A Quinine-activated Cationic Conductance in Vertebrate Taste Receptor Cells

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ABSTRACT The chincona alkaloid quinine is known to be a bitter tasting substance for various vertebrates. We examined the effects of quinine on isolated taste receptor cells from the bullfrog (*Rana catesbeiana*). Membrane currents were recorded by whole-cell recording, while quinine hydrochloride was applied extracellularly from a puffer pipette. At the resting potential (-77 ± 9 mV, mean ± SD, $n = 49$ cells), taste cells generated inward currents in response to quinine stimulation (>1 mM), indicating a depolarizing response in the taste cells. Two types of current responses were observed; a newly found quinine-activated cationic conductance and a previously reported blocking effect of quinine on K^+ conductances. The cationic current was isolated from the K^+ current by using a Cs^+-containing patch pipette. The relative permeabilities (P_{ion}) of the quinine-activated cationic conductance were: $P_{Na}/P_K/P_{Cs} = 1:0.5:0.42$. The quinine dose-response relation was described by the Hill equation with the $K_{1/2}$ of 3.6 mM and Hill coefficient of 5.3. When extracellular [Ca^{2+}] (1.8 mM) was reduced to nominally free, the conductance was enhanced by about sixfold. This property is consistent with observations on quinine responses recorded from the gustatory nerve, in vivo. The quinine-induced cationic current was decreased with an application of 8-bromo-cAMP. We conclude that the bitter substance quinine activates a cation channel in taste receptor cells and this channel plays an important role in bitter taste transduction.

KEY WORDS: bitter • gustatory • bullfrog • cation channel • patch-clamp

INTRODUCTION

Taste substances are detected by specialized epithelial receptor cells called taste receptor cells. Taste stimuli are transduced into electrophysiological signals in the taste cells, and the signals are transmitted to the gustatory nerves via synapses. It is now well known that taste receptor cells use multiple transduction pathways depending on the taste quality (Kinnamon and Cummings, 1992; Margolskee, 1993). Among these, bitter taste, which is one of the most important sensations for avoiding poisons, is thought to be mediated by several pathways. For example, it has been shown that certain bitter substances activate an enzymatic cascade involving a G protein and phospholipase C, leading to an increase in inositol 1,4,5-trisphosphate (IP_3) concentration; IP_3 is then thought to release Ca^{2+} from the endoplasmic reticulum, which in turn directly or indirectly triggers transmitter release from the synapse to second order neurons (Spielman et al., 1992). Also, very recently, it has been proposed that a G protein–mediated cyclic nucleotide phosphodiesterase (PDE) may mediate bitter via membrane excitation (Kolesnikov and Margolskee, 1995; Ruiz-Avila et al., 1995). A bitter-activated PDE would decrease cyclic nucleotide concentration in the taste receptor cell (Price, 1973; Ruiz-Avila et al., 1995), and this could cause excitation because taste receptor cells have been shown to contain depolarizing ionic channels that are closed by intracellular cyclic nucleotides (Kolesnikov and Margolskee, 1995). It is therefore possible that the reduction in cytoplasmic cAMP concentration leads to the opening of ionic channels causing cell depolarization.[1]

In electrophysiological experiments, it has been shown that quinine stimulation can suppress K^+ channels in taste cells, causing depolarizing responses (Avenet and Lindemann, 1987; Kinnamon and Roper, 1988; Sugimoto and Teeter, 1991; Cummings and Kinnamon, 1992). It has also been reported that quinine activates an active chloride transport of frog taste cells (Okada et al., 1988; Sato et al., 1994), again explaining the depolarizing response.

Portions of the present study were reported previously in abstract form (Tsunenari, Hayashi, Orita and Mori. 1995. *Chem. Senses.* 20: 356; Tsunenari, Hayashi, Orita, Kurahashi, Kaneko and Mori. 1996. *Chem. Senses.* 21:278).

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[1]After the submission of this report, Wong et al. (1996) has reported the study of transgenic mice that are missing a taste cell-specific G protein, gustducin. They revealed the involvement of gustducin in transduction of bitter and sweet. Their report strongly supports the cyclic nucleotide hypothesis.

The present study was carried out to investigate the possibility of additional transduction mechanisms for the bitter substance quinine in the bullfrog, *Rana catesbeiana*. Solitary taste receptor cells were studied using the whole-cell patch clamp configuration while they were stimulated with quinine under various ionic conditions. The present study also shows that quinine stimulation activates a novel cationic conductance which exhibits a similar Ca^{2+}-dependency to that seen in taste cells, in situ. Moreover, the application of cAMP analogue decreased the conductance, consistent with the hypothesis of the cyclic nucleotide-mediating cascade. We therefore conclude that this cationic conductance plays an important role in bitter transduction and perception.

METHODS

Cell Isolation

Bullfrogs (*Rana catesbeiana*) were obtained from commercial suppliers and maintained in tap water. They were fed bovine liver weekly. The animal was anesthetized in cold water (0°C) and pithed. The tongue was removed and washed with normal saline solution. Fungiform papillae on the tongue were cut off at the stem with fine scissors under a dissecting microscope (10–40×). The tissue was incubated in Ca^{2+}-free saline solution containing 2 mM EGTA for 30 min at 25°C, and then treated with 0.2% collagenase/dispase (Boehringer Mannheim Biochemica, Mannheim, Germany) in normal saline solution for 20 min at 25°C. After rinsing twice with Ca^{2+}-free saline solution (4°C), the tissue was dissociated by trituration with a glass pipette (opening diameter: 0.2 mm) in normal saline (4°C). Dissociated cells were stored in normal saline solution at 4°C for up to 11 h. Three types of dissociated cells could be identified by their characteristic morphology; taste receptor cells, associated cells, and ciliated cells. Taste receptor cells were flask- or spindle-shaped (30–70 μm in length and 5–15 μm in diameter at the cell body), and their dendrites were occasionally still attached with associated cells (Avenet and Lindemann, 1987; Miyamoto et al., 1988). Associated cells and ciliated cells could be also identified by their spherical shapes (10–20 μm in diameter). In some experiments, we examined the effect of quinine stimulation in these spherically shaped cells. However, none of these cells showed any voltage-dependent transient inward Na^+ current ($n = 7$ cells), which is a consistent feature of taste receptor cells. In these associated cells, no evidence of a quinine-activated conductance was found.

Whole-cell Recording

Isolated taste cells were plated onto a culture dish (35 mm/Collagen-Coated Dish; Corning, Iwaki Glass, Tokyo, Japan). A silicon ring made with Sylgard 184 (Dow Corning Corp., Midland, MI) was put into the dish to reduce the dead volume of the chamber (to ~0.3 ml). A dish containing isolated taste receptor cells was mounted on the stage of an inverted microscope (DIAPHOT-TMD; Nikon, Tokyo, Japan) with Hoffman Modulation Contrast (Modulation Optics, Inc., Greenvale, NY). The dish solution was changed at a rate of 1 ml/min to change the extracellular medium.

Membrane current was recorded by using the whole-cell configuration of the patch-clamp technique (Hamill et al, 1981). Patch pipettes were fabricated from borosilicate capillaries (GC 150F-10; Clark Electromedical Instruments, Reading, UK) using a two-stage vertical patch electrode puller (PP-83; Narishige, Tokyo, Japan). The tip of a patch pipette was fire-polished by using a microforge (MF-83; Narishige). The electrode resistance was 6–10 MΩ when the pipettes were filled with saline.

The recording pipette was connected to a patch clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo, Japan). The signal was low pass filtered at 1 kHz and sampled at 0.05–10 kHz by a 12-bit analog-to-digital converter connected to a microcomputer (PC-9801DA; NEC, Tokyo, Japan), by using laboratory-made programs. Command voltages were generated by a 12 bit digital-to-analog converter with a time resolution of 01./ms, and command voltage resolution at membrane was 0.098 mV.

An Ag-AgCl indifferent electrode was connected to the bath solution via an agar bridge containing Ca^{2+}-free saline solution. Liquid junction potentials at the tip of the patch pipette or the agar bridge were measured with a microelectrode containing 3 M KCl (Neher, 1992). All data reported here have been corrected for junction potentials. All experiments were performed at room temperature (20–26°C).

Solutions

Normal saline solution contained (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 2 Na-HEPES, 2 glucose, pH 7.2. The Ca^{2+}-free solution was normal saline solution without added $CaCl_2$. The Na^+-free solution contained (in mM): 115 *N*-methyl-D-glucamine, 2.5 KCl, 1.8 $CaCl_2$, 2 HEPES, 2 glucose, pH 7.2 (adjusted with HCl). The 120 mM NaCl solution contained (in mM): 120 NaCl, 2 Na-HEPES, 2 glucose, pH 7.2. The Cl^--free solution contained (in mM): 120 Na-gluconate, 2 Na-HEPES, 2 glucose, pH 7.2.

Two types of the patch-pipette-filling solution were used. The K^+ pipette solution contained (in mM): 115 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 2 K_2-EGTA, 2 K_2-ATP, 10 K-HEPES, pH 7.2, and the Cs^+ pipette solution contained (in mM): 120 CsCl, 5 Na_2-EGTA, 2 Na-HEPES, 2 Na_2-ATP, pH 7.2.

All solutions contained 0.0005% (wt/vol) phenol red.

Application of a Taste Stimulus

Quinine hydrochloride (Q-1125; Sigma Chemical Co., St. Louis, MO) was used as bitter stimulant. It was dissolved in the bath solution at 10 mM unless otherwise indicated (pH readjusted to 7.2) and ejected by pressure (20 kPa) from a glass pipette (1 μm opening) placed within 40 μm from the dendrite of the taste receptor cell. The puffer pipette was connected to a microinjector (IM-200; Narishige) controlled by a microcomputer. During the puff application, drugs included in the puffer pipette are thought to be diluted due to mixing with the bath solution. This dilution effect was calibrated by using cGMP-activated channels of rod photoreceptor (Fesenko et al., 1985; Yau and Baylor, 1989). Dose-response curves of cGMP-activated channels were compared between two perfusion protocols; pressure ejection from puffer pipettes (2 μm opening) and bath superfusion. Membrane currents from inside-out patches excised from bullfrog rod photoreceptor cells were monitored at +50 mV (membrane potential) under low Ca^{2+} and Mg^{2+} conditions, while cGMP was applied to the cytoplasmic side of the membrane. The tip of the recording pipette was placed 40 μm away from the tip of the puffer pipette, and puffing duration was 2 s, as is the case for quinine stimulation of solitary taste cells. For changing the stimulus concentration, the puffer pipette was manually exchanged as mentioned below. The dose-response relations were fitted by the Hill equation and the $K_{1/2}$ (stimulant concentration needed to activate half the maximal current) values were compared in data obtained for two perfusion protocols. The stimulant dilution under these conditions was found to be 1.6-fold.

When the ionic composition of the bath solution was changed by superfusion, the puffer pipette was also changed to one containing the same ionic composition as the bath. For changing the stimulant solutions during recordings, a puffer pipette was manually exchanged for a different puffer pipette containing a known concentration of quinine. In order to avoid losing a recorded cell during handling, the cell was lifted from the bottom of the culture dish. The relative positions between the puffer pipette and the recorded cell were kept constant throughout the experiments by adjusting the positions displayed on a CRT monitor.

For an application of cAMP analogue, 2 mM 8-Br-cAMP (B-7880; Sigma Chemical Co.) and 5 mM quinine hydrochloride dissolved in normal saline solution were ejected by pressure (30 kPa) from a puffer pipette, while a taste cell was stimulated with 5 mM quinine by bath-application.

RESULTS

K^+ Currents and Their Suppression by Internal Cs^+

Isolated taste receptor cells showed a resting membrane potential of -77 ± 9 mV (mean ± SD; $n = 49$ cells) when it was recorded in a normal saline solution and with a K^+ pipette solution. Under voltage clamp, depolarizing pulses triggered time- and voltage-dependent currents (Fig. 1, *A* and *B*). It has been reported in amphibian taste receptor cells that the fast transient inward current is an Na^+ current and the slow outward current is a mixture of K^+ currents (Avenet and Lindemann, 1987; Miyamoto et al., 1988; Kinnamon and Roper, 1988; Sugimoto and Teeter, 1991). We confirmed that our cell preparation shows essentially the same properties; the fast transient inward current was blocked entirely and reversibly by 1 μM tetrodotoxin ($n = 5$ cells) (Fig. 1 *C*) and was also eliminated by replacing external Na^+ with *N*-methyl-D-glucamine$^+$ (Na^+-free solution), and the slow component was suppressed by the addition of 5 mM tetraethylammonium chloride and 5 mM $BaCl_2$ to the bath solution. This K^+ component could also be suppressed by using a Cs^+ solution in the whole-cell patch pipette (Fig. 1 *D*). Further, both the fast and the slow component were sensitive to quinine as reported previously (Avenet and Lindemann, 1987; Kinnamon and Roper, 1988; Sugimoto and Teeter, 1991); quinine (0.1 mM) suppressed 78 ± 5% of the voltage-dependent Na^+ current and 60 ± 6% of outward K^+ currents of bullfrog taste cells (mean ± SD; $n = 4$ cells).

Quinine-induced Responses in Solitary Taste Cells

Whole-cell recordings under current-clamp and voltage-clamp were made from isolated taste receptor cells, while the bitter taste stimulus was applied via a puffer pipette. All taste cells tested responded to quinine ($n = 68$ cells, with Cs^+ or K^+ in the pipette solution). However, the response amplitude varied from cell to cell, ranging from 80 to 2,340 pA (470 ± 620 pA, mean ± SD, at −84 mV; $n = 21$ cells, using the Cs^+ pipette solution). During current-clamp recording, quinine stimulation caused a depolarizing response in the taste cells (Fig. 2 *A*). 2-s stimulus application caused transient depolarization, in this case by ~60 mV, which exceeded 0 mV. Under voltage-clamp with the K^+-containing pipette, quinine stimulation elicited an inward current at −84 mV, which was close to the resting membrane potential (Fig. 2 *B*). The inward current began 90 ms after the onset of stimulation and reached a peak value at ~1.3 s. (The delay of 90 ms includes the time from the valve opening of the microinjector to the solution ex-

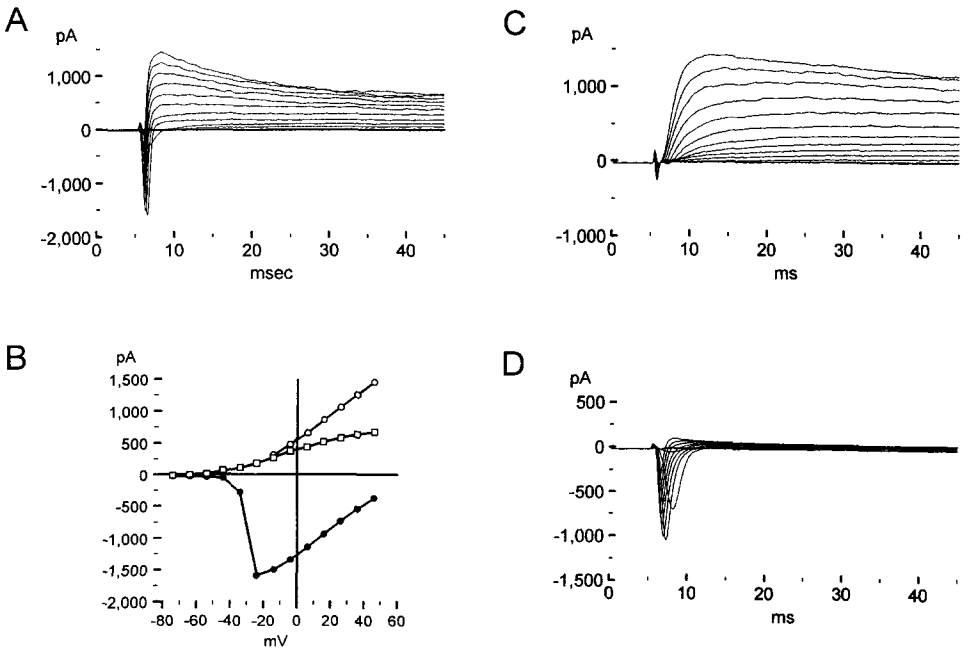


FIGURE 1. Whole-cell currents recorded from taste cells in response to depolarizing voltage pulses. Voltage pulses were in 10-mV steps from −74 to + 56 mV. The holding potential was −84 mV. (*A*) Time-course of whole-cell currents during a sequence of depolarizing voltage pulses. (*B*) Current-voltage (*I-V*) relations for peak inward (*filled circles*), peak outward (*open circles*), and steady-state outward (*open squares*) currents. The relations were plotted from *A*. (*C*) Suppression of transient inward current by 1 μM tetrodotoxin. The K^+ pipette solution was used. (*D*) Suppression of the outward currents by using the Cs^+ pipette solution. The bath solution was normal saline.

change around the cell.) The amplitude and polarity of the quinine-induced current were voltage dependent (Fig. 2, *B* and *C*). Near the resting potential, quinine induced a monotonic inward current. The amplitude of this current decreased as the holding potential was shifted toward positive, and the current polarity reversed to outward at membrane potentials of +36 to +56 mV (Fig. 2 *B*). This reversal potential was variable among cells (Fig. 2 *C*). Furthermore, around the reversal potential, current responses frequently exhibited a biphasic shape (e.g., Fig. 2 *B*, +56 mV). In earlier patch-clamp studies from taste receptor cells (Avenet and Lindemann, 1987; Kinnamon and Roper, 1988; Sugimoto and Teeter, 1991; Cummings and Kinnamon, 1992), it has been shown that quinine stimulation suppresses voltage-gated K^+ channels. Since the blockage of an outward K^+ current would appear as an inward current, part of the inward-directed current in the biphasic responses may be explained by this mechanism. However, the outward-directed currents observed at positive potentials cannot be explained by K^+ channel blockage. Moreover, quinine stimulation induced an inward current even at and near the resting potential (at $-84 \sim -64$ mV in Fig. 2, *B* and *C*) although voltage-dependent K^+ currents must be very small near the resting potential (see Fig. 1 *B* and the baseline current before stimulation at −64 and −84 mV in Fig. 2 *B*). This and above considerations suggest that another quinine-evoked conductance contributes to the quinine response. We therefore used a Cs^+ pipette solution to observe quinine-evoked conductances in the absence of currents due to K^+ channel blockage.

With the Cs^+ solution in the pipette, bitter-induced responses became monophasic at all voltages tested (Fig. 3 *A*), indicating that the current due to K^+ channel blockage was effectively eliminated by the Cs^+ substitution. The inward current began to be induced 80 ms after the onset of the stimulus and reached a peak value at ~2.3 s. The peak current-voltage (*I-V*) relation for the quinine-induced response recorded with the Cs^+ pipette solution was nearly linear throughout the physiological range and reversed at +16.4 ± 2.3 mV (n = 12 cells) (Fig. 3 *B*). In contrast to cells recorded with the K^+ pipette solution, the reversal potential was very constant among cells. These results show that the quinine response was mediated by a depolarizing conductance increase in addition to K^+ channel blockage. The conductance induced by quinine was not suppressed by 100 μM amiloride, which blocks Na^+ currents in frog taste cells (n = 4 cells) (Avenet and Lindemann, 1988).

After the cessation of stimulation, the current at −84 mV was increased by 40 pA, shaping an "off-response"-like current (Fig. 3 *A*). The off-response-like currents were shown in 2 of 21 cells in this condition and were also observed in cells recorded with K^+ pipettes (3 of 7 cells, the cell in Fig. 2 *B* did not show off-response). This small current component reversed at the same potential (+16 mV) as the quinine-activated conductance (Fig. 3 *A*), suggesting that both components of the off-

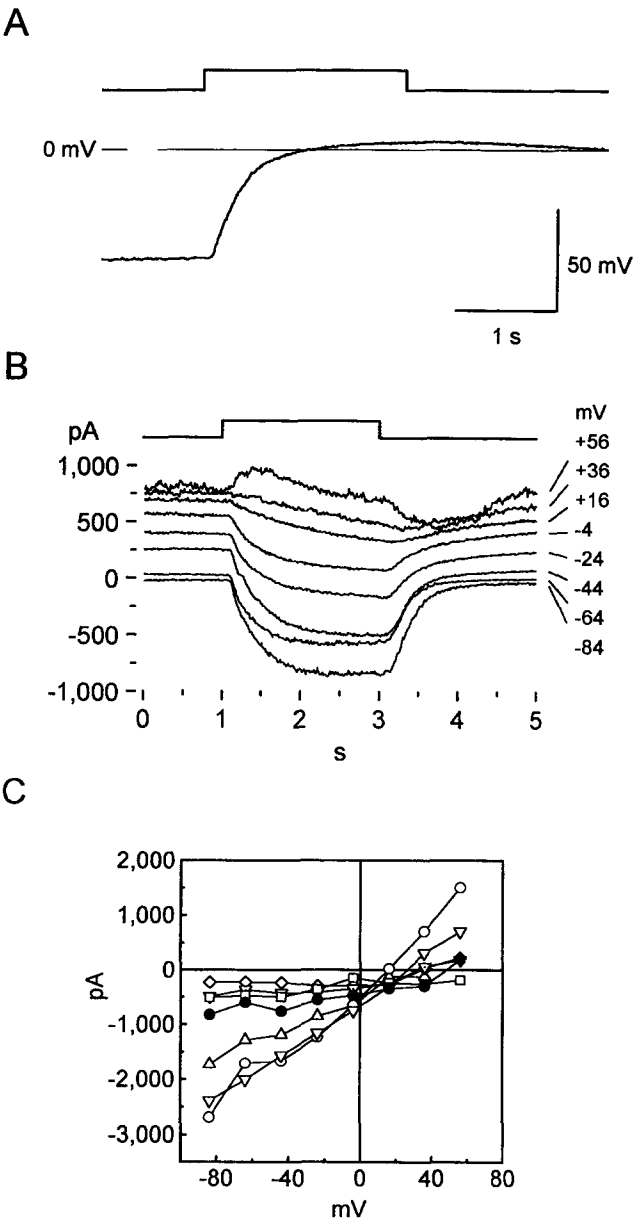


FIGURE 2. Responses to quinine stimulation in taste receptor cells bathed in normal saline solution. The recording pipette was filled with the K^+ solution. The square steps above the current traces in *A* and *B* show the timing of stimulation. Quinine stimulation was applied from a puffer pipette containing 10 mM quinine solution. (*A*) Voltage response evoked by quinine under current-clamp. (*B*) Quinine-induced currents recorded with various holding potentials. A downward current deflection indicates an inward membrane current. (*C*) *I-V* relations of the quinine-induced currents recorded from seven cells. Filled circles are data replotted from *B*. Open symbols are data from six other cells.

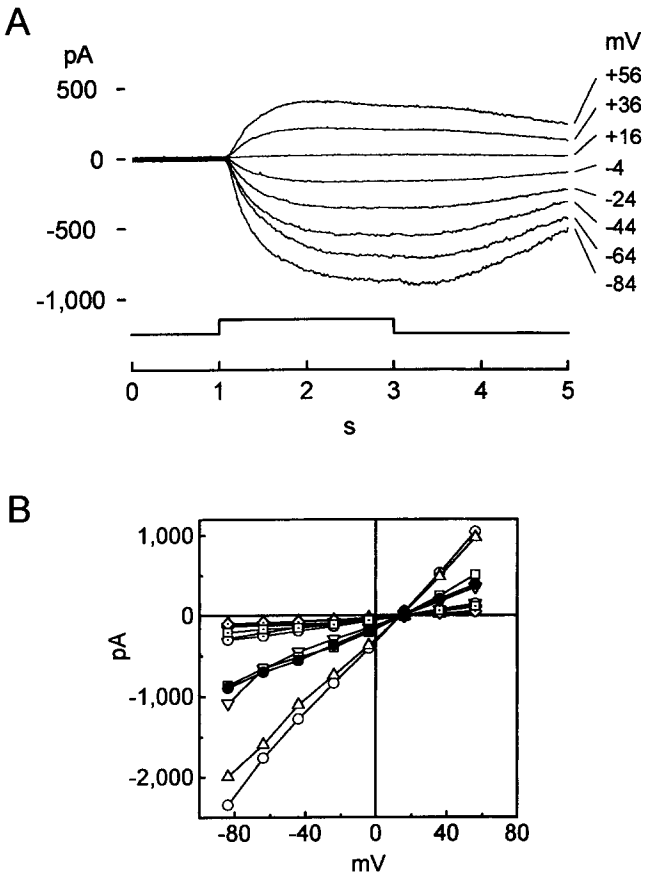


FIGURE 3. Voltage dependence of the quinine-induced current recorded with a pipette containing the Cs^+ solution. Cells were bathed in normal saline. Quinine stimulation was performed as in Fig. 2. (*A*) Quinine-induced currents recorded with various holding potentials. Timing of quinine stimulation is indicated by a square step below the current traces. (*B*) *I-V* relations of the quinine-induced currents recorded from 12 cells. Filled circles are replotted from *A*. Open symbols are from 11 other cells.

response-like current and the quinine-activated conductance were caused by the same ionic flux.

Concentration Dependence of the Quinine Response

The response amplitude evoked by quinine stimulation was concentration dependent (Fig. 4 *A*). Fig. 4 *B* shows a semilogarithmic plot of the relative peak amplitude of the quinine-induced current as a function of the concentration of quinine. This dose-response curve was sigmoidal-shaped and could be fitted by the Hill equation with a Hill coefficient of 5.3 and a $K_{1/2}$ of 3.6 mM.

At low quinine concentration (e.g., 2.5 and 3.7 mM in Fig. 4 *A*), the response frequently showed a biphasic shape: an initial small positive current followed by a negative current. The initial positive current might be explained by a nonspecific blocking effect of quinine on a basal inward current. At high concentrations (e.g., 9.3 mM in Fig. 4 *A*), an off-response-like current was seen.

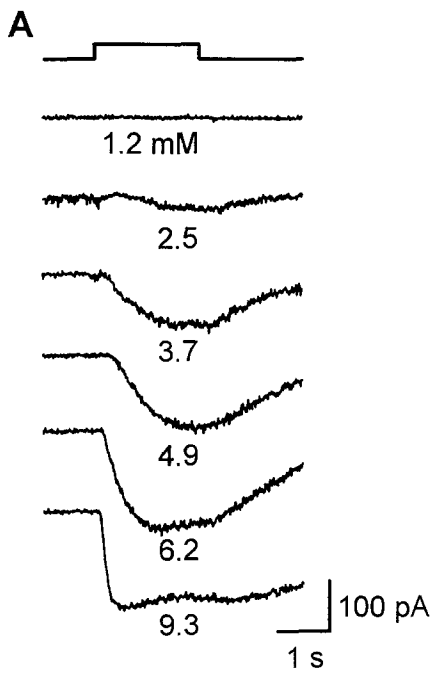
Effects of Extracellular Ions on the Quinine-activated Conductance

To identify the ionic species carrying the quinine-activated currents, we examined the quinine response in various bathing solutions. Under voltage clamp, the reversal potential of the quinine response was +16 mV in cells bathed in normal saline solution containing 115 mM NaCl (Fig. 3 *A*). When the major cation in the external solution, Na^+, was replaced with *N*-methyl-D-glucamine$^+$ (using Na^+-free solution), the reversal potential shifted negatively to between −49 and −29 mV (Fig. 5).

Since the amplitude of quinine responses tended to decrease gradually during the repetitive application of stimulants (time to half reduction was 15–30 min), the *I-V* relation was obtained more efficiently by using a ramp voltage command. In such experiments, the reversal potential was +16.0 ± 1.4 mV (n = 23 cells) and −39.9 ± 5.0 mV (n = 10 cells) in the normal saline solution and in the Na^+-free solution, respectively (Fig. 6 *A*). This result demonstrates that the quinine-activated conductance is highly permeable to Na^+.

We next tested the effect of changing the concentration of Cl^-, which is the major anion in our experimental environment. When extracellular Cl^- was replaced with gluconate$^-$ (the Cl^--free solution), the reversal potential was +13.6 ± 1.9 mV (n = 3 cells) (Fig. 6 *B*), which is very similar to that seen in 120 mM NaCl solution (+14.4 ± 1.5 mV, n = 6 cells, Fig. 6 *C*). This indicates that the quinine-induced conducting channels are cation selective.

The reversal potential obtained in 120 mM NaCl solution (+14 mV) was considerably more negative than the calculated equilibrium potential for Na^+ (+48 mV) for the solutions used in our experiments. It is therefore natural to think that internal Cs^+ can also pass through the quinine-activated ionic channels. Consistent with this possibility, the relative permeability derived from the Goldman-Hodgkin-Katz equation to Cs^+ versus Na^+ (P_{Cs}/P_{Na}) was 0.42. We also recorded the reversal potential using K^+ in the pipette solution (Fig. 2). Since, with K^+ in the pipette, quinine application caused K^+ channel blockage in addition to the opening of ionic channels, it was not possible to identify the true reversal potential for the ionic channels opened by quinine. In fact, the reversal potentials of relatively small induced currents (<1 nA at −84 mV) were very positive (>+32 mV) and were not constant among cells (Fig. 2 *C*) when K^+ was included in the pipette solution. Occasionally, however, we found cells which showed very large responses (e.g., 2.7 nA at −84 mV), so that the quinine-activated current would be expected to dominate. The reversal potential from one such cell was +15 mV, giving a P_K/P_{Na} of 0.5.



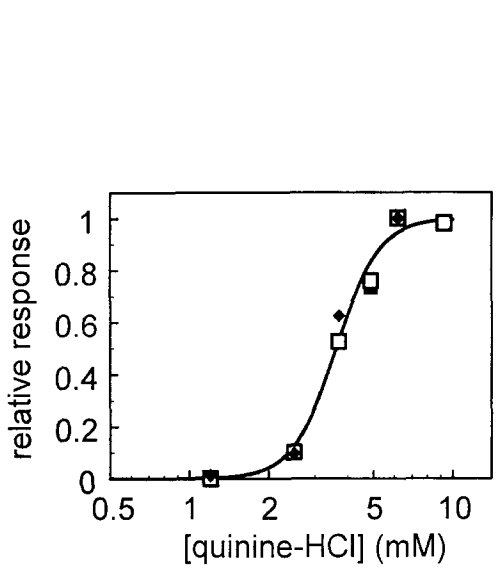


FIGURE 4. (*A*) Concentration dependence of the current evoked by quinine. The Cs^+ pipette solution was used. The holding potential was −64 mV. (*B*) Dose-response relation of the quinine-induced current. Relative peak amplitudes of the quinine-induced currents were plotted as a function of quinine concentration. The response with 6.2 mM quinine (200, 220, 260 pA in three cells) was taken as 1.0. The concentration values were calibrated for the dilution of the pipette solution after ejection from the puffer pipette (see METHODS). The solid curve was fitted by the Hill equation, $I/I_{max} = c^n/(c^n + K_{1/2}^n)$, with $n = 5.3$ and $K_{1/2} = 3.6$ mM, where c is the concentration.

Effects of External Ca^{2+} on the Quinine-induced Conductance

It has been reported that gustatory nerve responses to quinine are facilitated by reducing Ca^{2+} concentration at the interstitial solution to 0.2 mM by perfusing the lingual artery of the bullfrog with a low Ca^{2+} saline (Nagahama et al., 1982). However, the existing K^+-channel blockage model for bitter transduction cannot explain this phenomenon. We therefore examined the effect of extracellular Ca^{2+} on the quinine-activated cationic conductance. Removal of external Ca^{2+} enhanced the response amplitude and prolonged the time course of the quinine-induced current. A typical result is shown in Fig. 7, *A* and *B*. The maximal amplitude of the current induced by quinine stimulation increased by 5.3 times (at −84 mV), and the duration of the response was prolonged to >5 s (Fig. 7, *A* and *B*). Fig. 7 *C* shows the *I-V* relations of the quinine-activated conductance in the absence and presence of 1.8 mM Ca^{2+}. Removal of external Ca^{2+} increased the slope of the *I-V* curve, but did not affect the reversal potential. All cells tested showed the same Ca dependence (5.8 ± 1.8-fold increase, $n = 4$ cells).

These results suggest that Ca^{2+}, at least at a physiological concentration, is not a major charge carrier of the quinine-induced current, but acts as a modulator of this current. Although the molecular mechanism of Ca^{2+} action remains to be determined, the aforementioned Ca^{2+} effect on the bitter response in situ might be explained by this modulatory effect of Ca^{2+} on the bitter-induced cationic channel.

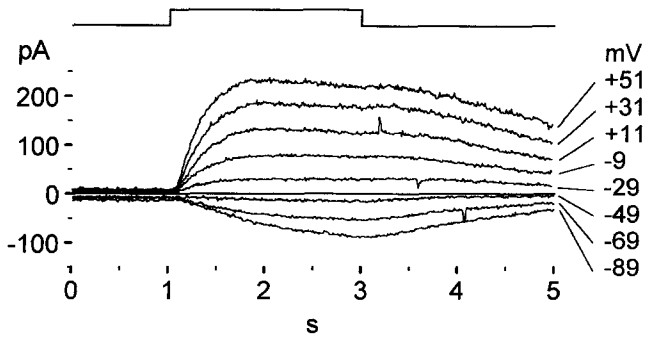


FIGURE 5. Voltage dependence of the quinine-induced current recorded from a cell bathed in Na^+-free solution. The Cs^+ pipette solution was used.

Effects of Cyclic AMP Analogue on the Quinine-induced Conductance

Kolesnikov and Margolskee (1995) have reported that bullfrog taste cells have an ionic conductance which is suppressed by intracellular cyclic nucleotides. Since the taste cell expresses a reduction of cytoplasmic cyclic nucleotides in response to bitter substances (Price, 1973; Ruiz-Avila et al., 1995), they claim that this ionic conductance could mediate bitter transduction. To examine this hypothesis, we investigated an effect of cytoplasmic cAMP on the quinine-activated current. A steady response induced by 5 mM quinine was suppressed by pressure-ejection of a membrane-permeable cAMP analogue, 8-Br-cAMP, by 24 ± 5% ($n = 6$ cells) within 10 s (Fig. 8 *A*). Suppression by 8-Br-cAMP was partial. Fig. 8 *B* shows the *I-V* relations produced by ramp changes in holding potential. When 8-Br-cAMP was applied, the

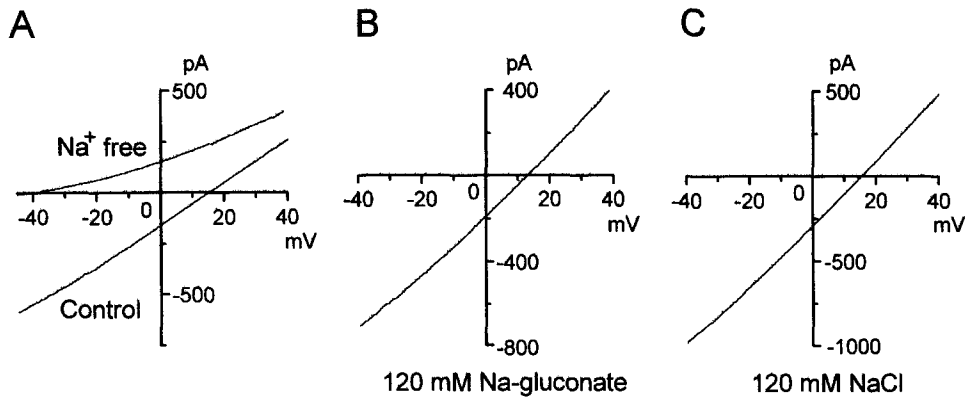


FIGURE 6. *I-V* relations of the quinine-activated conductance in various external solutions. The *I-V* relations were obtained by a ramp voltage command. Each curve represents the average of the data for the two currents in response to rising and falling voltage ramps (ramp rate = 195 mV/s). The Cs^+ pipette solution was used. (*A*) The conductance of a single cell in normal saline solution (*Control*) and in the Na^+-free solution. When the ionic composition of the bath solution was changed by superfusion, the stimulus puffer pipette was replaced by one containing the same ionic composition as the bath solution. (*B*) The conductance recorded in the Cl^--free solution. (*C*) The conductance was recorded in the 120 mM NaCl saline solution. *A*, *B*, and *C* are from three different cells.

slope of the ramp *I-V* curve became shallow without affecting the reversal potential (+18 mV), indicating that the current reduction was actually a conductance decrease.

DISCUSSION

The present study showed that a bitter taste stimulant, quinine, activates a cationic conductance in the plasma membrane of isolated bullfrog taste receptor cells and induces an inward current at the resting potential. The magnitudes of the quinine-induced current responses varied from cell to cell, although all taste cells responded to quinine stimulation. Similar results have also been reported using microelectrode recordings in situ, that is, a depolarizing response to 10 mM quinine was observed in nearly all taste cells, and the response amplitude varied from one cell to another (Akaike et al., 1976). This heterogeneity of responsiveness may result from the different taste specificities of individual cells, and therefore, may be involved in the discrimination of taste qualities. The effective quinine concentration range examined in this study (2–10 mM) is comparable to that used previously during intracellular recordings from bullfrog taste receptor cells, in situ (Akaike et al., 1976; Sato et al., 1994) and for behavioral experiments on a living amphibian (axolotl) (Takeuchi et al., 1994). The quinine concentration used in the present study is therefore physiologically relevant to taste perception in these species.

The present study demonstrates that quinine activates a cationic conductance and blocks K^+ channels. Quinine concentration higher than 1 mM was needed to activate a cationic conductance, although 0.1 mM

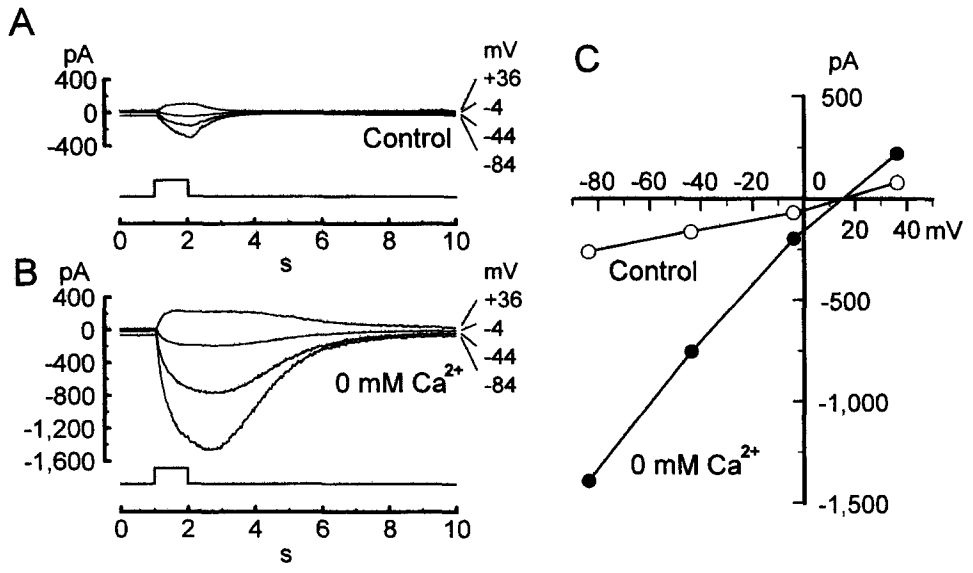


FIGURE 7. Effect of external Ca^{2+} on the quinine-induced response. The Cs^+ pipette solution was used. (*A*) Currents recorded in normal saline solution containing 1.8 mM Ca^{2+}. (*B*) Currents recorded in Ca^{2+}-free saline solution (see METHODS). The current recordings in *A* and *B* were from the same cell. (*C*) *I-V* relations of the quinine-induced current with 1.8 mM Ca^{2+} (*Control*) and 0 added Ca^{2+} (*0 mM Ca^{2+}*) conditions. Replotted from *A* and *B*. The currents were initially recorded in normal saline solution and next in Ca^{2+}-free saline solution. When the bathing Ca^{2+} concentration was changed by superfusion, the stimulus puffer pipette was replaced by one containing the same ionic composition as the bath.

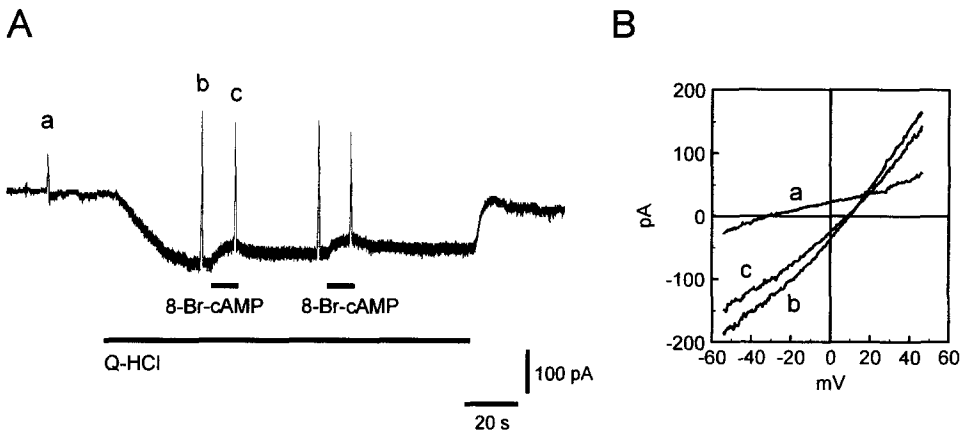


FIGURE 8. Effect of cAMP analogue on the quinine-activated conductance. (*A*) Suppression of the quinine-induced inward current by 8-Br-cAMP application. Quinine (5 mM; *Q-HCl*) was applied by a bath superfusion. Cyclic AMP analogue was applied by pressure ejection from the puffer pipette containing 2 mM 8-Br-cAMP. The puffer pipette also contained 5 mM quinine hydrochloride to make a quinine concentration around a cell steady. The cell was voltage clamped at −54 mV. Large deflections on the current trace indicate the currents evoked by ramp pulses (between −54 and +46 mV, ramp rate = 200 mV/s). The Cs^+ pipette solution was used for the intracellular solution. (*B*) The *I-V* relations recorded in normal saline (*a*), 5 mM quinine (*b*), and 5 mM quinine with 2 mM 8-Br-cAMP (*c*). Curves were reproduced from the current traces shown in *A*. Each curve represents the average of the data for the two currents in response to rising and falling voltage ramps.

quinine could block K^+ currents. We used the taste cells with modest quinine response to make a dose-response curve, and they had a similar threshold to quinine. However, it is not clear whether all taste cells have a similar threshold to quinine because a previous report shows that the threshold to a same odorant in the olfactory cells varies from cell to cell (Firestein et al., 1993). In fact, we found a couple of cells that showed extremely large responses (>1.5 nA at −64 mV, see Fig. 3 *B*).

The Hill coefficient calculated from the dose-response relation was 5.3. A similar high value of the Hill coefficient has been reported in odorant-induced currents of isolated olfactory cells (up to 4.4; Firestein and Werblin, 1989; Firestein et al., 1993). A high Hill coefficient in the olfactory system seems to be determined by multiple mechanisms (Kurahashi and Yau, 1994). Therefore, it is probable that the observed Hill coefficient of the cationic channels could indicate a presence for the multistep signal transduction cascade.

At a high concentration of quinine, taste cells showed small excitatory responses at the cessation of the stimulation. This response is very similar to the off-response which was reported in olfactory receptor cells (Takagi and Shibuya, 1959; Kurahashi et al., 1994). The mechanism of the olfactory off-response was recently shown to be caused by a suppressive effect of odorants on the transduction current (Kurahashi et al., 1994). Similarly, the off-response observed here may be explained by the fact that quinine has a nonselective blocking effect on several ionic channels (Walden and Speckmann, 1981; Gögelein and Capek, 1990; Gray and Argent, 1990); therefore, we propose that quinine may partially block the quinine-activated cation channels.

There are two types of cation-selective channels in frog taste receptor cells: the nonselective cation channel observed in excised membrane patches (P_{Cs}/P_{Na} = 1.43) (Fujiyama et al., 1993), and the acid-activated conductance observed during whole-cell recording (P_{Cs}/P_{Na} = 1.01) (Okada et al., 1994). However, the cationic channel found in our experiments (P_{Cs}/P_{Na} = 0.42) is significantly different from those reports in terms of ionic selectivity. The only other known example of a quinine-activated conductance is a gap junctional channel in retinal horizontal cells (Malchow et al., 1994), while it is well known that quinine can block various types of ionic channels in a variety of cells (Walden and Speckmann, 1981; Gögelein and Capek, 1990; Gray and Argent, 1990). The cationic channels presented in this study, however, are different from the gap junctional channels in terms of ionic selectivity; gap junctional channels having a large conductance do not generally discriminate between cations and anions.

A membrane-permeable cAMP analogue, 8-Br-cAMP, suppressed the quinine-induced cationic conductance, while 100 μM amiloride had no effects on the quinine-induced conductance. This result indicates that the quinine-induced conductance is sensitive to the intracellular cAMP. In the bullfrog taste receptor cells, a reported ionic conductance suppressed by intracellular cyclic nucleotides is also insensitive to amiloride, although a detailed relationship between the ionic channel and bitter substances has not been studied (Kolesnikov and Margolskee, 1995). From these pharmacological similarities of both conductances, it is likely that quinine activates the reported cyclic-nucleotide-suppressible conductance.

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