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Off-Depolarization and Off-Hyperpolarization after Termination of Quinine-HCl Stimulation in Frog Taste Cells

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ABSTRACT—Off-responses in frog taste cells evoked by a Ringer rinse following 1 mM quinine-HCl (Q-HCl) stimulation were investigated with an intracellular recording technique. Three types of off-responses were found; a transient off-depolarization, a rebound-type off-depolarization and a transient off-hyperpolarization. The time to peak and duration of off-responses were in the order of rebound type-off-depolarization > transient off-depolarization > transient off-hyperpolarization. The reversal potential for the rebound-type off-depolarization existed in more positive level than the resting potential. The reversal potential for the transient off-depolarization was around 0 mV, and that for the transient off-hyperpolarization was –58 mV. These three off-responses in frog taste cells may be initiated by an increase in permeability of the apical receptive membrane to Na^+, K^+ and Cl^-. A kind of principal ion is dependent on off-response types.

INTRODUCTION

It is known that a gustatory nerve gives rise to an off-response when a taste stimulus applied to the tongue is rinsed with a rinsing solution (Sato, 1976, 1978). The gustatory nerve in some animals shows the response to pure water applied to the tongue (Zotterman, 1956; Sato, 1978). When the taste receptors have sensitivity to pure water, the tongue is usually adapted to and rinsed with salt solutions including a Ringer solution to remove a contamination of the so-called water response (Sato, 1978).

In case of frogs, a large off-response is generated in the gustatory nerve by a Ringer rinse after the tongue is stimulated by bitter stimuli such as quinine-HCl (Q-HCl), quinine-H_2SO_4, picric acid and brucine (Sato, 1976, 1978). It has been found that the off-response in the frog gustatory nerve following bitter taste stimulation is generated by an NaCl component of the Ringer saline (Sato, 1976, 1978). An off-depolarization, which is related to generation of off-response in the gustatory nerve, has been obtained in frog taste cells by the Ringer rinse following bitter taste stimulation (Sato, 1978). It has been postulated that the off-response after bitter substances in the gustatory nerve and cell is due to bitter substance-induced conformational change in the apical receptive membrane of taste cells (Sato, 1978).

In this study we examined the relationships between membrane potentials and off-responses to understand ionic mechanisms of off-depolarization and off-hyperpolarization appearing following Q-HCl stimulation in frog taste cells.

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MATERIALS AND METHODS

Twenty-one bullfrogs (*Rana catesbeiana*) of 175–390 g were employed in the experiments. The animals were deeply anesthetized with an intraperitoneal injection of a 50% urethane-Ringer solution (3 g/kg body weight). The hypoglossal nerves and the hyoglossal and geniohyoid muscles were bilaterally severed to avoid the neurally driven contraction of tongue muscles. The animal was positioned in the supine position, and the tongue was pulled out from the mouth as long as possible and fixed on a cork plate with insect pins.

Intracellular recordings were made from single taste cells within the taste disk located at the top of the fungiform papillae. Glass capillary microelectrodes, filled with 3 M KCl and having a resistance of 40–80 MΩ were used for intracellular recordings. An indifferent capillary electrode, filled with 3 M KCl-3% agar and having a tip outer diameter of 80–120 μm, was put on the tongue surface. The membrane potentials led off from the taste cell were amplified with a microelectrode amplifier (DPZ-16A, Dia Medical System, Tokyo), displayed on an oscilloscope and recorded on a pen recorder. When measuring the reversal potential for an off-depolarizing or off-hyperpolarizing response in a taste cell, a bridge circuit was used for simultaneous current injection and membrane potential recording. The bridge was balanced completely before the penetration of the cell to cancel the resistance of the microelectrode. The measurement of the membrane potential was performed by recording the potential difference between an intracellular microelectrode and an indifferent electrode. The intensity of the current injected through the microelectrode was monitored by the potential drop across a resistor.

The tongue surface was usually adapted to a normal Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES; pH=7.2) and rinsed with the Ringer after Q-HCl stimulation of the tongue. Taste stimuli were Q-HCl solutions which were mostly prepared with deionized water (regent grade water, Millipore, MA). Taste and rinsing Ringer solutions were applied to the tongue at 0.129 ml/sec with a semiautomatically controlled gustatory stimulator. The pH of 1 mM Q-HCl dissolved in deionized water was 6.0.

All experiments were carried out at a room temperature of 22–25°C.

RESULTS

When a frog taste cell was stimulated for 10 sec by 1 mM Q-HCl dissolved in water and rinsed by a Ringer solution, an off-response evoked by a Ringer rinse following an on-response to 1 mM Q-HCl is classified into five types as shown in Figure 1. A transient off-depolarization induced by the Ringer rinse appeared following termination of a Q-HCl-induced sustained depolarization (A) or of that preceded by an initial hyperpolarization (B). A transient off-hyperpolarization was evoked by a Ringer rinse following Q-HCl-induced hyperpolarization (C). Sometimes the transient off-hyperpolarization did not follow the Q-HCl-induced hyperpolarization, but a rebound-type slow off-depolarization appeared after Q-HCl-induced hyperpolarization (D). In some taste cells, Q-HCl-induced hyperpolarization was followed by both a transient off-hyperpolarization and a rebound-type off-depolarization (E). Of 162 taste cells examined, the type A and B responses were seen in 15 cells (9%) and 13 cells (8%), respectively, and the type C, D and E responses were found in 72 cells (45%), 19 cells (12%) and 27 cells (17%), respectively. No off-responses appeared in 16 taste cells (10%) examined.

Application of 1 mM Q-HCl dissolved in Ringer sometimes

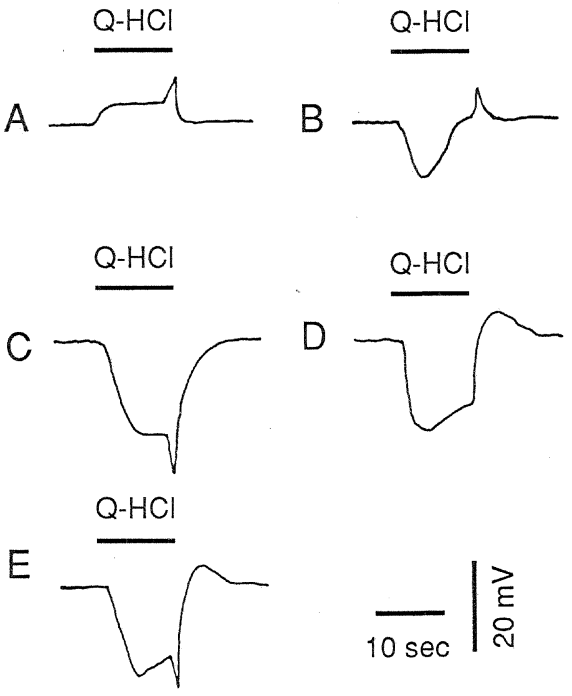

Fig. 1. Types of frog taste cell responses evoked by a Ringer rinse following 1 mM Q-HCl stimulation. The data were obtained intracellularly from 5 different taste cells. Horizontal bars above records are the period of 1 mM Q-HCl stimulation. A and B show transient off-depolarizations. C and D show transient off-hyperpolarization and rebound-type off-depolarization, respectively, and E shows both transient off-hyperpolarization and rebound-type off-depolarization. The resting potentials were –24 mV in A, –24 mV in B, –22 mV in C, –32 mV in D and –23 mV in E.

Table 1. Amplitude of off-responses after 1 mM Q-HCl

Response type in Figure 1	Off-response	Amplitude (mV)	(n)
A	Transient off-depolarization	3.2±0.6	(15)
B	Transient off-depolarization	4.4±0.8	(13)
C	Transient off-hyperpolarization	– 4.5±0.5	(72)
D	Rebound-type off-depolarization	3.7±0.5	(19)
E	Transient off-hyperpolarization	– 4.6±0.9	(27)
	Rebound-type off-depolarization	3.3±0.3	(27)

Off-response amplitudes are given in mean±SE.

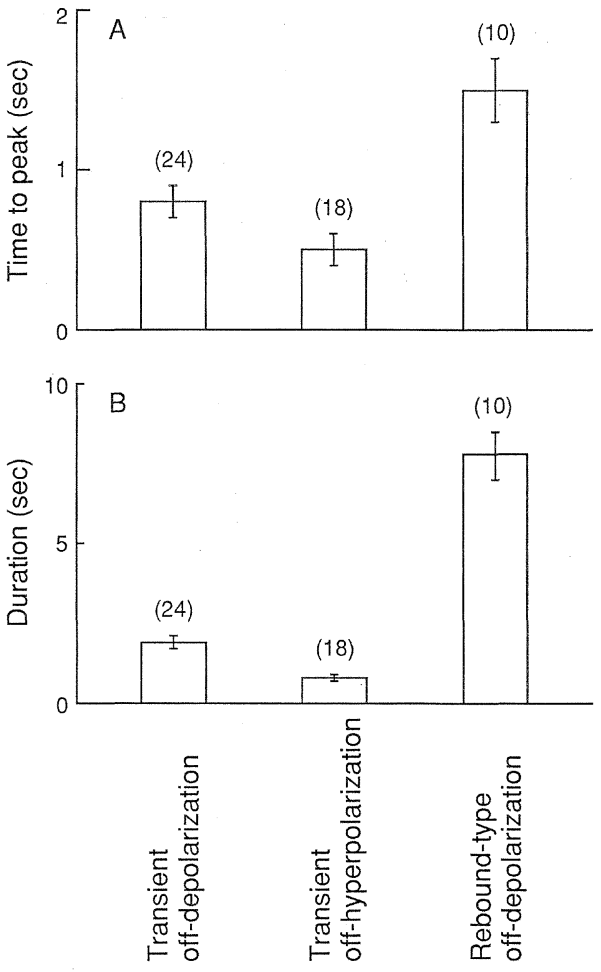

Fig. 2. Time to peak and duration of three types of off-responses evoked by Ringer rinse after 1 mM Q-HCl stimulation. Number of taste cells examined is shown within parenthesis. Vertical bars are SE.

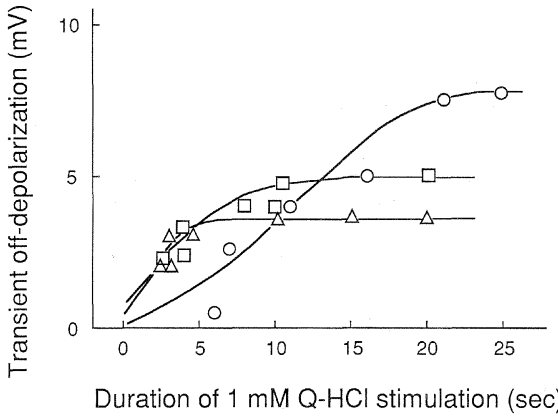

Fig. 3. Relationship between amplitude of transient off-depolarization and duration of 1 mM Q-HCl stimulation. Data were obtained from three taste cells.

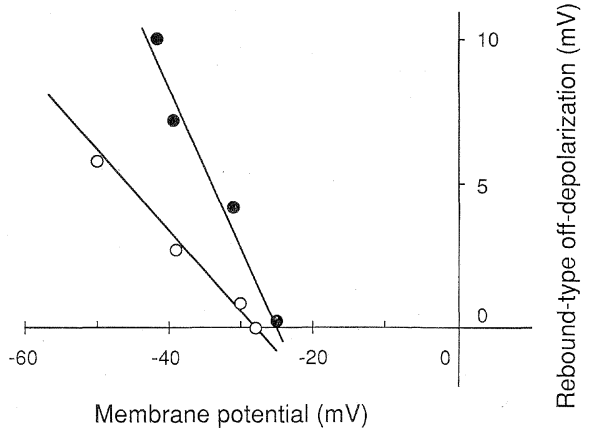

Fig. 5. Relationship between level of membrane potential and amplitude of rebound-type off-depolarizations evoked by Ringer rinse after 1 mM Q-HCl. Data were from two taste cells. The resting potential was –30 (○) and –32 mV (●).

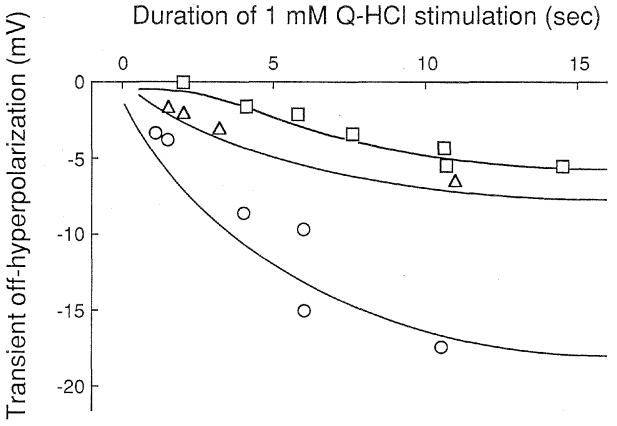

Fig. 4. Relationship between amplitude of transient off-hyperpolarization and duration of 1 mM Q-HCl stimulation. Data were from three taste cells.

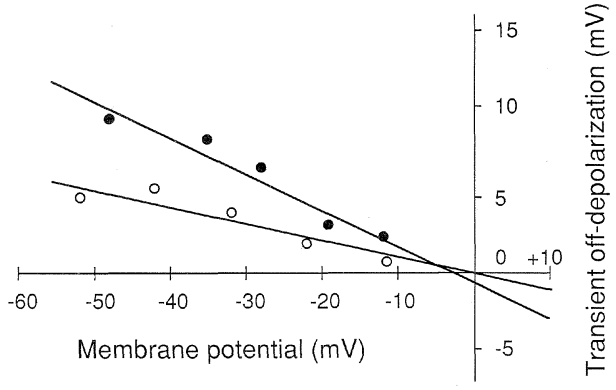

Fig. 6. Relationship between level of membrane potential and amplitude of transient off-depolarizations evoked by Ringer rinse after 1 mM Q-HCl. Data were from two taste cells. The resting potential was –28 (●) and –32 mV (○).

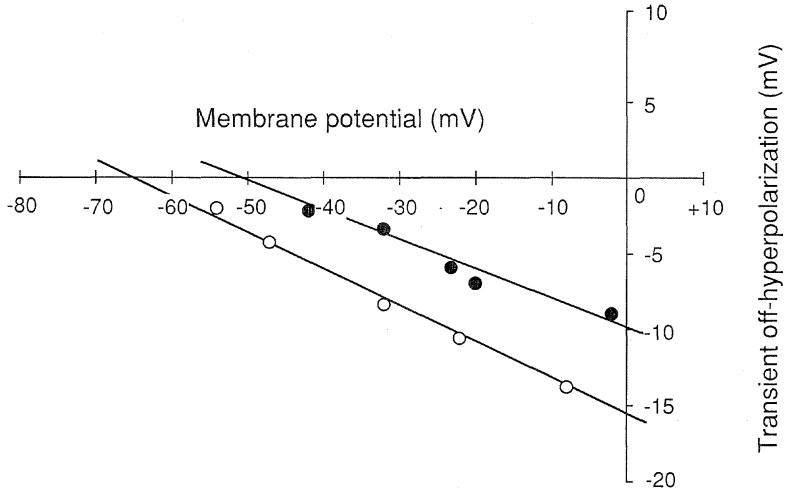

Fig. 7. Relationship between level of membrane potential and amplitude of transient off-hyperpolarizations evoked by Ringer rinse after 1 mM Q-HCl. Data were from two taste cells. The resting potential was –32 mV in either cell.

induced sustained depolarizations, but did not hyperpolarizations. No types of off-responses were evoked by a Ringer rinse after application of Q-HCl in Ringer.

The mean amplitudes of off-responses in five response types of Figure 1 are shown in Table 1. No significant differences in amplitude were found among off-depolarizations in types A, B, D and E ($P>0.05$). Also no significant difference was seen between off-hyperpolarizations in types C and E ($P>0.05$).

The mean time to peak after onset of off-responses was 0.8 sec in transient off-depolarizations in the types A and B of Figure 1 (Fig. 2A). The mean time to peak was 0.5 sec in transient off-hyperpolarizations in the types C and E and 1.5 sec in rebound-type slow off-depolarizations in the types D and E (Fig. 2A). The peak time of rebound-type off-depolarizations was significantly the longest ($P<0.01$). On the other hand, the mean duration of off-responses was 1.9 sec in the transient off-depolarizations, 0.8 sec in the transient off-hyperpolarizations and 7.8 sec in the rebound-type off-depolarizations (Fig. 2B). The duration of rebound-type off-responses was much longer than that of the other off-responses ($P<0.01$).

As shown in Figures 3 and 4, either transient off-depolarization or transient off-hyperpolarization increased in amplitude with increasing period of Q-HCl stimulation, and the maximum off-responses appeared mostly at 10 sec of the stimulation.

While the membrane potential of a taste cell was changed by injecting current, the amplitude of rebound-type off-depolarization following 1 mM Q-HCl stimulation was measured. As shown in data from two taste cells of Figure 5, the off-depolarization increased with increasing membrane potential. The reversal potential for the rebound-type off-depolarization existed in several millivolts more positive level than the resting potential. In the two cells of Figure 5, the reversal point was –25 and –28 mV. The input resistance of a taste cell during the rebound-type off-depolarization was observed to decrease by 5–8% in three taste cells.

The transient off-depolarization was increased in amplitude as the membrane potential became larger. The example is shown in Figure 6, where the reversal potential for the off-depolarizations in two taste cells was 0 mV and –2 mV. During generation of the transient off-depolarization the input resistance decreased by 5–10% in four taste cells.

On the other hand, the amplitude of transient off-hyperpolarization decreased with increasing membrane potential. The reversal potential for the off-hyperpolarizing responses in two taste cells was –65 mV and –52 mV in Figure 7. The input resistance of a taste cell decreased by 5-15% in three cells during the off-hyperpolarization evoked by a Ringer rinse.

DISCUSSION

The off-response is elicited in the frog gustatory nerves and cells by rinsing the tongue with a Ringer saline after the gustatory cells are stimulated with Q-HCl and related bitter substances (Sato, 1978). The Ringer-induced off-response in the taste nerve following Q-HCl is suggested to be due to an NaCl component of rinsing Ringer solution, sensitivity of whose receptors is enhanced during Q-HCl stimulation (Sato, 1976, 1978).

Ringer-induced off-responses in taste nerve and cell after application of Q-HCl dissolved in water change in amplitude depending on Q-HCl concentration (Sato, 1979). Therefore, the off-responses after Q-HCl solution are due to the Q-HCl solute but not to the water solvent. However, no off-responses are induced after application of Q-HCl dissolved in Ringer. It is likely that Ringer in a Q-HCl solution depresses Q-HCl molecule-induced conformational change of taste receptor sites, as previously suggested (Sato, 1978).

In the present experiments we found three kinds of off-responses in frog taste cells following termination of Q-HCl stimulation (Fig. 1): a transient off-depolarization; a rebound-type off-depolarization; a transient off-hyperpolarization. The time to peak and duration of rebound-type off-depolarizations are significantly longer than those of transient off-depolarizations (Fig. 2).

As shown in Figure 5, the reversal potential for a rebound-type off-depolarization induced by a Ringer rinse exists in more positive level than the resting membrane potential (mean, –27 mV). From the reversal point it is estimated that the rebound-type off-depolarization is induced by outflow of Cl^- and inflow of Na^+ through the apical membrane of taste cells. The rebound-type off-depolarization evoked by termination of injected hyperpolarizing current has been reported in frog taste cells (Kashiwayanagi *et al.*, 1983). This off-depolarization lasts about 10 msec and the amplitude is dependent on the amplitude of hyperpolarized level. The rebound-type off-depolarization induced by the so-called anode break is suggested to originate from Na^+ and Ca^{2+} current through the basolateral membrane (Kashiwayanagi *et al.*, 1983). Therefore, the rebound-type off-depolarization in the present study is quite different from the off-depolarization evoked by an anode break.

As shown in Figure 6, the reversal potential for the transient off-depolarization elicited by a Ringer rinse after Q-HCl stimulation is close to zero membrane potential. Probably the permeability of the apical receptive membrane of frog taste cell to Na^+, K^+ and Cl^- is related to generation of the off-depolarization, since generation of depolarizing receptor potential in response to a NaCl stimulus is proposed to be related to these ions (Sato, 1980; Miyamoto *et al.*, 1989, 1993; Sato *et al.*, 1994). However, during generation of the transient off-depolarization the permeability of the apical membrane in a frog taste cell to Cl^- may be very small as previously suggested (Miyamoto *et al.*, 1989). It is also known that the permeability of apical receptive membrane of frog taste cell to Na^+ and K^+ is relatively high in the resting state (Sato *et al.*, 1984; Okada *et al.*, 1986).

The reversal potential for a transient off-hyperpolarization evoked by a Ringer rinse after Q-HCl is about –60 mV (Fig.

7). When the apical receptive membrane of the frog taste cell is covered with normal Ringer solution, the equilibrium potential of Cl^- is calculated to be −53 mV, assuming that the intracellular Cl^- is 15 mM (Miyamoto *et al.*, 1993). This suggests that the transient off-hyperpolarization in a frog taste cell is mainly generated by entry of Cl^- in Ringer solution through the apical receptive membrane. Since the transient off-hyperpolarizing response is elicited only after termination of Q-HCl-induced hyperpolarization, it is likely that the off-hyperpolarization evoked by a Ringer rinse is due to Cl^- entry *via* hyperpolarization-activated Cl^- channels in the apical membrane. The hyperpolarization-gated Cl^- channels have been reported in some cells (Schwiebert *et al.*, 1994).

Ion replacement experiments are necessary to reveal ionic mechanisms of the off-responses in the taste cells.

Since transient off-depolarization and off-hyperpolarization increased with increasing period of Q-HCl stimulation (Figs. 3 and 4), these off-responses are related to the conformational change of salt receptor sites which is induced by irritant action of Q-HCl. This suggestion has already been given in detail (Rollo, 1970; Sollmann, 1957; Sato and Sugimoto, 1979, 1995).

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