Changes in Outward K^+ Currents in Response to Two Types of Sweeteners in Sweet Taste Transduction of Gerbil Taste Cells

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Abstract

Using the whole cell patch clamp technique, we measured changes in outward K^+ currents of gerbil taste cells in response to different kinds of sweeteners. Outward K^+ currents of the taste cell induced by depolarizing pulses were suppressed by sweet stimuli such as 10 mM Na-saccharin. The membrane-permeable analog of cAMP, cpt-cAMP, also decreased outward K^+ currents. On the other hand, the K^+ currents were enhanced by amino acid sweeteners such as 10 mM D-tryptophan. The outward K^+ current was enhanced by external application of Ca^{2+}-transporting ionophore, 5 μM ionomycin, and intracellular application of 5 μM inositol-1,4,5-trisphosphate (IP_3). The outward K^+ currents were no longer suppressed by 10 mM Na-saccharin containing 20 μM gurmarin, but were still enhanced by 10 mM D-tryptophan containing 20 μM gurmarin. These results suggest that sweet taste transduction for one group of sweeteners such as Na-saccharin in gerbils is concerned with an increase of the intracellular cAMP level, and that the transduction for the other group of sweeteners such as D-tryptophan is concerned with an increase of the intracellular IP_3 level which releases Ca^{2+} from the internal stores. **Chem. Senses 22: 163–169, 1997.**

Introduction

Usually, a depolarization in taste cells induced by sweeteners elicits gustatory neural information in the primary taste neurons (Sato and Beidler, 1982; Kinnamon, 1988; Kinnamon and Cummings, 1992; Cummings *et al.*, 1993; Sato *et al.*, 1994). Some sweet substances bind to specific receptor sites in taste cell membrane coupled to GTP-binding proteins, leading to an increase in the cytosolic cAMP level. Then, the activated protein kinase A in the taste cell closes a class of K^+ channels, resulting in a depolarization of the taste cell (Avenet *et al.*, 1988). A taste cell in frog and mouse is depolarized by an electrophoretical injection of cAMP or cGMP into the cell (Okada *et al.*, 1987; Tonosaki and Funakoshi, 1988). The cyclic nucleotide-induced depolarization accompanied with a conductance decrease in mouse taste cells is similar to that elicited by sugar stimuli (Tonosaki and Funakoshi, 1988). On the other hand, it is known that sugar stimuli applied to frog taste cells induce the depolarization accompanied with a conductance increase (Okada *et al.*, 1992). Béhé *et al.* (1990) reported that application of a Na-saccharin stimulus suppresses the outward K^+ currents in rat taste cells. Cummings *et al.* (1993, 1996) have suggested that either cAMP or cGMP mediates the sweet taste transduction by blocking outward K^+ currents in taste cells of hamsters.

Mammals used in the previous gustatory studies, excepting mouse, are low-sensitive to various types of sweeteners. Since the neurophysiological and behavioral studies indicate that taste cells in Mongolian gerbils are high-sensitive to various sweeteners (Jakinovich and Oakley, 1975; Jakinovich, 1976, 1981), we used isolated gerbil taste cells to study sweet taste transduction mechanisms. We report that taste cells in gerbil have two differential pathways for sweet taste transduction cascades.

This study has been published previously in abstract form (Uchida *et al.*, 1994, 1995, 1996).

Materials and methods

Isolation of the taste cell

Mongolian gerbils (*Meriones unguiculatus*) from 12 to 15 weeks old were used in the present experiments. Taste cells were isolated by the same procedure as reported by Akabas *et al.* (1988). After anesthetizing the gerbil with pentobarbital (30 mg/kg), the animal was killed by dislocating the cervical vertebrae, and the tongue was quickly removed and placed in a cold Tyrode solution oxygenated with 100% O_2. The tongue was washed with and incubated in a Ca^{2+}-free Tyrode solution containing 2 mM EGTA for 30 min at 30°C. Elastase 1 mg/ml (Boehringer Mannheim, Mannheim, Germany) in a normal Tyrode solution was injected through the cut proximal end into the whole muscular surface below the dorsal lingual epithelium with a fine needle (25 G; Terumo, Tokyo, Japan), and the tongue was incubated in an oxygenated divalent cation-free Tyrode solution for 10 min at 30°C. The dorsal epithelium was gently peeled free from the tongue muscles. Taste buds of the fungiform papillae were mechanically dissociated, while the epithelial sheet containing the papillae was triturated several times in a Tyrode solution. Taste cells were mechanically dissociated from taste buds after incubating them in 0.25% trypsin (Gibco, Gaithersburg, MD) in Ca^{2+}-free Tyrode for 5 min at 32°C. After taste cells were attached to an experimental chamber of 0.5 ml, they were thoroughly washed with a Tyrode solution to remove debris. All experiments were performed at a room temperature of 20–23°C.

Electrical recordings

Changes in the membrane currents of taste cells in response to sweet stimuli were measured using the whole cell patch clamp technique (Hamill *et al.*, 1981). The currents were amplified with a patch clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo, Japan). The current signals were low-pass filtered at 1 kHz, digitized at 125 kHz, acquired at a sampling rate of 10 kHz using a pCLAMP software (Axon Instruments, Foster City, CA) and stored on an IBM-PC computer. The membrane currents were usually induced in taste cells by depolarizing pulses of –40 to +60 mV from a holding potential of –60 mV. The leak currents flowing through the membrane were automatically subtracted from records by computer (Kinnamon and Roper, 1988). Patch pipettes were pulled from borosilicate glass capillaries (1.5 mm outer diameter; Clark Electromedical Instruments, Pangbourne, UK) with a two stage vertical puller (PP83; Narishige, Tokyo, Japan). The resistance of a pipette filled with a high K^+ solution ranged between 5 and 10 MΩ. Cell attached seal resistances were in the range of 10–20 GΩ. A patch pipette was manipulated with a hydraulic 3-D manipulator (WR-88; Narishige) using an inverted microscope (IMT-2; Olympus, Tokyo, Japan). Isolated taste cells were settled on the chamber filled with a Tyrode solution. The initial sealing of a pipette to a taste cell surface was facilitated by applying weak suction, and the patch membrane was ruptured by applying strong suction and a rapid train of negative electrical pulses. Changes in outward K^+ currents in response to sweeteners were usually measured within 1 min after a sweet stimulus was applied to the bath.

Solutions

The composition of the pipette solution was: 140 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA and 10 mM HEPES–KOH (pH = 7.2). The Tyrode solution contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES–NaOH (pH = 7.4), 10 mM glucose and 10 mM pyruvate. Sweet stimuli of 10 mM Na-saccharin, 10 mM D-tryptophan and 10 mM D-phenylalanine (Nacalai, Kyoto, Japan) and drugs of 5 mM 8-(4-chlorophenylthio)-adenosine 3′-5′-cyclic monophosphate (cpt-cAMP) (Sigma, St Louis, MO) and 10 mM tetraethylammonium chloride (TEA-Cl) (Nacalai) were prepared in the Tyrode solution and stored at 4°C. Solutions of Na-saccharin 10 mM and D-tryptophan induce half-maximal chorda tympani responses in gerbils (Jakinovich, 1981). Inositol-1,4,5-trisphosphate (IP_3) 5 μM (Sigma) was dissolved in the pipette solution. Ionomycin (Carbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide (DMSO; Sigma) at a

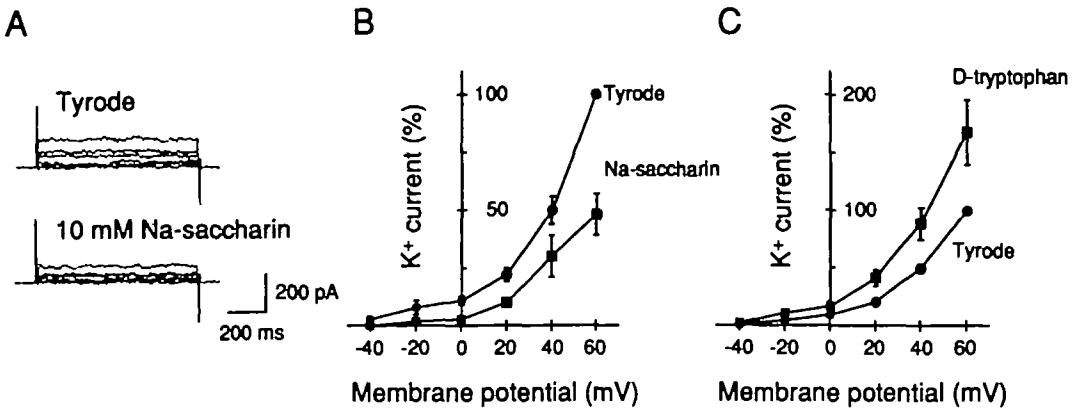

Figure 1 Effects of 10 mM Na-saccharin and 10 mM D-tryptophan on the outward K^+ currents in gerbil taste cells. **(A)** Outward K^+ currents in normal Tyrode solution and 10 mM Na-saccharin solution in Tyrode. Data were from one taste cell. The currents were induced by depolarizing voltage pulses of –40, –20, 0, 20, 40 and 60 mV in 20 mV increments from a holding potential of –60 mV in this and the other figures. The current measurement in Na-saccharin was made 2 min after that in Tyrode. Leak currents were subtracted in this and subsequent figures. **(B)** Relations between K^+ currents (I) and membrane potentials (V) in Tyrode and 10 mM Na-saccharin in Tyrode. From five taste cells. **(C)** I–V relations in Tyrode and 10 mM D-tryptophan in Tyrode. Data are from seven taste cells. The amplitudes of outward K^+ currents were normalized in this and the other figures. The current evoked at 60 mV in normal Tyrode was taken as 100%. Vertical bars are SEM.

concentration of 1 mM and this solution was added to the Tyrode solution to give a final concentration of 5 μM. Gurmarin, which was isolated from leaves of *Gymnema sylvestre*, was kindly supplied by Dr Imoto (Tottori University, Japan) (Imoto *et al.*, 1991). Gurmarin was dissolved in deionized water at 1 mM and this solution was added to the Tyrode solution to give a final concentration of 20 μM (pH 7.4). Elastase 1 mg/ml (Boehringer Mannheim) which was used to peel off the tongue epithelium was dissolved in the Tyrode solution. Trypsin (Gibco) was dissolved in a Ca^{2+}-free Tyrode solution containing 2 mM EDTA. Sweet stimuli and drugs were applied to the bath in a chamber with a fine pipette in the earlier stage of the experiments, but were slowly superfused in the later stage of the experiments.

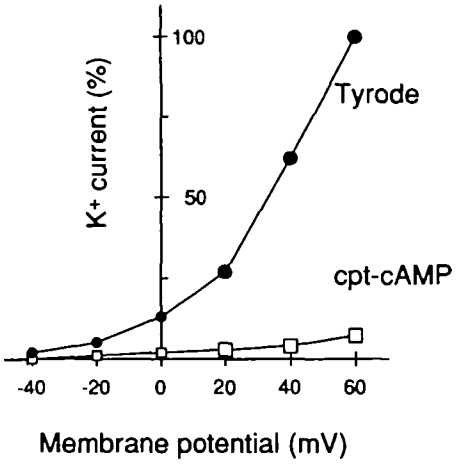

Figure 2 Relationships between K^+ currents and membrane potentials from a holding potential of –60 mV in Tyrode and 5 mM cpt-cAMP in Tyrode. Effect of cpt-cAMP was measured 3 min after its bath application. Each point is mean from two taste cells.

Results

As shown in Figure 1A, outward currents were always induced in taste cells by depolarizing pulses of –40, –20, 0, 20, 40 and 60 mV from a holding potential of –60 mV. The currents were identified as outwardly rectifying K^+ currents because the currents were completely blocked by adding 10 mM TEA to the bathing solution or by replacing K^+ in the pipette with Cs^+. Outward K^+ currents were suppressed by applying 10 mM Na-saccharin to the bath. This example is shown in lower traces of Figure 1A. Soon after being returned to Tyrode, the K^+ currents in response to the depolarizing pulses were recovered to the control levels. In Figure 1B, the relationships between the mean magnitudes of outward K^+ currents and the membrane potential levels from a holding potential of –60 mV are shown. The amplitude of outward K^+ currents was variable from cell to cell, so normalized amplitudes were compared between the cells. It is seen that the K^+ currents above –40 mV were significantly suppressed during stimulation of taste cells with 10 mM Na-saccharin ($P < 0.05$ at –40–0 mV; $P < 0.01$ at 20–60 mV). The suppression of K^+ currents was observed in five of seven taste cells sampled. The similar suppression of the K^+ currents was observed when a membrane

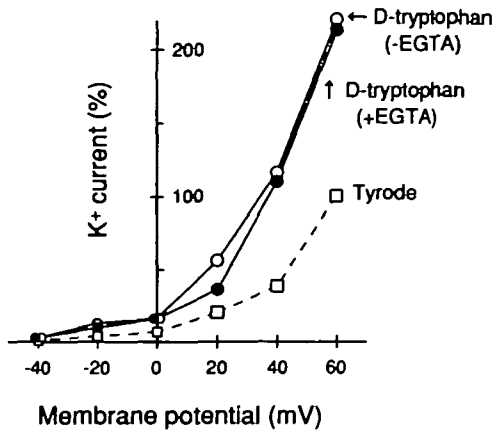

Figure 3 Effect of Ca^{2+} on tryptophan-induced responses. Relations between K^+ currents and membrane potentials in Tyrode solution and in 10 mM D-tryptophan solution with (–EGTA) and without 2 mM Ca^{2+} (+EGTA). 2 mM EGTA was added to Ca^{2+}-free Tyrode to remove Ca^{2+}. Each point is mean from two cells.

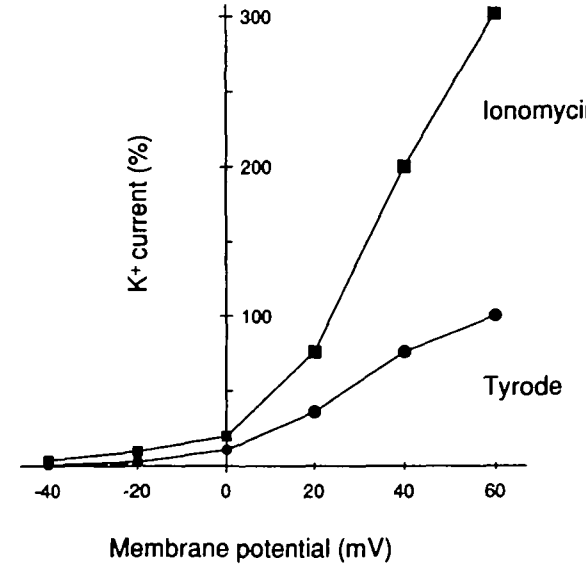

Figure 4 Effect of 5 μM ionomycin on K^+ currents. Ionomycin 5 μM in normal Tyrode was added to the bath. Each point is mean from two taste cells.

permeable cpt-cAMP (5 mM) of cAMP analog was applied to the bath (Figure 2). cAMP is well known as one of the second messengers.

On the other hand, outward currents were enhanced when 10 mM D-tryptophan of an amino acid sweetener was applied to the bath (Figure 1C). The amplitudes of the outward currents at the membrane potentials above –40 mV under stimulation with D-tryptophan were significantly larger than those under normal Tyrode ($P < 0.05$ at –40 to –0 mV; $P < 0.01$ at 20–60 mV). The enhancement of outward currents was observed in all seven taste cells tested. The outward currents were mostly depressed after the mixture of 10 mM D-tryptophan and 10 mM TEA had been applied to the bath. This indicates that enhanced outward currents are a family of K^+ currents. As shown in Figure 3, the enhancement of outward K^+ currents occurred in the same degree regardless of the presence of Ca^{2+} in the D-tryptophan solution. The outward K^+ currents were also enhanced even when the D-tryptophan in Ca^{2+}-free Tyrode was applied to the taste cells bathed in Ca^{2+}-free Tyrode. The K^+ currents were enhanced during stimulation with 10 mM D-phenylalanine.

The outward K^+ currents were greatly enhanced by application of 5 μM ionomycin in a Tyrode solution which is one of the Ca^{2+}-ionophores (Figure 4). This enhancement occurred even when 5 μM ionomycin was prepared in Ca^{2+}-free Tyrode containing 2 mM EGTA. This suggests that the enhancement of the K^+-currents is related to release of Ca^{2+} from internal Ca^{2+} stores where Ca^{2+} ionophores were formed.

We measured the effect of IP_3 on the outward K^+ currents with a patch pipette containing 5 μm IP_3. As shown in the upper traces of Figure 5A, the amplitudes of K^+ currents measured immediately after the rupture of the patch membrane with the IP_3 pipette were the same as the mean K^+ currents measured with a normal K^+ pipette. Therefore, K^+ currents immediately after the membrane rupture were considered to be unaffected by IP_3. On the other hand, the outward K^+ currents 3 min after the rupture were greatly enhanced (Figure 5B), suggesting that an enhancement of outward K^+ currents is concerned with Ca^{2+}-dependent K^+ currents which are activated by IP_3-released Ca^{2+} from the internal stores.

Gurmarin, isolated from the leaves of *Gymnema sylvestre*, is known to suppress the gustatory neural responses elicited by sweet stimuli (Imoto *et al.*, 1991; Ninomiya and Imoto, 1995). Outward K^+ currents were no longer suppressed by a 10 mM Na-saccharin solution when 20 μM gurmarin was added to it, while the K^+ currents were still enhanced by a 10 mM D-tryptophan solution containing 20 μM gurmarin. Plain gurmarin in a normal Tyrode solution did not affect the outward K^+ currents. Figure 6 summarizes effects of gurmarin. It is seen that the magnitudes of outward K^+ currents above 0 mV under control Tyrode are greatly enhanced by 10 mM D-tryptophan regardless of the presence of 20 μM gurmarin (B), but are almost the same as those by 10 mM Na-saccharin containing 20 μM gurmarin (A), suggesting that receptors for Na-saccharin are gurmarin-sensitive, whereas those for D-tryptophan are gurmarin-insensitive.

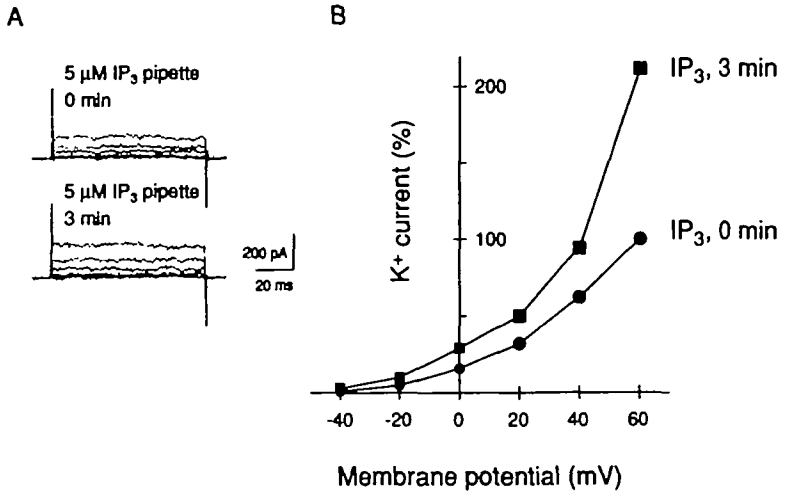

Figure 5 Effect of IP_3 on K^+ currents in taste cells. **(A)** Outward K^+ currents in a taste cell were measured immediately after rupture of the patch membrane with a pipette containing 5 μM IP_3 (upper traces) and 3 min after the rupture (lower traces). K^+ currents were induced by depolarizing pulses of –40 to –60 mV from a holding potential of –60 mV. **(B)** Relations between K^+ currents and membrane potentials. Times are 0 min and 3 min after rupture of membrane with a 5 μM IP_3 pipette. Each point is mean from two taste cells.

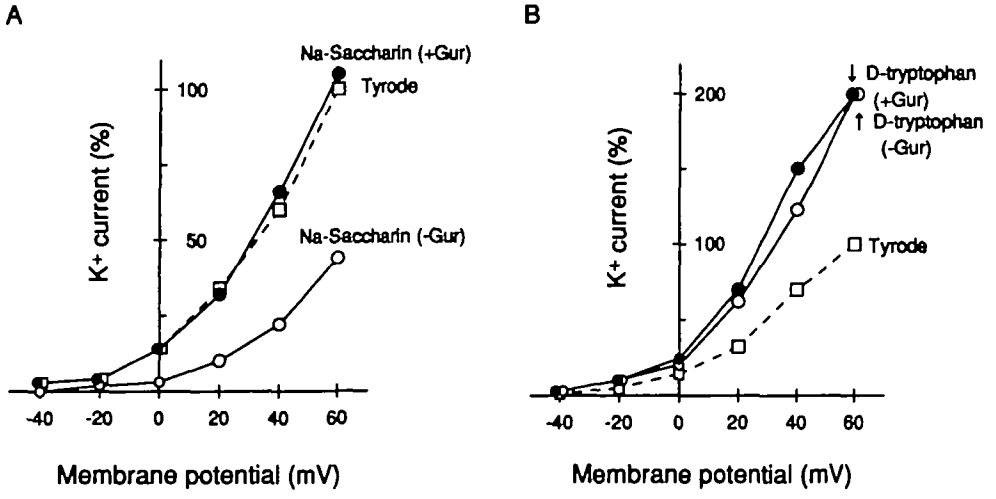

Figure 6 Effects of gurmarin on the K^+ currents during stimulation of taste cells with 10 mM Na-saccharin in Tyrode and 10 mM D-tryptophan in Tyrode. **(A)** I–V relation in 10 mM Na-saccharin solution in Tyrode with (+Gur) and without 20 μM gurmarin (–Gur). Each point is mean from three taste cells. **(B)** I–V relations in 10 mM D-tryptophan solution in Tyrode with (+Gur) and without 20 μM gurmarin (–Gur). Each point is mean from two taste cells. In **(A)** and **(B)**, I–V relations were measured 2 min after the saccharin or D-tryptophan solution with gurmarin was applied to the bath.

Discussion

It has been reported that a depolarizing receptor potential in a rat or hamster taste cell induced by Na-saccharin originates from a decrease of outward K^+ currents which is due to closing of K^+ channels by a cytosolic increase of cAMP (Béhé *et al.*, 1990; Cummings *et al.*, 1996). In the present study we confirmed the inhibition of outward K^+ currents by Na-saccharin. Outward K^+ currents were also suppressed by bath application of 5 mM cpt-cAMP, which is a membrane-permeable analog of cAMP. On the other hand, the outward K^+ currents were enhanced by stimulation with an amino acid sweetener D-tryptophan and bath application of 5 μM ionomycin of a Ca^{2+} ionophore. The enhancement by the ionomycin may be related to an activation of Ca^{2+}-dependent K^+ channels by Ca^{2+} which may be carried from the bath solution and the internal stores through Ca^{2+} transporting ionophores formed at the external and internal membranes. The outward K^+ currents were similarly enhanced by intracellular application of IP_3. The enhancement of outward K^+ currents by D-tryptophan and ionomycin occurred even when these stimulus and drug

in Ca^{2+}-free Tyrode were applied to a taste cell in a Ca^{2+}-free bathing solution. These facts suggest that non-sugar sweet stimuli such as D-tryptophan may increase an intracellular level of IP_3 which releases Ca^{2+} from internal stores, and that in consequence the released Ca^{2+} activates Ca^{2+}-dependent K^+ channels. We have studied changes in cytosolic Ca^{2+} in gerbil taste cells in response to amino acid sweeteners using the fura-2 fluorescence imaging method (Uchida *et al.*, 1996). Our finding indicates that external application of D-phenylalanine in Ca^{2+}-free Tyrode causes an increase in cytosolic Ca^{2+} which is capable of releasing a neurotransmitter from the taste cell without a depolarization (Uchida *et al.*, 1996). A similar mechanism has been proposed in bitter taste transduction (Akabas *et al.*, 1988; Hwang *et al.*, 1990).

The present study indicates that differential second messenger pathways are activated by different types of sweet stimuli. At least two second messenger systems appear to be involved in sweet taste signal transductions as has been proposed by Bernhardt *et al.* (1996). One type of sweet stimuli has the potency for stimulating adenylate cyclase which forms cAMP, while the other type of sweet stimuli can activate the phospholipase C which increases IP_3 level.

Ninomiya and Imoto (1995) and Miyasaka and Imoto (1995) have reported that gurmarin, a specific inhibitor of sweet taste responses, selectively and significantly suppresses the chorda tympani responses to sugars, sweet amino acids and artificial sweeteners in the C57BL strain of mouse and rat. However, gurmarin hardly affects these responses in the BALB strain of mouse (Ninomiya and Imoto, 1995). They suggest that the mouse and rat taste cells have gurmarin-sensitive and -insensitive sweet receptors. In the present experiments, gurmarin did not suppress the gustatory responses to tryptophan in gerbils. This may be a species difference. In the present study, the disinhibitory effect of gurmarin on Na-saccharin-induced K^+ current decrease, as well as no effect of gurmarin on D-tryptophan-induced K^+ current increase, strongly suggests that the action of gurmarin occurs at the receptor level. It is assumed that in gerbil taste cells one group of sweeteners such as Na-saccharin and sucrose may bind to some sweet receptor sites and that the other group of sweeteners such as D-tryptophan may bind to other sweet receptor sites. We suppose that cAMP is a second messenger in the transduction for gurmarin-sensitive sweeteners such as Na-saccharin, and that IP_3 is a second messenger in the transduction for gurmarin-insensitive sweeteners such as D-tryptophan. A recent study on sweet taste transduction with the circumvallate taste buds shows that IP_3 is a second messenger in Na-saccharin-sensitive taste cells in rats (Bernhardt *et al.*, 1996). This is in contrast to our data in gerbil taste cells. However, the discrepancy between second messengers for saccharin transduction may be due to a species difference or a lingual papilla difference.

In frog and mouse taste cells, sugar stimuli induce a depolarization accompanied by a conductance increase (Tonosaki and Funakoshi, 1984; Okada *et al.*, 1992). Therefore, multiple transduction mechanisms for sweeteners may exist in taste cells. Since we used the conventional whole cell patch clamp method in this experiment, we could not deny the possibility that some indispensable intracellular substances for a depolarization in a taste cell were washed out. Further study needs to elucidate this problem using the perforated-patch clamp technique.

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