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# Intracellular Calcium Increase in Gerbil Taste Cell by Amino Acid Sweeteners

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## Abstract

Gustatory transduction mechanisms for sucrose and amino acid sweeteners in gerbil taste cells were studied with $Ca^{2+}$ imaging and whole cell recording techniques. A 100 mM sucrose stimulus with $Ca^{2+}$ increased the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in sweet-sensitive taste cells of the taste bud, but the sucrose stimulus without $Ca^{2+}$ did not change the $[Ca^{2+}]_i$. A 10 mM D-phenylalanine sweet stimulus with or without $Ca^{2+}$ similarly increased the $[Ca^{2+}]_i$ in the taste bud. The addition of 5 μM ionomysin, a $Ca^{2+}$-ionophore, without $Ca^{2+}$ greatly increased the $[Ca^{2+}]_i$ in the taste bud. The application of 10 mM D-phenylalanine stimulus without $Ca^{2+}$ enhanced the outward $K^+$ current in isolated taste cells. These results suggest that a sugar sweetener such as sucrose induces a depolarization in gerbil taste cells which activates voltage-dependent $Ca^{2+}$ channels and that a non-sugar sweetener such as D-phenylalanine releases $Ca^{2+}$ from the internal stores without a depolarization. **Chem. Senses 22: 83–91, 1997.**

## Introduction

Bovine taste buds possess proteins which can bind sweet substances (Dastoli and Price, 1966). The application of a proteolytic enzyme on the human tongue surface selectively suppresses only sugar-induced taste responses (Hiji, 1975). This suggests that the receptor sites for sugar stimuli are different from the sites for other types of taste stimuli. Electrophysiological studies show that sweet stimuli elicit the receptor potential in taste cells in various species of animals (Kimura and Beidler, 1961; Ozeki, 1971; Sato and Beidler, 1982; Tonosaki and Funakoshi, 1988; Okada *et al.*, 1992). Taste cells in frog and mouse are depolarized by an electrophoretic injection of cAMP into the cells (Okada *et al.*, 1987; Tonosaki and Funakoshi, 1988). Involvement of $K^+$ channels in a sweet taste transduction cascade was evidenced by the electrophysiological finding that a cAMP-dependent protein kinase closes a class of $K^+$ channels in isolated frog taste cells, resulting in the depolarization of the cells (Avenet *et al.*, 1988). This depolarization may activate voltage-dependent $Ca^{2+}$ channels which increase the intracellular $Ca^{2+}$ level, leading to a release of the neurotransmitter from taste cells (Béhé *et al.*, 1990). Biochemical studies confirmed that sugar stimuli increase the cAMP level in the lingual epithelia (Striem *et al.*, 1989). Using a noninvasive recording technique, Cummings *et al.* (1993) have suggested that cAMP and cGMP mediate the transduction of sweet taste stimuli in hamster taste cells. On the other hand, based on biochemical studies, Naim *et al.* (1994) have proposed that there is another possibility that sweet substances other than sugars activate G-proteins directly without binding to the receptor proteins.

Recently, it has been shown that stimulation with

non-sugar sweeteners increases inositol 1,4,5-trisphosphate ($IP_3$) in rat taste cells (Bernhardt *et al.*, 1996) but increases cAMP in hamster taste cells (Cummings *et al.*, 1996). These two results seem to be controversial.

Neurophysiological and behavioral experiments on taste in gerbils have shown that the taste cells are very sensitive to various types of sweetener (Jakinovich and Oakley, 1975; Jakinovich, 1976, 1981). In the present study we show that application of amino acid sweeteners to the gerbil taste bud causes an increase in an intracellular $Ca^{2+}$ level in the taste cell which is related to $IP_3$.

## Materials and methods

### Preparations

Gerbils (*Meriones unguiculatus*), 12–15 weeks old, were used for the experiments. After anesthesia with pentobarbital (30 mg/kg), the animal was killed by dislocating cervical vertebrae and its tongue was removed. Elastase (1 mg/ml; Boehringer Mannheim, Mannheim, Germany) in a Tyrode solution was injected between the dorsal epithelial and muscular layers from the cut end of the tongue. In this case, the tip of an injection needle was carefully moved to the distal tip of the tongue while injecting the elastase (Miyamoto *et al.*, 1996). After incubating the tongue in Tyrode solution for 10 min at 30°C, the dorsal lingual epithelium with filiform and fungiform papillae was peeled off from the underlying muscular layer and kept in a normal Tyrode solution. Small pieces of the peeled epithelium were employed to measure changes in $[Ca^{2+}]_i$ in taste bud cells.

Single taste bud cells were used for electrophysiological investigation. They were isolated by modifying the method described by Akabas *et al.* (1988). The removed lingual epithelium was washed and incubated in a $Ca^{2+}$-free Tyrode solution containing 2 mM EGTA for 30 min at 30°C to loosen the tight junctions, then triturated several times in the $Ca^{2+}$-free Tyrode. Taste buds alone were mechanically detached from the fungiform papillae scattering on the epithelial sheet. Single taste bud cells were also mechanically isolated from the taste buds treated with 0.25% trypsin (Gibco, Gaithersburg, MD) for 5 min at 32°C. Cells having a dendritic process were regarded as taste cells. After taste cells were attached to a chamber, they were washed thoroughly with a Tyrode solution to remove debris. All experiments were carried out at a room temperature of 23–26°C.

### Solutions

An external Tyrode solution contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 pyruvate and 10 HEPES–NaOH (pH 7.4). A recording pipette solution contained (in mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES–KOH (pH 7.2). A 100 mM $K^+$ solution containing $Ca^{2+}$ was prepared by replacing 95 mM NaCl in normal Tyrode with 95 mM KCl, and a 100 mM $K^+$ solution without $Ca^{2+}$ did not contain the 2 mM $CaCl_2$.

Sweet taste stimuli such as sucrose (Nacalai, Kyoto, Japan), D-phenylalanine (Nacalai), D-tryptophan (Nacalai) and aspartame (Sigma, St Louis, MO) were usually dissolved in a $Ca^{2+}$-free Tyrode solution containing 2 mM EGTA. D-Mannitol (Nacalai) was dissolved in normal Tyrode solution. Ionomycin (1 mM; Carbiochem, La Jolla, CA) was prepared in dimethyl sulphoxide (DMSO; Sigma) and added to the $Ca^{2+}$-free Tyrode solution to give a final concentration of 5 μM. The acetoxymethyl ester of fura-2 (fura-2/AM, Dojindo Laboratories, Kamamoto, Japan) was first dissolved in DMSO, premixed with the surfactant cremophore EL (Nacalai) and finally dissolved in a normal Tyrode solution to make a 5 μM solution. In earlier experiments of $Ca^{2+}$ imaging and patch clamp recording, a taste solution was slowly applied to the experimental chamber with a fine pipette, but in the later experiments the stimulus solution was superfused with an inlet–outlet system.

### Measurement of $[Ca^{2+}]_i$

Changes in the intracellular calcium concentration, $[Ca^{2+}]_i$, in taste bud cells located at the top of the fungiform papillae were measured using a fluorescent membrane-permeable probe which was an acetoxymethyl ester of fura-2 (fura-2/AM) (Grynkiewicz *et al.*, 1985; Tsien, 1989). Part of the lingual epithelium (3 × 3 mm) possessing a few fungiform papillae was loaded in a Tyrode solution containing 5 μM fura-2/AM and 0.025% cremophore EL (Nacalai) saturated with 95% $O_2$ and 5% $CO_2$ for 30 min. The fura-2/AM-loaded epithelium was postincubated in the same medium for 30 min and washed with the medium twice. Finally the epithelium was rinsed with a $Ca^{2+}$-free Tyrode solution containing 2 mM EGTA. The mucosal side of epithelial sheet was adhered to a thin coverslip (Matsunami, Kishiwada, Japan; >0.12 mm thick) which had been coated with a layer of adhesive (Cell-Tak, Collaborative Research Inc., Bedford, MA), forming the bottom of the experimental chamber. There were a number

of the filiform papillae on the epithelial sheet whose height was much higher than that of the fungiform papillae. Therefore, spine-like tips of the filiform papillae were fixed to the layer of adhesive to make a taste stimulus-accessible space between the coverslip and the pore of the taste buds. Taste stimuli and drugs were applied to the whole epithelial sheet so that both the receptive and the basolateral membranes of taste bud cells were stimulated. The fura-2 loaded epithelium in a chamber was placed on the stage of an inverted microscope (TMD-300, Nikon, Tokyo, Japan) equipped with a high-resolution differential interference contrast imaging and imaged with a ×40 objective lens. The fluorescence was imaged using a silicon intensified target (SIT) camera (C2400-08, Hamamatsu) interfaced with an Argus 50 image processor system (Hamamatsu) and digitized to 8 bits per pixel. The filter and intensifier gains were controlled by a personal computer (C4468, Hamamatsu). The imaging data were stored on a magnetic optical disk. Paired images of the fluorescence (510 nm) emitted from excitation at 340 and 380 nm light pulses were used to calculate pixel ratios. A shutter was used to minimize the exposure of cells to UV light to prevent bleaching of the fura-2 dye. Fluorescence signals were corrected for background by digital subtraction. The excitation light emitted by a 100 W Xenon lamp was passed through a neutral density filter and was reflected by a dichroic mirror, and the fura-2 fluorescence was focused on the SIT camera. A time course of change in fluorescence intensity ratios from excitation at 340 and 380 nm was calculated before and during taste stimulation. Usually fluorescence images were obtained from the horizontal plane of the taste bud ~30 μm away from the pore, and the intracellular $Ca^{2+}$ concentration in the taste bud cells was measured as the mean from the taste bud area of 15 × 15 μm.

The $[Ca^{2+}]_i$ in taste bud cells was calibrated by measuring the ratio of 340/380 nm intensities of 5 μM fura-2 solution whose $Ca^{2+}$ concentration was already known using a calcium calibration buffer kit (Molecular Probes Inc., Eugene, OR).

### Patch clamp recording

The membrane currents of single gerbil taste cells were measured using the conventional whole cell patch clamp technique (Hamill *et al.*, 1981) and were amplified with a patch clamp amplifier (CEZ-2300, Nihon Kohden, Tokyo, Japan). The current signal was low-pass-filtered at 1 kHz, digitized at 125 kHz, acquired at a sampling rate of 10 kHz and stored on an IBM-PC compatible personal computer. The pulse protocol was generated by pCLAMP software (Axon Instruments, Foster City, CA). Recording pipettes were pulled from borosilicate glass capillaries (1.5 mm outer diameter, Clark Electromedical Instruments, Pangbourne, UK) with a two-stage puller (PD-5, Narishige, Tokyo, Japan). The recording pipettes had a resistance of 5–10 MΩ and the seal resistances were in the range of 10–20 GΩ. The pipette were manipulated with a hydraulic manipulator (WR-88, Narishige). Membrane rupture in a taste cell was achieved by brief suction pulses or short negative voltage pulses. The electrical recordings were finished within 2 h after cell dissociation.

### Statistics

Averages of experimental data were given in a mean ± SEM. Significance of a difference between means was assessed using the Student's *t*-test. A $P$ value of 0.01 was considered to be statistically significant.

## Results

### Effect of $K^+$ solution on $[Ca^{2+}]_i$ in the taste bud

When 100 mM $K^+$ solution was applied to the taste bud for 4 min, the mean $[Ca^{2+}]_i$ in the taste bud cells was measured. There was no significant difference in average $[Ca^{2+}]_i$ in the taste bud between a $Ca^{2+}$-free Tyrode solution (60 ± 5 nM, $n$ = 18) and a $Ca^{2+}$-free, 100 mM $K^+$ solution (67 ± 6 nM, $n$ = 18). On the other hand, a 100 mM $K^+$ solution with $Ca^{2+}$ significantly increased $[Ca^{2+}]_i$ in the taste bud from a control level of 56 ± 4 nM ($n$ = 12) to 70 ± 5 nM ($n$ = 12). The relatively small rise in the $[Ca^{2+}]_i$ was due to the incomplete replacement of interstitial fluid in the taste bud by the high-concentration $K^+$ solution with $Ca^{2+}$ because of the short period of solution application. In these and the other experiments, the control measurement of $[Ca^{2+}]_i$ in the taste bud after the lingual epithelium was bathed in $Ca^{2+}$-free Tyrode was finished within 2 min because of avoidance of depletion of intracellular $Ca^{2+}$ stores prior to taste stimulation. The result indicates that high-concentration $K^+$-induced depolarization in taste cells in the presence of external $Ca^{2+}$ causes a $Ca^{2+}$ entry through the taste cell membrane.

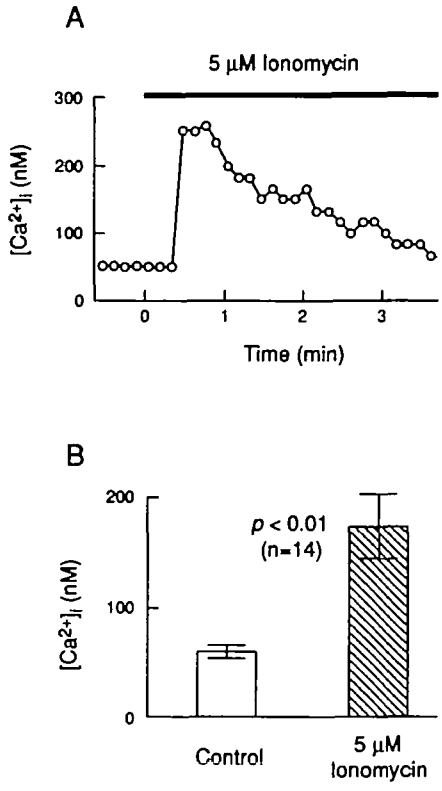


**Figure 1** Effect of 5 μM ionomycin ($Ca^{2+}$-ionophore) on the $[Ca^{2+}]_i$ inthetaste bud. **(A)** Time course of the $[Ca^{2+}]_i$ before and after applicationof ionomycin. **(B)** Mean $[Ca^{2+}]_i$ in 14 taste buds in $Ca^{2+}$-freeTyrode solution (control) and 5 μM ionomycin in $Ca^{2+}$-free Tyrodesolution. The horizontal bars are the SEM in this and the other figures.

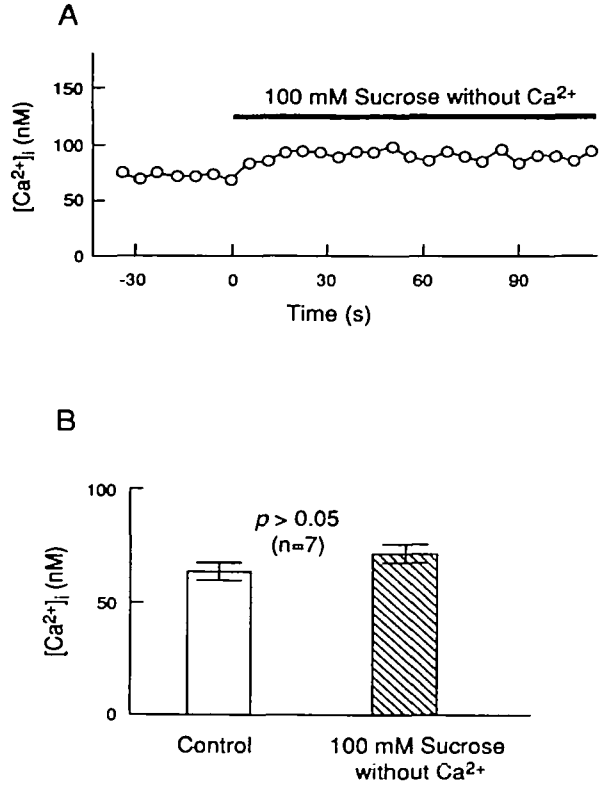


**Figure 2** Effect of 100 mM sucrose without $Ca^{2+}$ on $[Ca^{2+}]_i$ in the taste bud. **(A)** Time course of the $[Ca^{2+}]_i$. **(B)** Mean $[Ca^{2+}]_i$ in seven taste buds in $Ca^{2+}$-free Tyrode (control) and $Ca^{2+}$-free 100 mM sucrose.

## Effect of ionomycin on $[Ca^{2+}]_i$ in the taste bud

As shown in Figure 1A, external application of 5 μM ionomycin, a $Ca^{2+}$-ionophore, in a $Ca^{2+}$-free Tyrode solution induced a rapid increase in $[Ca^{2+}]_i$ in the taste bud followed by a gradual decrease. When changes in $[Ca^{2+}]_i$ were compared at the peaks, ionomycin significantly increased the $[Ca^{2+}]_i$ from a control level of 60 ± 6 nM ($n$ = 14) to 173 ± 30 nM ($n$ = 14) ($P < 0.01$) (Figure 1B). This shows that the elevated $[Ca^{2+}]_i$ level might be due to a release of $Ca^{2+}$ through the ionophores formed in the internal $Ca^{2+}$ store membranes.

## Effect of sucrose on $[Ca^{2+}]_i$ in the taste bud

Gerbils are sensitive to sweet stimuli. A 100 mM solution of sucrose will produce a sweet taste to a gerbil (Jakinovich, 1976). When 100 mM sucrose without $Ca^{2+}$ was applied to the taste bud bathed in $Ca^{2+}$-free Tyrode, a slight increase in $[Ca^{2+}]_i$ was sometimes observed in the taste bud (Figure 2A). However, the increased $[Ca^{2+}]_i$ returned to the control level within a period of 3–5 min. No statistically significant difference was found in the $[Ca^{2+}]_i$ before and after stimulation of the bud with 100 mM sucrose in a $Ca^{2+}$-free Tyrode solution (Figure 2B). On the other hand, 100 mM sucrose in a normal Tyrode solution induced an increase of $[Ca^{2+}]_i$ in sweet-sensitive taste buds, as exemplified in Figure 3A, but not in sweet-insensitive buds. The $[Ca^{2+}]_i$ rise was due to $Ca^{2+}$ entry from the taste cell membrane. Sucrose-sensitive buds were observed in 6/17 taste buds examined. There was a significant difference in the $[Ca^{2+}]_i$ of the bud between 58 ± 2 nM ($n$ = 6) in the $Ca^{2+}$-free Tyrode control and 80 ± 3 nM ($n$ = 6) in the sucrose solution containing 2 mM $Ca^{2+}$ (Figure 3B). When taste buds were bathed in normal Tyrode, the $[Ca^{2+}]_i$ was 59 ± 3 nM ($n$ = 6). A significant rise in $[Ca^{2+}]_i$ was also seen between normal Tyrode and 100 mM sucrose with $Ca^{2+}$ ($P < 0.01$). Since a rise in $[Ca^{2+}]_i$ is assumed to be affected by osmotic pressure, the effect of osmotic pressure on the $[Ca^{2+}]_i$ in the taste bud was examined by applying non-sweet D-mannitol (100 mM) in normal Tyrode. However, it did not change the $[Ca^{2+}]_i$ ($n$ = 3), indicating that such a rise in osmotic pressure was not related to a $[Ca^{2+}]_i$ rise in response to the sucrose stimulus.

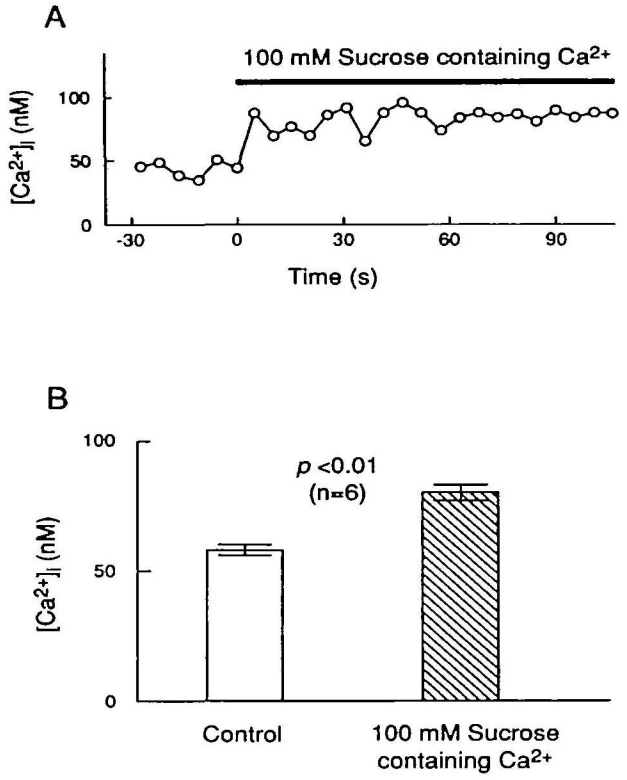


**Figure 3** Effect of 100 mM sucrose containing 2 mM $Ca^{2+}$ on $[Ca^{2+}]_i$ in the taste bud. **(A)** Time course of the $[Ca^{2+}]_i$. **(B)** Mean $[Ca^{2+}]_i$ in six taste buds in $Ca^{2+}$-free Tyrode (control) and 100 mM sucrose with 2 mM $Ca^{2+}$.

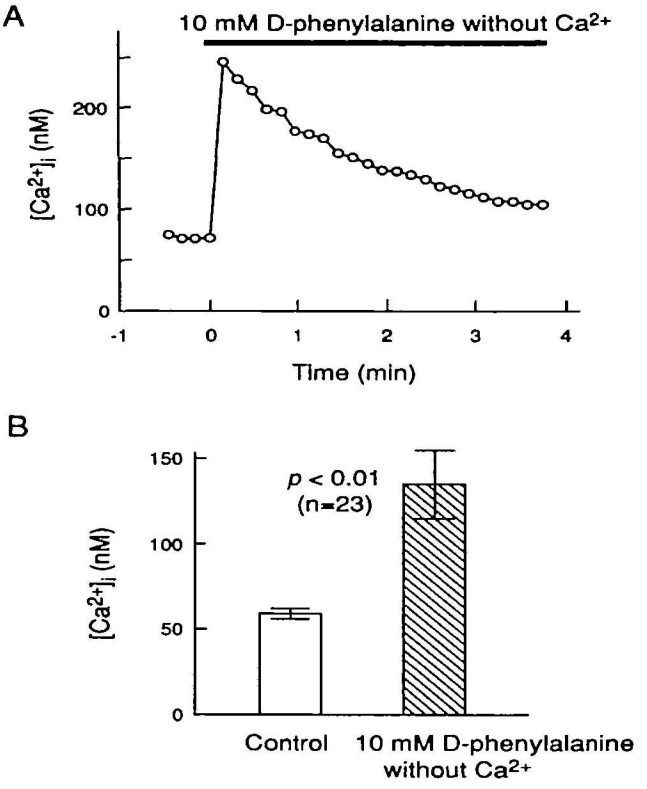


**Figure 4** Effect of 10 mM D-phenylalanine without $Ca^{2+}$ on $[Ca^{2+}]_i$ in the taste bud. **(A)** Time course of the $[Ca^{2+}]_i$. **(B)** Mean $[Ca^{2+}]_i$ in 23 taste buds in $Ca^{2+}$-free Tyrode (control) and 10 mM D-phenylalanine in $Ca^{2+}$-free Tyrode.

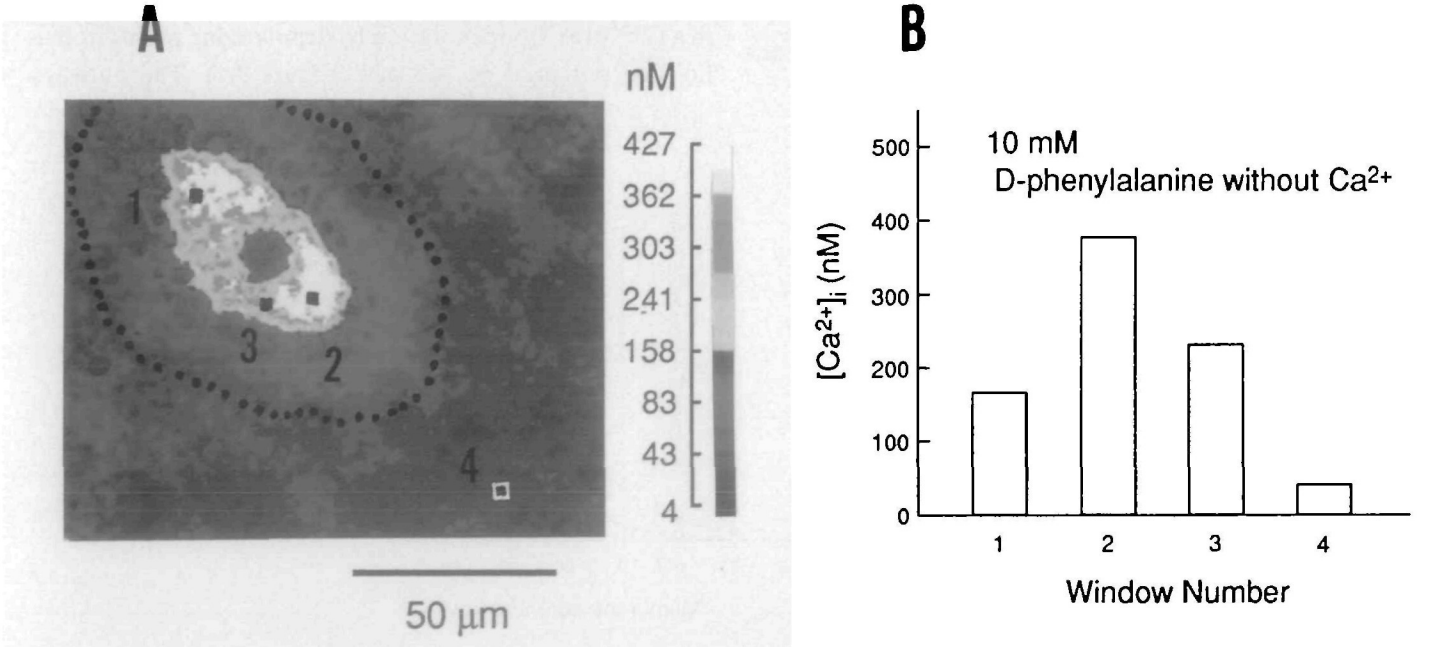


**Figure 5** Change in $[Ca^{2+}]_i$ in different regions of a taste bud during stimulation with 10 mM D-phenylalanine without $Ca^{2+}$. **(A)** Pseudo-color image inside and outside the taste bud. An outline of a fungiform papilla is shown by a dotted line. The photomicrograph was taken 30 s after application of the D-phenylalanine and shows a horizontal taste bud plane 35 µm away from the taste pore. Four small windows numbered denote 2 × 2 µm areas where $[Ca^{2+}]_i$ was measured. Windows nos 1–3 were located inside the taste bud of a fungiform papilla, but window no. 4 was inside a filiform papilla. **(B)** $[Ca^{2+}]_i$ in four windows in (A).

## Effect of amino acid sweetener on the $[Ca^{2+}]_i$

As shown in Figure 4A, application of a non-sugar sweetener (10 mM D-phenylalanine) in $Ca^{2+}$-free Tyrode to the epithelium induced a rapid rise in the $[Ca^{2+}]_i$ in the taste bud followed by a gradual fall. The mean amplitude of peak $[Ca^{2+}]_i$ in response to D-phenylalanine without $Ca^{2+}$ measured from 23 taste buds was 137 ± 18 nM, which was significantly larger than the control (59 ± 3 nM) (Figure 4B). All 23 taste buds examined showed a $[Ca^{2+}]_i$ rise, indicating that they are all D-phenylalanine-sensitive.

Figure 5A shows a pseudo-color ratio image of the lingual epithelial sheet loaded with fura-2 when 10 mM phenylalanine without $Ca^{2+}$ was applied to it. One fungiform papilla bearing a taste bud and its surround are shown. The fungiform papilla whose border is shown by a dotted curve indicates the horizontal focal plane ~35 μm away from the top surface. Figure 5B indicates changes in $[Ca^{2+}]_i$ in three small windows (square nos 1–3 in Figure 5A) within the taste bud and one small window (square no. 4 in Figure 5A) within a filiform papilla. It is clear that the magnitude of $[Ca^{2+}]_i$ in the taste bud changed depending on the loci. The $[Ca^{2+}]_i$ in window no. 2 was the highest and that in window no. 4 in the filiform papilla was the lowest.

Figure 6 shows a time course of the $[Ca^{2+}]_i$ in response to 10 mM D-phenylalanine in a normal Tyrode. Compared with the $[Ca^{2+}]_i$ level produced with the D-phenylalanine without $Ca^{2+}$, a sustained but fluctuating increase in $[Ca^{2+}]_i$ was induced by application of 10 mM D-phenylalanine containing 2 mM $Ca^{2+}$. The mean $[Ca^{2+}]_i$ rise was 145 ± 15 nM ($n$ = 3), which was similar to the value obtained by the D-phenylalanine without $Ca^{2+}$. An increase in the $[Ca^{2+}]_i$ in the taste bud by another non-sugar sweetener, 10 mM D-tryptophan, without $Ca^{2+}$ was observed in nine taste buds (data not shown). The artificial sweetener 10 mM aspartame in $Ca^{2+}$-free Tyrode also elicited a transient response in $[Ca^{2+}]_i$ in the taste bud.

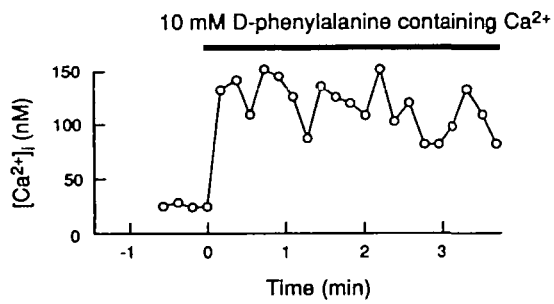


**Figure 6** Effect of 10 mM D-phenylalanine containing 2 mM $Ca^{2+}$ on $[Ca^{2+}]_i$ in the taste bud.

## Enhancement of outward $K^+$ current by D-phenylalanine

The membrane currents of isolated taste cells were measured using the whole cell recording under the voltage clamp. Outward currents were always induced in taste cells in a $Ca^{2+}$-free Tyrode solution by depolarizing pulses from a holding potential of –60 mV (Figure 7A). The outward

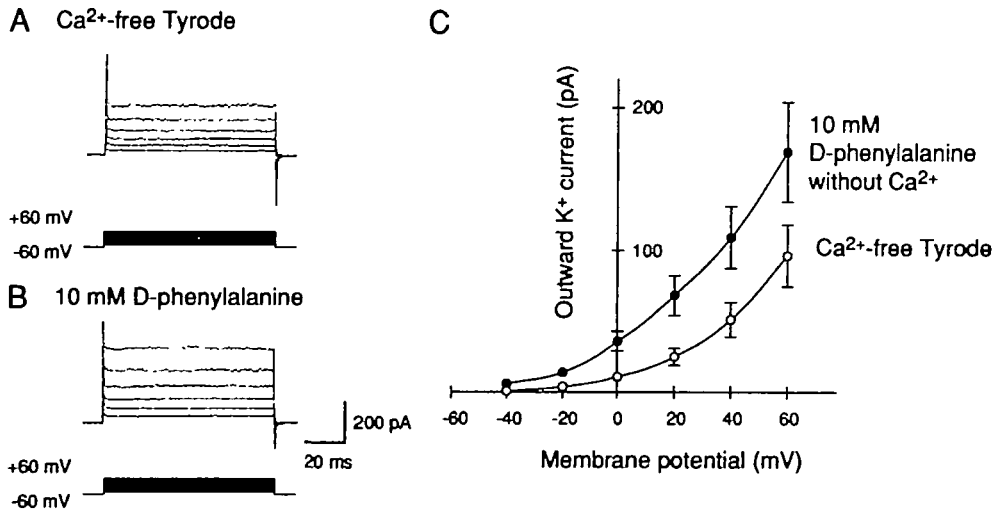


**Figure 7** Voltage-dependent outward $K^+$ currents recorded from isolated gerbil taste cells. **(A)** The $K^+$ currents in $Ca^{2+}$-free Tyrode. **(B)** The $K^+$ currents in 10 mM D-phenylalanine in $Ca^{2+}$-free Tyrode. Depolarizing voltage pulses were applied from -40 mV to +60 mV in 20 mV increments from a holding potential of –60 mV. Records A and B were obtained from the same cell. Resting potential was –40 mV. **(C)** *I–V* relationships from nine taste cells in $Ca^{2+}$-free Tyrode and 10 mM D-phenylalanine in $Ca^{2+}$-free Tyrode. Vertical bars are the SEM. The $K^+$ currents at the membrane potentials >–20 mV were significantly larger during 10 mM D-phenylalanine stimulation ($P < 0.01$). Resting potentials were in the range of –35 to –50 mV.

currents were identified as outwardly rectifying $K^+$ currents because they were completely blocked by adding 10 mM tetraethylammonium to the bathing solution and by replacing $K^+$ in the pipette solution with $Cs^+$ (Uchida *et al.*, 1994). In each experiment the measurement of the control $K^+$ currents from the taste cell after the cell was bathed in $Ca^{2+}$-free Tyrode was finished within 2 min to avoid depletion of intracellular $Ca^{2+}$ stores prior to sweet stimulation. The outward $K^+$ currents were enhanced by applying 10 mM D-phenylalanine in a $Ca^{2+}$-free Tyrode solution containing 2 mM EGTA (Figure 7B and C). The control outward $K^+$ current of 96 ± 22 pA ($n$ = 9) at +60 mV under a $Ca^{2+}$-free Tyrode solution was increased to 169 ± 35 pA ($n$ = 9) during stimulation with 10 mM D-phenylalanine (Figure 7C). The enhancement of the $K^+$ currents was observed in all nine taste cells tested. The outward $K^+$ currents were also augmented by other non-sugar sweeteners such as 10 mM D-tryptophan. We were unable to measure changes in outward $K^+$ currents during stimulation with sucrose since the whole cell recording could not be kept under a viscous sucrose solution.

## Discussion

Recent studies have demonstrated that the $[Ca^{2+}]_i$ of isolated taste cells in rats and guinea pigs is increased by denatonium, which is a bitter substance (Akabas *et al.*, 1988; Orola *et al.*, 1992). In the present experiment we used fluorescence imaging with fura-2 to measure the $[Ca^{2+}]_i$ in the gerbil taste bud *in situ*. Application of a non-sugar sweetener amino acid in a $Ca^{2+}$-free Tyrode solution increased the $[Ca^{2+}]_i$ in the taste bud. It is likely that the increased $[Ca^{2+}]_i$ is due to a release from the internal $Ca^{2+}$ stores in a taste cell, as suggested by Akabas *et al.* (1988). It is well known that $IP_3$ can release $Ca^{2+}$ from the internal stores of a cell in response to a variety of external substances (Berridge, 1993). Intracellularly applied $IP_3$ enhances the outward $K^+$ currents in gerbil taste cells (Uchida *et al.*, 1995). In the present experiments a similar enhancement of outward $K^+$ current in the taste cells by amino acid sweeteners may be attributed to an increase of $Ca^{2+}$ release from the internal stores. The increased $Ca^{2+}$ level may activate $Ca^{2+}$-dependent $K^+$ channels of the taste cell membrane, resulting in an enhancement of outward $K^+$ currents. It was estimated that the outward $K^+$ current of a taste cell induced by released $Ca^{2+}$ occurs at the resting membrane potential level. However, this was not the case (Figure 7). It is suggested that closure of the leak-type $K^+$ channels in taste cells by protein kinase C occurs following stimulation with non-sugar sweeteners (Bernhardt *et al.*, 1996). Another possibility is that $Ca^{2+}$-dependent non-selective cation channels may be activated by non-sugar sweeteners. These possible mechanisms may be concerned with small outward $K^+$ currents at the resting potential levels.

No rise in $[Ca^{2+}]_i$ in the gerbil taste bud occurred even after a depolarization in a taste cell had been induced by a high-concentration $K^+$ solution without $Ca^{2+}$. A similar result has been reported in guinea pig taste cells (Orola *et al.*, 1992). The rise in $[Ca^{2+}]_i$ in the taste bud by a high-concentration $K^+$ solution with $Ca^{2+}$ is due to $Ca^{2+}$ carried through voltage-dependent $Ca^{2+}$ channels activated by a 100 mM $K^+$-induced depolarization. Electrophysiological studies have shown that taste cells in various species of animals have voltage-gated $Ca^{2+}$ channels (Kashiwayanagi *et al.*, 1983; Kinnamon and Roper, 1987; Béhé *et al.*, 1990). In our experiment 100 mM sucrose without $Ca^{2+}$ did not increase $[Ca^{2+}]_i$, but 100 mM sucrose with $Ca^{2+}$ did increase $[Ca^{2+}]_i$ significantly in the taste bud. This $[Ca^{2+}]_i$ rise is due to $Ca^{2+}$ carried through voltage-dependent $Ca^{2+}$ channels activated by sucrose-induced depolarization.

It is likely that sugar sweeteners such as sucrose may increase the intracellular cAMP level in a taste cell which activates protein kinase A, and that the activated protein kinase A may close $K^+$ channels, resulting in the depolarization of the taste cell accompanied by a decrease in conductance (Avenet *et al.*, 1988; Tonosaki and Funakoshi, 1988; Striem *et al.*, 1989; Béhé *et al.*, 1990). On the other hand, sugar stimulation of frog and mouse taste cells induces a depolarization accompanied by an increase in conductance (Tonosaki and Funakoshi, 1984; Okada *et al.*, 1992). The depolarization may activate the voltage-gated $Ca^{2+}$ channels in the taste cells.

Aspartame, an artificial sweetener, elicited only a small increase in $[Ca^{2+}]_i$ in the gerbil taste bud. Responsiveness of hamster taste cells to aspartame has been reported electrophysiologically and behaviorally (Nowlis *et al.*, 1980; Cummings *et al.*, 1993), though the gerbil does not exhibit chorda tympani responses to this sweetener (Jakinovich, 1981), Therefore, a slight increase of $[Ca^{2+}]_i$ in gerbil taste bud stimulated by aspartame suggests that some sweet-sensitive taste cells in the gerbil was activated by aspartame.

Neurophysiological studies have suggested that the

gustatory transduction mechanism for amino acid sweeteners in the mouse is different from that for sucrose (Ninomiya and Kajiura, 1993; Ninomiya *et al.*, 1993). Several psychophysical studies on humans (Schiffman *et al.*, 1981) and electrophysiological studies on gerbils (Jakinovich, 1981; Vlahopoulos and Jakinovich, 1986) have suggested that there is more than one type of receptor site for sweet substances. An electrophysiological study on gerbil taste cells using the whole cell patch clamp technique has indicated that gurmarin can inhibit Na-saccharin-induced responses but not amino acid-induced responses, suggesting the existence of at least two sweet receptor sites in gerbil taste cells (Uchida *et al.*, 1995). In addition, Uchida *et al.* (1995) suggest that there are two sweet taste transduction pathways mediating cAMP and $IP_3$ in gerbil taste cells. By combining our previous and present studies, it is likely that gerbil taste cells can respond to sugar sweeteners with a second messenger cAMP-mediated signal and to non-sugar sweeteners with a second messenger $IP_3$-mediated signal. Similar dual transduction cascades for sweet taste have been proposed in rat taste cells (Bernhardt *et al.*, 1996).

Similar dual pathways have been proposed in olfactory transduction mechanisms (Restrepo *et al.*, 1990; Breer and Boekhoff, 1991; Miyamoto *et al.*, 1992; Ronnett *et al.*, 1993). Calcium is a key regulatory messenger in a variety of cellular mechanisms.

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## REFERENCES

Akabas, M.H., Dodd, J. and Al-Awaqati, Q. (1988) A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. *Science*, **242**, 1047–1050.

Avenet, P., Hofmann, F. and Lindemann, B. (1988) Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature*, **331**, 351–354.

Béhé, P., DeSimone, J.A., Avenet, P. and Lindemann, B. (1990) Membrane currents in taste cells of the rat fungiform papilla. Evidence for two Ca currents and inhibition of K currents by saccharin. *J. Gen. Physiol.*, **96**, 1061–1084.

Bernhardt, S.J., Naim, M., Zehavi, U. and Lindemann, B. (1996) Changes in $IP_3$ and cytosolic $Ca^{2+}$ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. *J. Physiol.*, **490**, 325–336.

Berridge, M.J. (1993) Inositol trisphosphate and calcium signalling. *Nature*, **361**, 315–325.

Breer, H. and Boekhoff, I. (1991) Odorants of the same odor class activate different second messenger pathways. *Chem. Senses*, **16**, 19–29.

Cummings, T.A., Powell, J. and Kinnamon, S.C. (1993) Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. *J. Neurophysiol.*, **70**, 2326–2336.

Cummings, T.A., Daniels, C. and Kinnamon, S.C. (1996) Sweet taste transduction in hamster: Sweeteners and cyclic nucleotides depolarize taste cells by reducing a $K^+$ current. *J. Neurophysiol.*, **75**, 1256–1263.

Dastoli, F.R. and Price, S. (1966) Sweet-sensitive protein from bovine taste buds: Isolation and assay. *Science*, **154**, 905–907.

Grynkiewicz, G., Poenie, M. and Tsien R.Y. (1985) A new generation of $Ca^{2+}$ indicators with greatly improved fluorescence properties. *J. Biol. Chem.*, **260**, 3440–3450.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

Hiji, Y. (1975) Selective elimination of taste responses to sugars by proteolytic enzymes. *Nature*, **256**, 427–429.

Jakinovich, W., Jr (1976) Stimulation of the gerbil's gustatory receptors by disaccharides. *Brain Res.*, **110**, 481–490.

Jakinovich, W., Jr (1981) Stimulation of the gerbil's gustatory receptors by artificial sweeteners. *Brain Res.*, **210**, 69–81.

Jakinovich, W., Jr and Oakley, B. (1975) Comparative gustatory responses in four species of gerbilline rodents. *J. Comp. Physiol.*, **99**, 89–101.

Kashiwayanagi, M., Miyake, M. and Kurihara, K. (1983)

Voltage-dependent $Ca^{2+}$ channel and $Na^{+}$ channel in frog taste cells. *Am. J. Physiol.*, **244**, C82–C88.

Kimura, K. and Beidler, L.M. (1961) Microelectrode study of taste receptors of rat and hamster. *J. Cell. Comp. Physiol.*, **58**, 131–140.

Kinnamon, S.C. and Roper, S.D. (1987) Passive and active membrane properties of mudpuppy taste receptor cells. *J. Physiol.*, **383**, 601–614.

Miyamoto, T., Restrepo, D., Cragoe, E.J. and Teeter, J.H. (1992) $IP_3$- and cAMP-induced responses in isolated olfactory receptor neurons from the channel catfish. *J. Membr. Biol.*, **127**, 173–183.

Miyamoto, T., Miyazaki, T., Okada Y. and Sato, T. (1996) Whole-cell recording from non-dissociated taste cells in mouse taste bud. *J. Neurosci. Methods*, **64**, 245–252.

Naim, M., Seifert, R., Nürnberg, B., Grünbaum, L. and Schultz, G. (1994) Some taste substances are direct activators of G-proteins. *Biochem. J.*, **297**, 451–454.

Ninomiya, Y. and Kajiura, H. (1993) Enhancement of murine gustatory neural responses to D-amino acids by saccharin. *Brain Res.*, **626**, 287–294.

Ninomiya, Y., Kajiura, H. and Mochizuki, K. (1993) Differential taste responses of mouse chorda tympani and glossopharyngeal nerves to sugars and amino acids. *Neurosci. Lett.*, **163**, 197–200.

Nowlis, G. H., Frank, M.E. and Pfaffmann, C. (1980) Specificity of acquired aversions to taste qualities in hamster and rats. *J. Comp. Physiol. Psychol.*, **94**, 932–946.

Okada, Y., Miyamoto, T. and Sato, T. (1987) Depolarization induced by injection of cyclic nucleotides into frog taste cell. *Biochim. Biophys. Acta*, **904**, 187–190.

Okada, Y., Miyamoto, T. and Sato, T. (1992) The ionic basis of the receptor potential of frog taste cells induced by sugar stimuli. *J. Exp. Biol.*, **162**, 23–36.

Orola, C.N., Yamashita, T., Harada, N., Amano, H., Ohtani, M. and Kumazawa, T. (1992) Intracellular free calcium concentrations in single taste receptor cells in the guinea pig. *Acta Otolaryngol.*, **112**, 120–127.

Ozeki, M. (1971) Conductance change associated with receptor potentials of gustatory cells in rat. *J. Gen. Physiol.*, **58**, 688–699.

Restrepo, D., Miyamoto, T., Bryant, B.P. and Teeter, J.H. (1990) Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. *Science*, **249**, 1166–1168.

Ronnett, G. V., Cho, H., Hester, L.D., Wood, S.F. and Snyder, S.H. (1993) Odorants differentially enhance phosphoinositide turnover and adenylyl cyclase in olfactory receptor neuronal cultures. *J. Neurosci.*, **13**, 1751–1758.

Sato, T. and Beidler, L.M. (1982) The response characteristics of rat taste cells to four basic taste stimuli. *Comp. Biochem. Physiol.*, **73A**, 1–10.

Schiffmann, S.S., Sennewald, K. and Gagnon, J. (1981) Comparison of taste qualities and thresholds of D- and L-amino acids. *Physiol. Behav.*, **27**, 51–59.

Striem, B.J., Pace, U., Zehavi, U., Naim, M. and Lancet, D. (1989) Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes. *Biochem. J.*, **260**, 121–126.

Tonosaki, K. and Funakoshi, M. (1984) Effect of polarization of mouse taste cells. *Chem. Senses*, **9**, 381–387.

Tonosaki, K. and Funakoshi, M. (1988) Cyclic nucleotides may mediate taste transduction. *Nature*, **331**, 354–356.

Tsien, R.Y. (1989) Fluorescent probes of cell signaling. *Annu. Rev. Neurosci.*, **12**, 227–253.

Uchida, Y., Miyamoto, T. and Sato, T. (1994) Patch clamping of mammalian taste receptor cells. In Kurihara, K., Suzuki, N. and Ogawa, H. (eds), *Olfaction and Taste XI*. Springer-Verlag, Tokyo, p.112.

Uchida, Y., Miyamoto, T. and Sato, T. (1995) Dual sweet taste transduction pathways in gerbil taste cells. *Chem. Senses*, **20**, 121.

Vlahopoulos, V. and Jakinovich W., Jr. (1986) Antagonism of the gerbil's sucrose taste response by p-nitrophenyl-α-D-glucopyranoside and chloramphenicol. *J. Neurosci.*, **6**, 2611–2615.

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