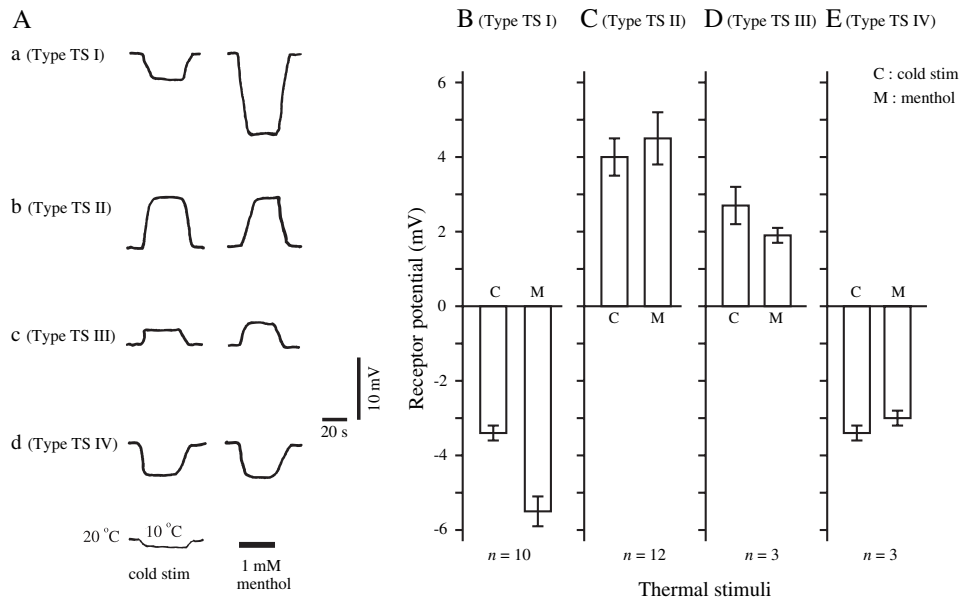


Figure 4 Receptor potentials of taste cells to cold and menthol stimuli. **(A)** Responses of 4 types of taste cells—**(a)** A Type TS I cell, **(b)** A Type TS II cell, **(c)** A Type TS III cell, and **(d)** A Type TS IV cell. **(B–E)** Depolarizing and hyperpolarizing responses of 4 taste cell types for cold stimulus at 10 °C and 1 mM menthol at 20 °C. **(B)** Type TS I cell. **(C)** Type TS II cell. **(D)** Type TS III cell. **(E)** Type TS IV cell.

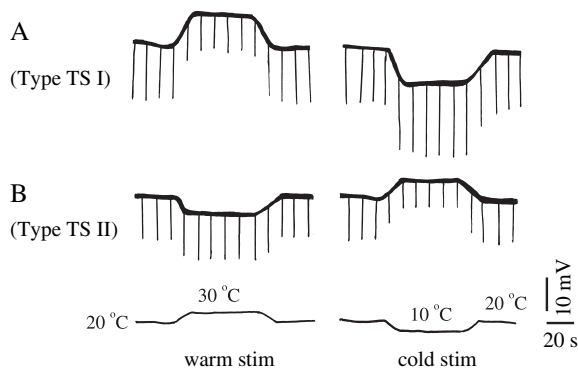


Figure 5 Membrane resistance change during responses for cold and warm stimuli. **(A)** and **(B)** Resistance changes during warm (left) and cold (right) stimulations in a Type TS I (A) and a Type TS II cell (B). Hyperpolarizing potential pulses were induced by injecting intracellularly constant current pulses. A decrease in the pulse amplitude means a decrease in the membrane resistance, and an increase of the pulse amplitude means an increase in the resistance. Input resistance at rest was 55 M Ω in the cell of (A) and 67 M Ω in the cell of (B).

depolarization and a 30% increase at 10 mV hyperpolarization during thermal stimulation. The data in Figure 6 imply that a number of thermal sensor channels open during adaptation of the tongue to an adapting solution of 10 mM NaCl in 4 types of taste cells.

Reversal potential

The reversal potentials of depolarizing and hyperpolarizing responses induced by cold and warm stimuli were measured while changing the membrane potential levels. Figure 7A

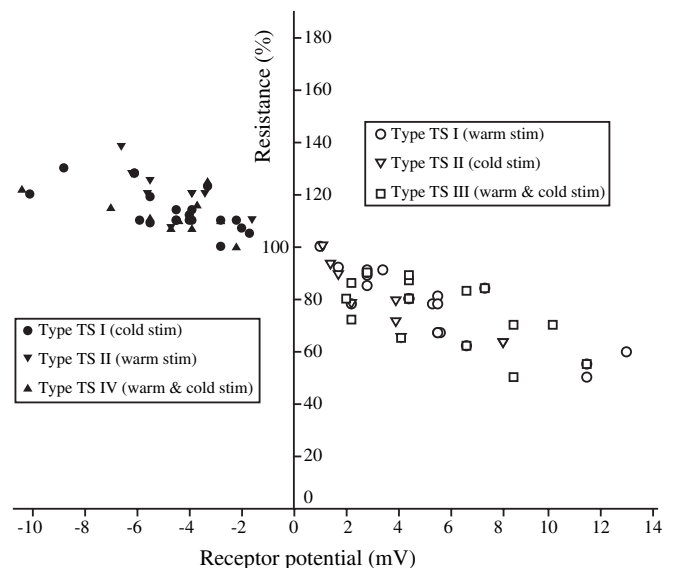


Figure 6 Relationship between the amplitudes of receptor potentials induced by cold and warm stimuli and the amplitudes of membrane resistances. Membrane resistance is expressed as percentage of the control resistance during adaptation of taste cells to 10 mM NaCl at 20 °C. Resistance was measured from Types TS I, TS II, TS III, and TS IV cells ($n = 46$). Open symbols show thermal stimuli-induced depolarizations and filled symbols thermal stimuli-induced hyperpolarizations.

shows an example of change in the amplitude and polarity of receptor potentials in a Type TS I cell induced by warm stimuli when the membrane potential of was altered. Figure 7B,C illustrates the relationships between warm responses and membrane potentials using a Type TS I (B) and Type TS II cell (C). Similar relationships between

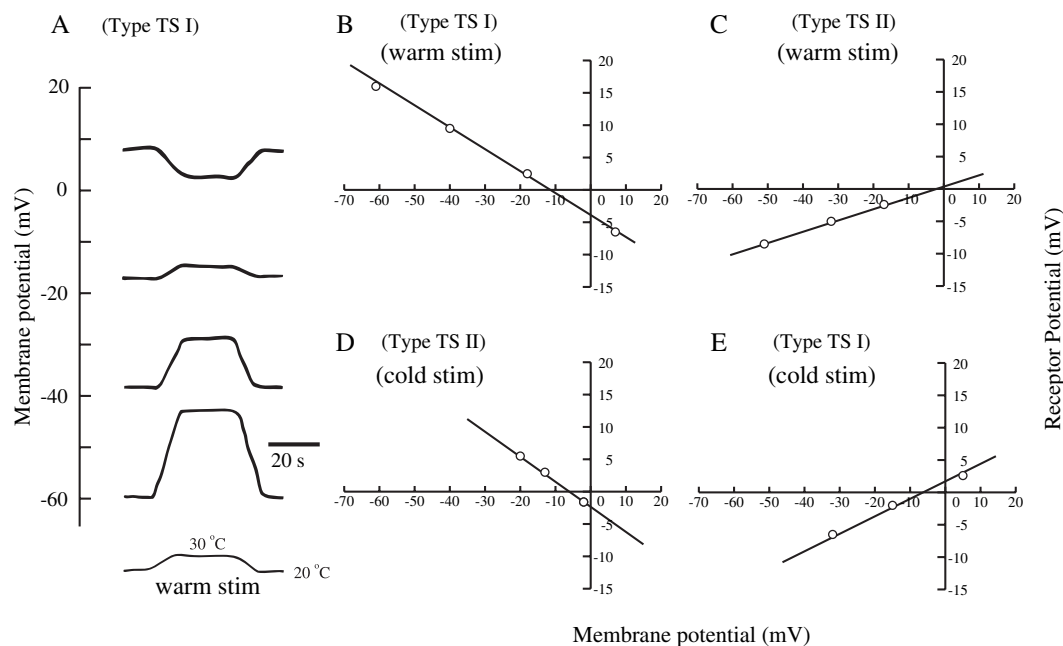


Figure 7 Measurement of reversal potentials of hyperpolarizing and depolarizing potentials for thermal stimuli. **(A)** Change in amplitude of depolarizing responses and response polarity for warm stimuli while altering membrane potential of a Type TS I cell. **(B)** and **(C)** Relationships between warm stimuli-induced responses and membrane potential levels in a Type TS I cell (**B**) and a Type TS II cell (**C**). **(D)** and **(E)** Relationships between cold stimuli-induced responses and membrane potential levels in a Type TS II cell (**D**) and a Type TS I cell (**E**). Data in **(A)** are plotted in **(B)**.

cold responses and membrane potentials are shown in a Type TS II (Figure 7D) and Type TS I cell (Figure 7E). The mean reversal potentials were -7.7 ± 1.8 mV ($n = 3$) for warm stimulus-induced depolarizing response in Type TS I cells and -5.8 ± 5.3 mV ($n = 6$) for warm stimulus-induced hyperpolarizing response in Type TS II cells. The cold stimulus-induced depolarization in Type TS II cells and hyperpolarization in Type TS I cells had the reversal potential of -3.0 ± 4.4 mV ($n = 4$) and -5.7 ± 3.5 mV ($n = 3$). No significant difference was found among the 4 reversal potentials ($P > 0.05$) in Types TS I and TS II cells.

Effect of cation channel blocker

A potent nonselective cation channel blocker, flufenamic acid (Hescheler and Schultz 1993; Sato et al. 2004) was intravenously injected at a dose of 5 mg/kg body weight. As shown in Figure 8, the blocker greatly reduced all the thermal stimuli-induced depolarizations and/or hyperpolarizations in Types TS I (A), TS II (B), TS III (C), and TS IV (D) cells. The amplitudes of thermal responses in all types of cells are expressed as percentage of the controls measured before the blocker injection.

Relation between thermal and gustatory responses

We investigated how Types TS I and TS II cells depolarized by thermal stimuli respond to gustatory stimuli. Examples of the receptor potentials induced by thermal and gustatory stimuli in 2 taste cells (Types TS I and TS II cells) are shown in Figure 9A,B. Correlations matrix between depolarizing

receptor potentials induced by pairs of thermal stimuli (cold and warm) and taste stimuli (100 mM NaCl, 10 mM Q-HCl, 1 M sucrose, and 0.3 mM acetic acid) is shown Table 1. Significant correlations were found between depolarizing receptor potentials induced by 3 pairs of warm and NaCl stimuli in Type TS I cells, cold and NaCl stimuli in Type TS II cells, and warm and sucrose stimuli in Type TS I cells ($P < 0.05$). These correlations are illustrated in Figure 10A–C. The correlation between warm and NaCl responses was negative but 2 other correlations were positive. The data of Figure 10B,C indicate that the amplitude of cold responses is almost proportionate to the amplitude of NaCl responses in Type TS II cells (B) and the amplitude of warm responses is almost proportionate to the amplitude of sucrose responses in Type TS I cells (C).

Discussion

Cold and warm sensations in the skin and oral mucosa of vertebrates are generated in receptors in the free nerve endings of cold and warm fibers of A δ and C types (Ganong 2005). Ogawa et al. (1968) found thermal sensitivities of gustatory neural fibers of A β type in the rat and hamster and suggested that temperature sensation will appear in taste cells. The present study clearly revealed that frog taste cells respond to thermal stimuli of 5–35 °C with depolarizing and hyperpolarizing receptor potentials.

Type TS I taste cells responded to warm stimulus of 30 °C with a depolarization, and Type TS II cells responded to cold stimulus of 10 °C with a depolarization. A menthol solution,

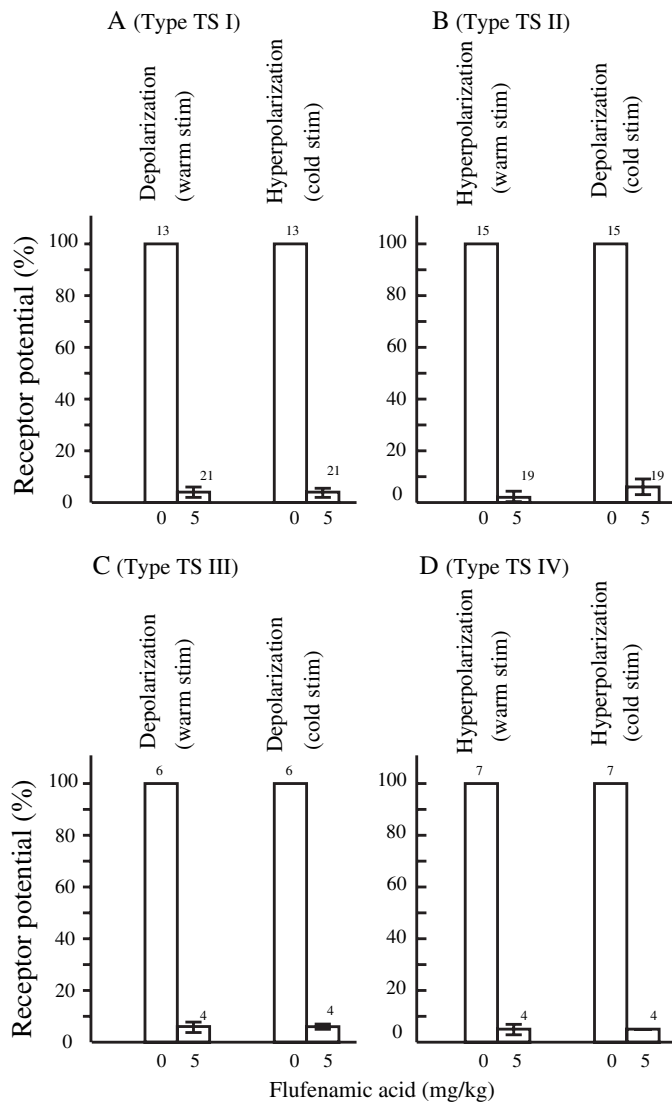


Figure 8 Effect of flufenamic acid on depolarizing and hyperpolarizing responses for thermal stimuli. (A) Type TS I cells. (B) Type TS II cells. (C) Type TS III cells. (D) Type TS IV cells. The amplitudes of thermal responses after intravenous injection of flufenamic acid (5 mg/kg) are expressed as percentage of the control thermal responses. Numerals above columns are the number of taste cells tested.

which is a selective activator of TRPM8 channels sensitive to cold stimuli, depolarized Type TS II cells. From these results, it is very likely that Type TS I cells have warm sensor channels alone and Type TS II cells have cold sensor channels alone. Because menthol caused a depolarization in cold-sensitive Type TS II cells, a candidate of cold sensors in Type TS II cell is TRPM8 channels. The 5 TS TRPs such as TRPM5, TRPM8, TRPV1, TRPV3, and TRPA1 are found in the tongue (Damann et al. 2008). TRPM5 alone has been identified in mammalian taste cells sensitive to bitter, sweet, and umami compounds (Pérez et al. 2002; Prawitt et al. 2003; Zhang et al. 2007). The other 4 TRPs are not identified in taste cells but found in the free nerve ending of tri-

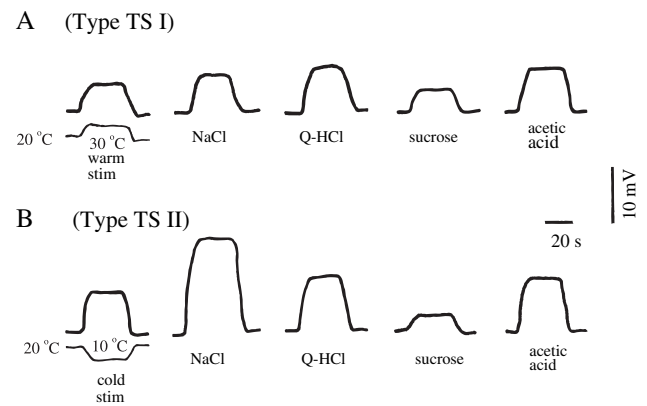


Figure 9 Receptor potentials induced by 2 thermal stimuli and 4 gustatory stimuli. (A) A Type TS I cell. (B) A Type TS II cell. Stimuli were cold (10 °C) and warm (30 °C) stimuli and 100 mM NaCl, 10 mM Q-HCl, 1 M sucrose, and 0.3 mM acetic acid solutions at 20 °C.

Table 1 Correlation matrix for pairs of depolarizing receptor potentials induced by thermal and gustatory stimuli

	100 mM NaCl	10 mM Q-HCl	1 M Sucrose	0.3 mM Acetic acid
Warm stim	−0.392 ^a	0.138	0.448 ^a	0.150
Cold stim	0.600 ^a	0.282	0.152	0.157

Cold stimulus (stim) was 10 mM NaCl solution at 10 °C and warm stimulus (stim) was 10 mM NaCl solution at 30 °C. Calculations of correlation coefficients between pairs of depolarizations induced by warm and taste stimuli were based on 31 Type TS I cells and those between the depolarizations induced by cold and taste stimuli were based on 22 Type TS II cells.

^aCorrelation is significant ($P < 0.05$).

geminal neurons innervating the tongue and palate (Damann et al. 2008). In the present experiment, a temperature sensitivity of depolarizing response in Type TS I cells for warm stimuli is a range of 20–35 °C. This temperature sensitivity is close to that of TRPM5. However, the evolutionary analysis of thermoTRP channels in vertebrates indicates that a loss of TRPM5 occurred in clawed frog (*Xenopus tropicalis*) during evolutionary process (Saito and Shingai 2006). If this is true in other frogs and toads of *Rana* and *Bufo*, the taste cells in the tongue of bullfrogs may not have TRPM5 channels. Clawed frogs have TRPM4 whose temperature sensitivity is 15–35 °C, which is very similar to sensitivity of TRPM5 (Saito and Shingai 2006). Therefore, it is supposed that Type TS I taste cells of bullfrogs have TRPM4-like channels whose warm sensitivity is at a temperature >20 °C.

The thermal responses of types TS III and TS IV cells (Figure 1) would be explained by considering the existence of 2 different TRPs such as TRPM4-like and TRPM8 channels in these cells. Thus, the responses in Types TS III and TS IV cells may come from a summation of the thermal responses induced by warm sensor TRPM4-like channels and cold sensor TRPM8 channels. The depolarizing responses evoked by

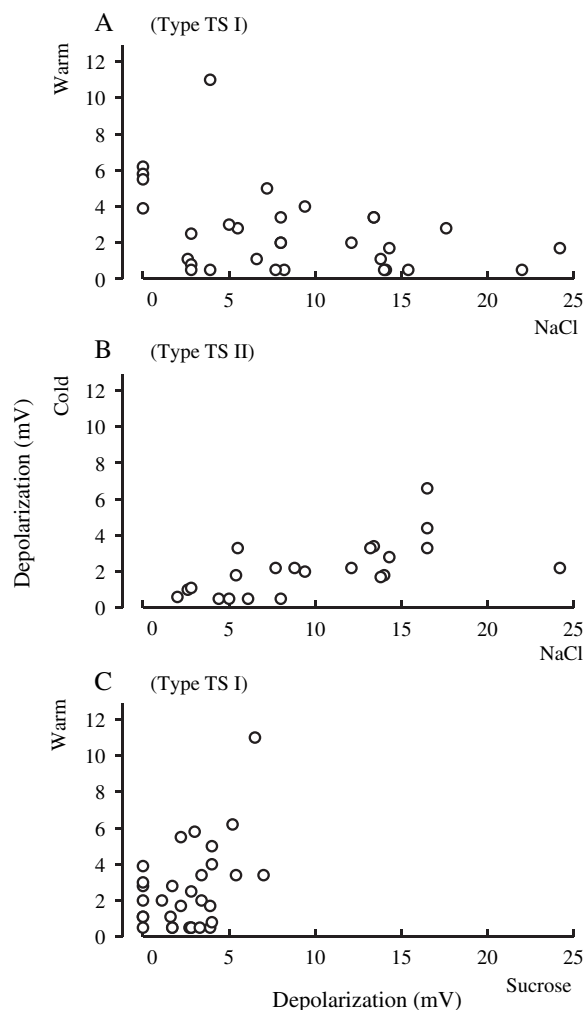


Figure 10 Relationships between gustatory responses and thermal responses. **(A)** Between 100 mM NaCl and warm stimulus in Type TS I cells. **(B)** Between 100 mM NaCl and cold stimulus in Type TS II cells. **(C)** Between 1 M sucrose and warm stimulus in Type TS I cells. $n = 31$ in Type TS I cells and 22 in Type TS II cells.

both cold and warm stimuli in a Type TS III cell might be a summation of the large amplitude of depolarizations induced by activating cold and warm sensors and the small amplitude of hyperpolarizations by inhibiting both sensors. On the other hand, the Type TS IV cell response exhibiting hyperpolarizations for both cold and warm stimuli might be a summation of large hyperpolarizations by inhibiting both cold and warm sensors and small depolarizations induced by activating both the sensors.

Because all responses in 4 kinds of Type TS cells were blocked by a nonselective cation channel blocker, estimated TRPM4-like and TRPM8 channels would be nonselective cation channels permeable to Na^+ and K^+ . The measurement of membrane resistance during thermal responses (Figure 6) suggests that a number of TRPM4-like and/or TRPM8 channels in taste cells are open in an unstimulated state and that depolarizations and hyperpolarizations are induced

by opening closed channels and by closing opened channels, respectively. In the Type TS I cell, a depolarization to warm stimulus is probably induced by Na^+ entry through the opened TRPM4-like channels and a hyperpolarization to cold stimulus might be due to a blockage of Na^+ entry by closing TRPM4-like channels. Also, a hyperpolarization of Type TS I cells by menthol might be due to a blockage of Na^+ entry by closing TRPM4-like channels. On the other hand, in the Type TS II cell, a hyperpolarization by warm stimuli would be due to a blockage of Na^+ entry by closing TRPM8 channels and a depolarization by cold and menthol stimuli would be due to opening TRPM8 channels permeable to Na^+ . This explanation is supported by the finding that the membrane resistance is decreased during thermal stimuli-induced depolarizations and increased during thermal stimuli-induced hyperpolarizations. The properties of these channels are similar to those of nonselective cation channels in generation of hyperpolarizing and depolarizing postsynaptic potentials in efferent synapses of frog taste cells (Sato et al. 2002, 2005, 2007). In conclusion, we propose that a depolarization of Type TS I taste cells in frogs for warm stimulus derives from warm-sensitive TRPM4-like channels and a depolarization of Type TS II cells for cold stimulus derives from cold-sensitive TRPM8 channels.

In gustatory researches with poikilothermal frogs, the tongue surface is usually adapted to an adapting solution of 20–25 °C and many important gustatory transduction mechanisms have been provided (Sato et al. 1994, 1995). The maximum responses of frog taste nerve for 4 basic gustatory solutions and water appear when the temperature of stimulating solutions is at 20–25 °C (Yamashita 1964). When the temperature of 0.5 M NaCl, 0.1 mM Q-HCl, and 5 mM acetic acid are reduced to 10 °C, frog taste responses for these stimuli are decreased to 10–50%. Increasing the temperature of these stimuli to 35 °C also reduce the taste nerve responses to 40–60% (Yamashita 1964). The temperature of tongue surface in anesthetized rats is at ~30 °C, and the maximum taste nerve responses are obtained by applying gustatory solutions of 30 °C (Yamashita and Sato 1965). The taste nerve responses are greatly reduced by applying taste stimuli >30 and <30 °C (Yamashita and Sato 1965). The maximum effect of taste solution temperature on a gustatory transduction of taste cells is at 20–25 °C in frogs and at 30 °C in rats (Yamashita 1964; Yamashita and Sato 1965). The relationship between lingual adapting temperature and thermal transduction of taste cells is unknown. The present experiment was performed under adaptation of the frog tongue to 20 °C and the large amplitude of depolarizing warm and cold responses was obtained in frog taste cells. If the effect of tongue surface temperature on both gustatory and thermal transductions is at the same, thermal transduction in frog taste cells might occur most effectively when the tongue surface is adapted to 20–25 °C. When the frog tongue surface is adapted to 30 °C, the amplitude of depolarizing response in Type TS I cell and hyperpolarizing response in Type

TS II cell to the warm stimuli of 40 °C would be greatly reduced. It is biologically tempting that the relationships between the level of lingual adapting temperature and the magnitude of thermal stimuli-induced receptor potentials are clarified in amphibian and mammalian taste cells. In humans, warming the tongue tip from 20 to 35 °C induces a weak thermal sweetness (Cruz and Green 2000). In Type TS I taste cells in frogs a significant correlation exists between depolarizations induced by warm stimuli of 30 °C and depolarizations induced by 1 M sucrose of 20 °C. Therefore, it is estimated that even frogs perceive thermal sweet responses during the warm stimulation of the tongue.

Funding

Japan Society for Promotion of Science (17570064).

References

- Akaike N, Noma A, Sato M. 1976. Electrical responses of frog taste cells to chemical stimuli. *J Physiol.* 254:87–107.
- Akaike N, Sato M. 1976. Water response in frog taste cells. *Comp Biochem Physiol A.* 54:149–156.
- Bartoshuk LM, Rennert K, Rodin J, Stevens JC. 1982. Effects of temperature on the perceived sweetness of sucrose. *Physiol Behav.* 28:905–910.
- Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS. 2006. The receptors and cells for mammalian taste. *Nature.* 444:288–294.
- Cruz A, Green BG. 2000. Thermal stimulation of taste. *Nature.* 403:889–892.
- Damann N, Voets T, Nilius B. 2008. TRPs in our senses. *Curr Biol.* 18:R880–R889.
- Ganong WF. 2005. Review of medical physiology. 22nd ed. New York: McGraw-Hill.
- Green BG, Frankmann SP. 1987. The effect of cooling the tongue on the perceived intensity of taste. *Chem Senses.* 12:609–619.
- Green BG, Frankmann SP. 1988. The effect of cooling on the perception of carbohydrate and intensive sweeteners. *Physiol Behav.* 43:515–519.
- Hescheler J, Schultz G. 1993. Nonselective cation channels: physiological and pharmacological modulations of channel activity. In: Siemen D, Hescheler J, editors. *Nonselective cation channels: pharmacology, physiology and biophysics.* Basel (Switzerland): Birkhäuser. p. 27–43.
- Lindemann B. 1996. Taste perception. *Physiol Rev.* 76:719–766.
- Miyamoto T, Okada Y, Sato T. 1988. Ionic basis of receptor potential of frog taste cells induced by acid stimuli. *J Physiol.* 405:699–711.
- Myers BR, Sigal YM, Julius D. 2009. Evolution of thermal response properties in a cold-activated TRP channel. *PLoS One.* 4:e5741.
- Ogawa H, Sato M, Yamashita S. 1968. Multiple sensitivity of chorda tympani fibres of the rat and hamster to gustatory and thermal stimuli. *J Physiol.* 199:223–240.
- Okada Y, Miyamoto T, Sato T. 1993. The ionic basis of the receptor potential of frog taste cells induced by water stimuli. *J Exp Biol.* 174:1–17.
- Ozeki M, Sato M. 1972. Responses of gustatory cells in the tongue of rat to stimuli representing four taste qualities. *Comp Biochem Physiol A.* 41:391–407.
- Patapoutian A, Peier AM, Story GM, Viswanath V. 2003. ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nat Rev Neurosci.* 4:529–539.
- Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, Mclyntre P, Bevan S, et al. 2002. A TRP channel that senses cold stimuli and menthol. *Cell.* 108:705–715.
- Pérez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H, Max M, Margolske RF. 2002. A transient receptor potential channel expressed in taste receptor cells. *Nature Neurosci.* 5:1169–1176.
- Prawitt D, Montell-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Pemner R. 2003. TRPM5 is a transient Ca²⁺-activated cation channel responding to rapid changes in [Ca²⁺]_i. *Proc Natl Acad Sci U S A.* 100:15166–15171.
- Saito S, Shingai R. 2006. Evolution in thermoTRP ion channel homologs in vertebrates. *Physiol Genomics.* 21:219–230.
- Sato T, Beidler LM. 1975. Membrane resistance change of the frog taste cells in response to water and NaCl. *J Gen Physiol.* 66:735–763.
- Sato T, Beidler LM. 1983. Dependence of gustatory neural response on depolarizing and hyperpolarizing receptor potentials of taste cells in the rat. *Comp Biochem Physiol A.* 75:131–137.
- Sato T, Miyamoto T, Okada Y. 1994. Comparison of gustatory transduction mechanisms in vertebrate taste cells. *Zool Sci.* 11:767–780.
- Sato T, Miyamoto T, Okada Y. 2002. Slow potentials in taste cells induced by frog glossopharyngeal nerve stimulation. *Chem Senses.* 27:367–374.
- Sato T, Nishishita K, Mineda T, Okada Y, Toda K. 2007. Depression of gustatory receptor potential in frog taste cell by parasympathetic nerve-induced slow hyperpolarizing potential. *Chem Senses.* 32:3–10.
- Sato T, Nishishita K, Kato Y, Okada Y, Toda K. 2006. Tonic activity of parasympathetic efferent nerve fibers hyperpolarizes the resting membrane potential of frog taste cells. *Chem Senses.* 31:307–313.
- Sato T, Ohkusa M, Okada Y, Sasaki M. 1983. Topographical difference in taste organ density and its sensitivity of frog tongue. *Comp Biochem Physiol A.* 76:233–239.
- Sato T, Okada Y, Miyamoto T. 1995. Molecular mechanisms of gustatory transductions in frog taste cells. *Prog Neurobiol.* 46:239–287.
- Sato T, Okada Y, Miyamoto T, Kato Y, Toda K. 2005. Taste cell responses in the frog are modulated by parasympathetic efferent nerve fibers. *Chem Senses.* 30:761–769.
- Sato T, Okada Y, Toda K. 2004. Analysis of slow hyperpolarizing potentials in frog taste cells induced by glossopharyngeal nerve stimulation. *Chem Senses.* 29:651–657.
- Talavera K, Yasumatsu KY, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolske RF, Nilius B. 2005. Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. *Nature.* 438:1022–1025.
- Yamashita S. 1964. Chemoreceptor response in frog, as modified by temperature change. *Jpn J Physiol.* 14:488–504.
- Yamashita S, Sato M. 1965. The effects of temperature on gustatory response of rats. *J Cell Comp Physiol.* 66:1–18.
- Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJP. 2003. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell.* 112:293–301.
- Zhang Z, Zhao Z, Margolske RF, Liman E. 2007. The transduction channel TRPM5 is gated by intracellular calcium in taste cells. *J Neurosci.* 27:5777–5786.