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Quinine-HCl-Induced Modification of Receptor Potentials for Taste Stimuli in Frog Taste Cells

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ABSTRACT—After frog taste cells were adapted to 1 mM quinine-HCl (Q-HCl) for 10 sec, modification of receptor potentials in the taste cells induced by salt, acid, sugar and bitter stimuli was studied with microelectrodes. The phasic component of receptor potentials induced by 0.1 M NaCl, KCl, NH_4Cl and $MgCl_2$ was enhanced following adaptation to Q-HCl. The rate of rise of receptor potentials in response to the salts was increased after Q-HCl adaptation. The amplitude and the rate of rise of receptor potentials induced by 1 mM acetic acid were larger after Q-HCl adaptation than after water adaptation. The amplitude of phasic component and rate of rise of receptor potentials for 0.5 M sucrose after Q-HCl were the same as those after water. The amplitudes of tonic receptor potentials for 1 mM Q-H_2SO_4, brucine and picric acid after Q-HCl adaptation were the same as those after 1 mM NaCl adaptation. Correlation coefficient between taste cell responses induced by 1 mM Q-HCl and 1 mM Q-H_2SO_4 was very high, but those between 1 mM Q-HCl and 1 mM brucine responses and between 1 mM Q-HCl and 1 mM picric acid responses were low. This indicates that Q-HCl and Q-H_2SO_4 bind to the same receptor site, but brucine and picric acid bind to different receptor sites to which Q-HCl does not bind.

INTRODUCTION

The amplitude of gustatory neural responses to taste stimuli changes depending on the temperature [2, 16] and flow rate [7] of stimulus solutions and the previously applied solutions [5, 8, 9, 12, 14, 15, 17, 18]. In our previous papers we showed that frog gustatory neural responses to salts, acids and sugars are enhanced when the tongue surface is beforehand adapted to bitter solutions of quinine-HCl (Q-HCl), quinine-H_2SO_4 (Q-H_2SO_4) and picric acid for a short period of time [5, 6, 8, 9, 14]. Enhancement of salt responses after adaptation of the tongue to Q-HCl has been demonstrated in gustatory neural responses of rat and hamster [17, 18].

The purpose of this study is to examine characteristics of intracellular receptor potentials in response to four basic stimuli after Q-HCl in frog taste cells in order to clarify the enhancing mechanisms of gustatory neural responses to tastants after Q-HCl adaptation.

MATERIALS AND METHODS

Twenty-one bullfrog (*Rana catesbeiana*) of 180–320 g were used in the experiments. The animals were anesthetized with an intrapritonial injection of a 50% urethane-Ringer solution (3 g/kg body weight). Bilateral hypoglossal nerves and bilateral hyoglossal and geniohyoid muscles were severed to remove the movement of the tongue. The animal was positioned in the supine position and the tongue was pulled out as long as possible and pinned on a cork plate in a lucite chamber.

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Intracellular recordings were made from single taste cells in the taste disc of the fungiform papillae scattered on the dorsal tongue surface. Glass capillary microelectrodes filled with 3 M KCl and having a resistance of 30–60 MΩ were employed. An indifferent electrode of a glass capillary (100 μm, tip outer diameter) filled with 3% agar-3 M KCl was put on the tongue surface.

Intracellular membrane potentials were amplified with a microelectrode amplifier (DPZ-10A, Diamedical System, Tokyo) and recorded on a pen-recorder.

The tongue surface was usually preadapted by a continuous flow of Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 5 mM HEPES, pH=7.2). The Ringer solution and taste solutions were delivered to the tongue surface using a semiautomatically controlled gustatory stimulator [4]. The solutions coming from delivery nozzles of the gustatory stimulator flowed at a rate of 0.129 ml/sec. The adapting solution was usually Ringer, 1 mM Q-HCl and deionized water, and was usually applied for 10 sec. The test solutions were of NaCl, KCl, NH_4Cl, $MgCl_2$, acetic acid, sucrose, Q-HCl, Q-H_2SO_4, brucine and picric acid. All chemicals were reagent-grade and unless otherwise stated all chemicals were dissolved in deionized water (Milli-Q, Millipore Corp., MA). The pH of 1 mM Q-HCl and 1 mM Q-H_2SO_4 in deionized water was 6.0 and the pH of those in Ringer was 7.2.

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

RESULTS

Salt responses in taste cells following Q-HCl adaptation

Resting potentials of taste cells under Ringer adaptation were -27.9 ± 1.0 mV (mean±SE, n=181; a range of -14.3—-53.5 mV). Out of 106 taste cells examined during 10 sec adaptation of the tongue to 1 mM Q-HCl 16%

showed a depolarization alone, 6% a depolarization preceded by a hyperpolarization and 78% a hyperpolarization alone. Water adaptation of the tongue for 10 sec caused a depolarization alone in 5% of 106 taste cells examined, a depolarization preceded by a hyper-polarization in 1% and a hyperpolarization alone in 94%.

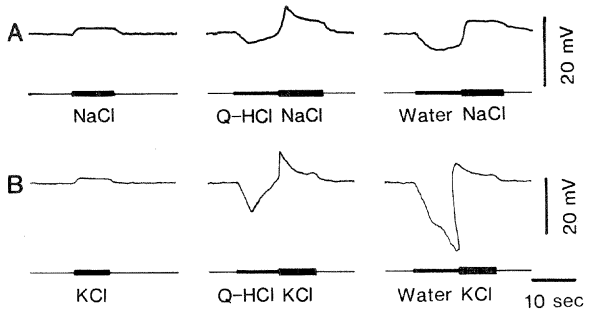

FIG. 1. Receptor potentials in response to 0.1 M NaCl (A) and 0.1 M KCl (B) after adaptation of frog taste cells to three different adapting solutions. Adapting solution was Ringer (left), 1 mM Q-HCl (middle) and deionized water (right). A and B were recorded from two different taste cells. Preadapting solution and rinsing solution excepting Figs. 11, 12 and 13 were a Ringer solution. The tongue was preadapted to Ringer before application of adapting Q-HCl solution and water and was rinsed with Ringer after test stimulation. When an adapting solution such as Q-HCl and water was not used as in the left trace, the preadapting Ringer was regarded as the adapting solution.

Figure 1 shows receptor potentials in response to 0.1 M NaCl (A) and 0.1 M KCl (B) after adaptation to Ringer (left), 1 mM Q-HCl (middle) and deionized water (right). Adaptation time for the Q-HCl and water was usually 10 sec.

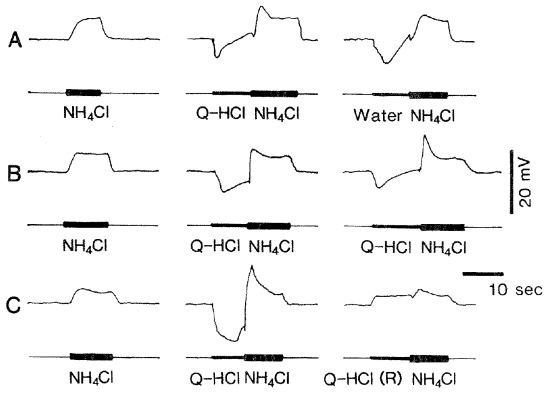

FIG. 2. Receptor potentials in response to 0.1 M NH_4Cl in frog taste cells. (A) 0.1 M NH_4Cl responses after adaptation to Ringer (left), 1 mM Q-HCl and deionized water. (B) 0.1 M NH_4Cl responses after adaptation to Ringer (left) and 1 mM Q-HCl. Adaptation time for 1 mM Q-HCl was 9 sec (middle) and 12 sec (right). (C) 0.1 M NH_4Cl responses after adaptation to Ringer (left), 1 mM Q-HCl (middle) and 1 mM Q-HCl dissolved in Ringer solution (R) (right). A-C were obtained from three different taste cells.

The responses to these salts were composed of phasic and tonic components. Figure 2 shows 0.1 M NH_4Cl responses in taste cells after adaptation to the three kinds of adapting solutions. The rate of rise of a depolarization to these salts was slower after Ringer adaptation, but increased after adaptation to 1 mM Q-HCl and water. Also the amplitude of the initial phasic component of the receptor potential increased after the Q-HCl and water adaptation compared with that after Ringer adaptation (Fig. 2A). The amplitude of phasic responses to salts after Q-HCl adaptation increased

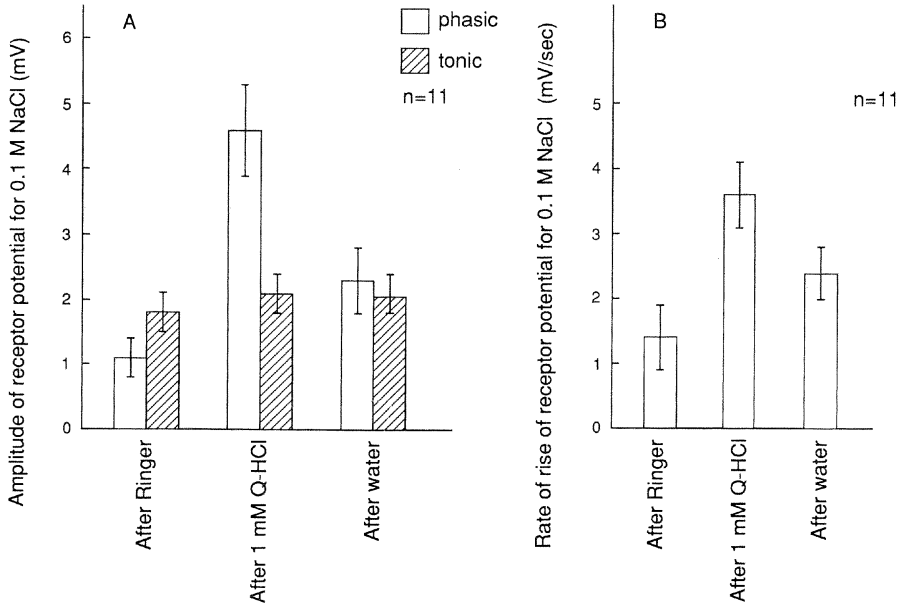

FIG. 3. The mean amplitude and the mean rate of rise of receptor potentials in response to 0.1 M NaCl in taste cells. (A) Phasic and tonic components of receptor potentials after adaptation to Ringer, 1 mM Q-HCl and deionized water. The amplitude of depolarizing receptor potential evoked by onset of test stimulation was measured as the phasic component and if a hyperpolarizing potential existed at the end of adapting solution, this hyperpolarization was excluded from the test stimulus-induced depolarization. (B) Rate of rise of receptor potential after adaptation to Ringer, 1 mM Q-HCl and water. Bars are SE in this and other figures.

depending on the adaptation time (Fig. 2B). Enhancing effect of the adapting Q-HCl solution was lost when Q-HCl was dissolved in Ringer (Fig. 2C).

In Figures 3–6 are shown the mean amplitude (A) and the mean rate of rise (B) of receptor potentials in response to 0.1 M NaCl, KCl, $MgCl_2$ and NH_4Cl after adaptation to Ringer, 1 mM Q-HCl and water. The amplitude of initial phasic receptor potential elicited by a test stimulus was measured as a potential change from the membrane potential level after termination of an adapting solution. When a depolarizing response for a test stimulus commenced from the hyperpolarized level and was superimposed on its returning to the base line, the magnitude of the hyperpolarization was excluded from the response. The rate of rise of receptor potential was measured by dividing the maximal amplitude of the response to a test stimulus by the peak time.

The amplitude of initial phasic responses in Figures 3–6 was of the order of 1 mM Q-HCl≧water>Ringer adapta-

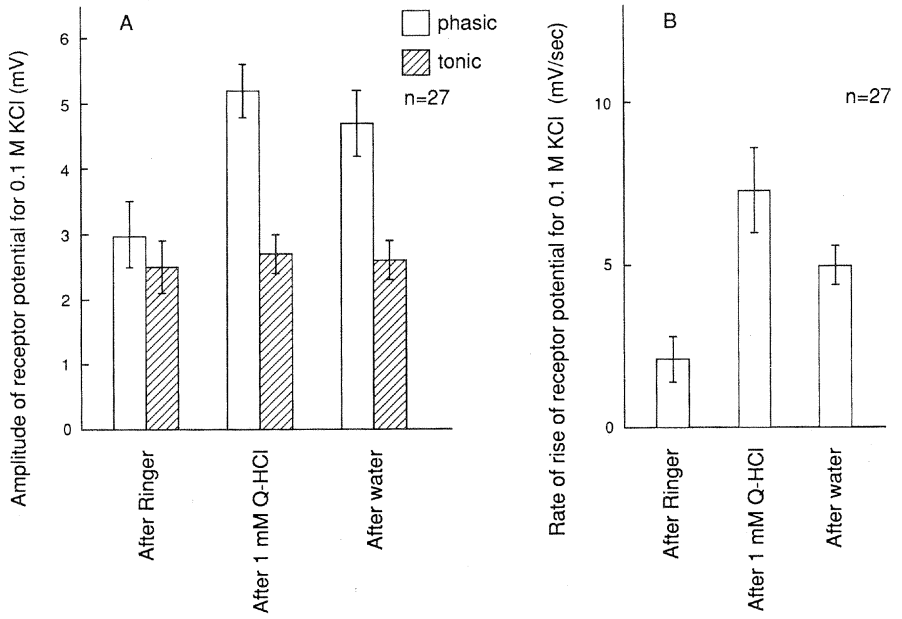

FIG. 4. The amplitude and rate of rise of receptor potentials in response to 0.1 M KCl in taste cells. (A) The amplitude of phasic and tonic components of receptor potentials after adaptation to Ringer, 1 mM Q-HCl and water. (B) The rate of rise of receptor potentials after adaptation to Ringer, 1 mM Q-HCl and water.

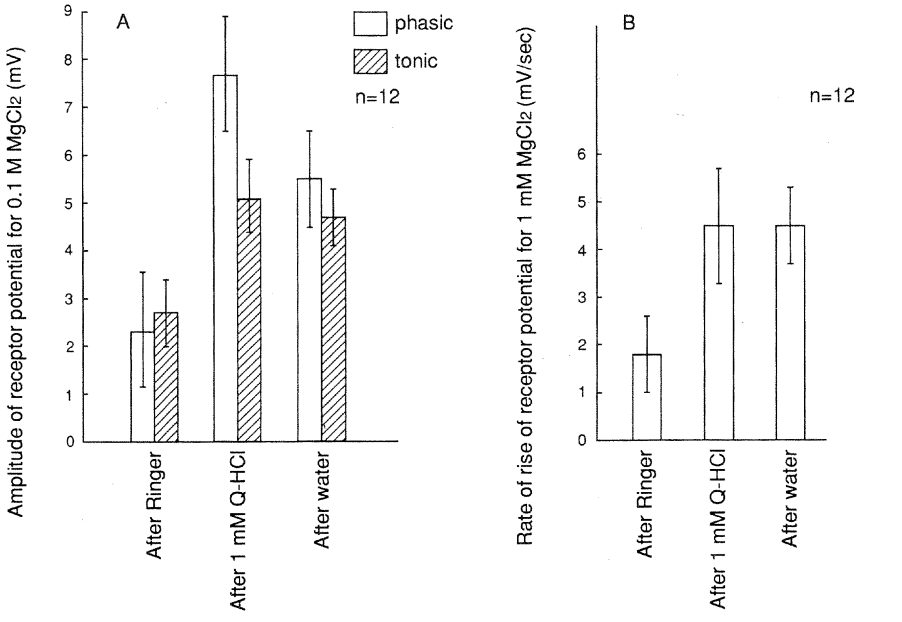

FIG. 5. The amplitude (A) and rate of rise (B) of receptor potentials in response to 0.1 M $MgCl_2$ in taste cells. Adapting solutions were Ringer, 1 mM Q-HCl and water.

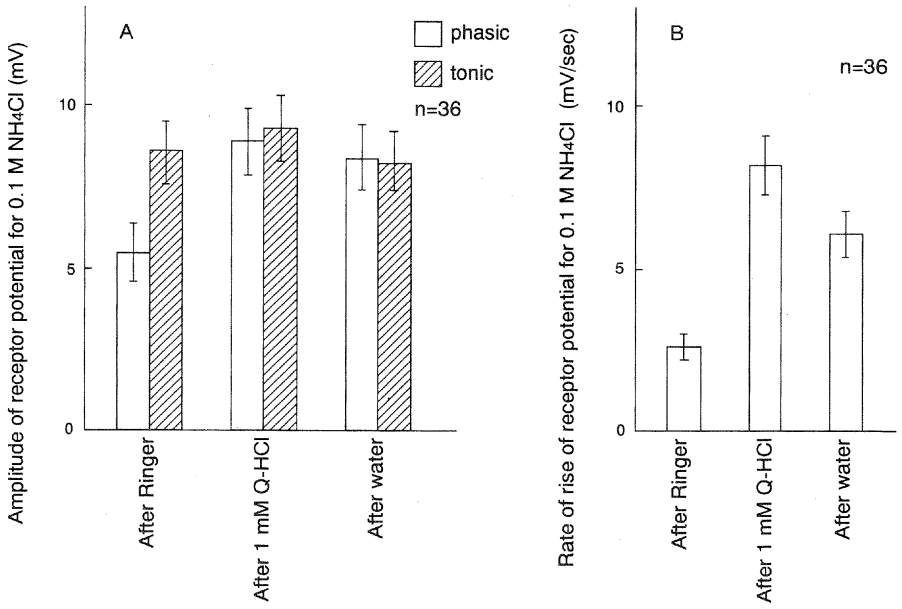

FIG. 6. The amplitude (A) and rate of rise (B) of receptor potentials in response to 0.1 M NH_4Cl in taste cells. Adapting solutions were Ringer, 1 mM Q-HCl and water.

tion. In case of 0.1 M KCl and 0.1 M NH_4Cl stimulations the amplitude of phasic responses did not significantly differ between Q-HCl and water adaptation (Figs. 4 and 6). Excepting 0.1 M $MgCl_2$ stimulation the amplitudes of tonic responses induced by all the salts examined did not change after the three different adapting solutions were applied. The rate of rise of phasic responses induced by salts excepting 0.1 M $MgCl_2$ was significantly increased in the order of Q-HCl>water>Ringer adaptation.

Acid responses in taste cells following Q-HCl adaptation

Figure 7 illustrates an example of depolarizing (A) and hyperpolarizing (B) receptor potentials induced by 1 mM acetic acid when the taste cell was adapted to Ringer, 1 mM Q-HCl and water. Of 44 taste cells examined the acetic acid responses after Ringer adaptation showed a depolarization in 57% and a hyperpolarization in 43%. The acid response was smaller under water adaptation than under Q-HCl and water adaptation. As shown in Figure 7B, a hyperpolarizing

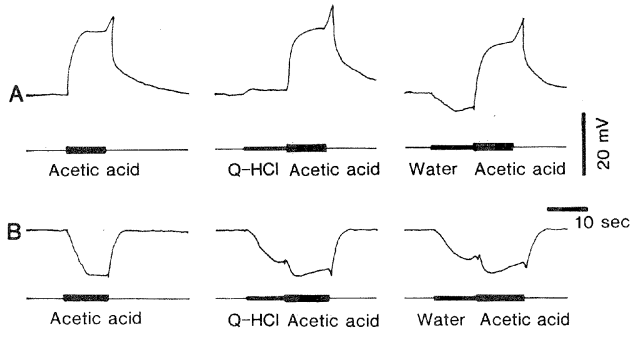

FIG. 7. Receptor potentials of taste cell induced by 1 mM acetic acid following adaptation to Ringer (left), 1 mM Q-HCl and water. A and B were obtained from two different taste cells.

response induced by the acetic acid was larger than that induced by water adaptation, indicating that acetic acid molecules themselves can exert a hyper-polarizing action on the taste receptive membrane. These phenomena were observed in all the taste cells hyperpolarized by the acid. Figure 8A illustrates the mean amplitudes of phasic and tonic depolarizing responses in taste cells induced by 1 mM acetic acid after Ringer, 1 mM Q-HCl and water adaptation. The acetic acid response after water was the smallest. The response amplitudes to acid did not change between Ringer and 1 mM Q-HCl adaptation. The rate of rise of initial phasic responses with acetic acid was much rapider after Ringer and Q-HCl than after water adaptation (Fig. 8B).

Sugar responses in taste cells following Q-HCl adaptation

In Figure 9 are shown responses to 0.5 M sucrose after Ringer, 1 mM Q-HCl and water adaptation. Of 27 taste cells examined the sugar responses after the Q-HCl adaptation were a depolarization (Fig. 9A) in 14 cells, a hyperpolarization (Fig. 9B) in 12 cells and nothing in one cell, and the sugar responses after water adaptation were a depolarization in 23 cells, a hyperpolarization in two cells and nothing in two cells. Figure 10A illustrates the mean amplitudes of depolarizing responses with 14 taste cells depolarized by 0.5 M sucrose after adaptation to both Q-HCl and water. Initial phasic and tonic depolarizations induced by the sucrose after Q-HCl and water were significantly larger than those after Ringer adaptation. The rate of rise of the phasic sucrose responses was much rapider after Q-HCl and water than after Ringer adaptation (Fig. 10B).

Bitter responses in taste cells following Q-HCl adaptation

Frog taste cell responses to bitter solutions were generally small under Ringer adaptation, so that the examination

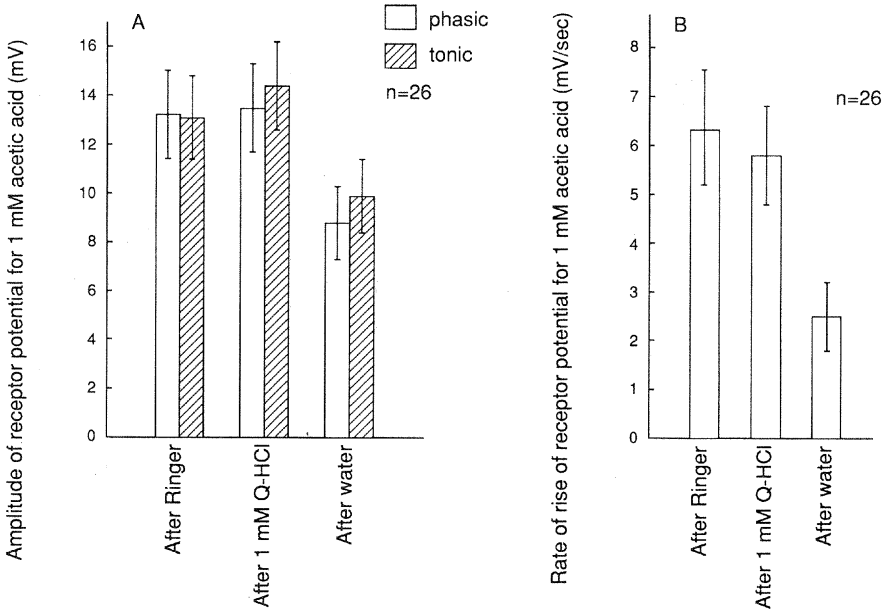

FIG. 8. The amplitude (A) and rate of rise (B) of receptor potentials in response to 1 mM acetic acid. Adapting solutions were Ringer, 1 mM Q-HCl and water.

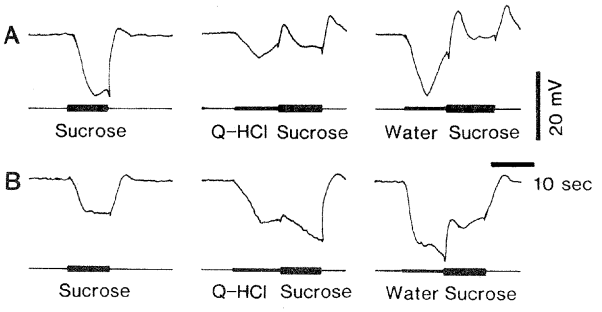

FIG. 9. Receptor potentials of taste cells induced by 0.5 M sucrose after adaptation to Ringer (left), 1 mM Q-HCl and water. A and B were obtained from different taste cells.

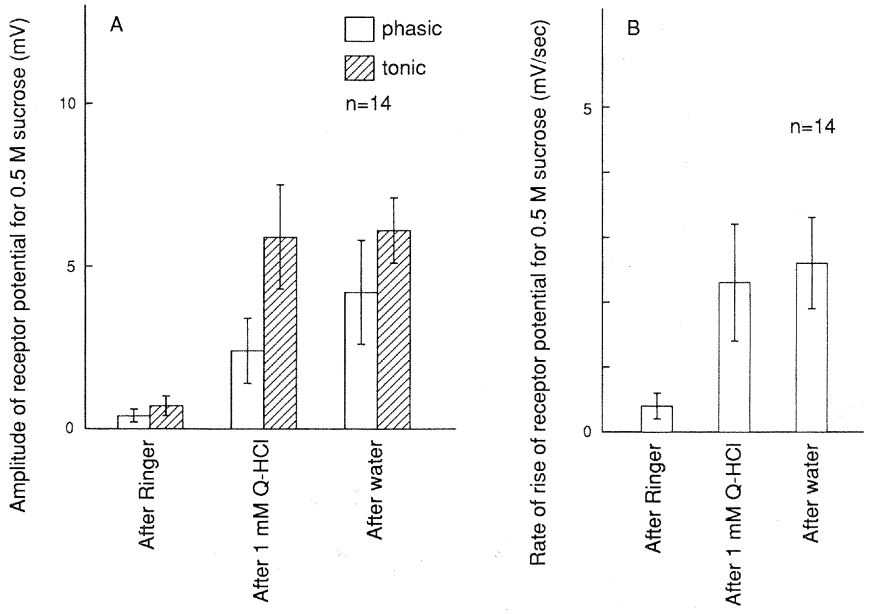

FIG. 10. The amplitude (A) and rate of rise (B) of receptor potentials induced by 0.5 M sucrose in taste cells. Adapting solutions were Ringer, 1 mM Q-HCl and water.

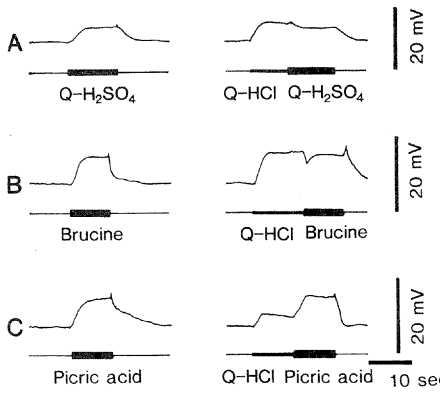

FIG. 11. Receptor potentials of taste cells induced by 1 mM Q-H_2SO_4 (A), 1 mM brucine (B) and 1 mM picric acid (C) after adaptation to 1 mM NaCl (left) and 1 mM Q-HCl (right). Preadapting and rinsing solution were 1 mM NaCl in this series of experiments (Figs. 11, 12, 13). A-C were obtained from three different cells.

of bitter responses was done under preadaptation of the tongue to 1 mM NaCl. The resting potential under 1 mM NaCl was -49.1 ± 1.3 mV (n=50). Figure 11 shows receptor potentials in response to 1 mM Q-H_2SO_4 (A), brucine (B) and picric acid (C) after the tongue was adapted to 1 mM NaCl (left) and 1 mM Q-HCl (right). The response amplitudes to each of the three bitter substances did not change after taste cells were adapted to either 1 mM NaCl or 1 mM Q-HCl (Figs. 11 and 12). In Figure 13 are shown relationships between response amplitudes induced by a pair of 1 mM Q-HCl and 1 mM Q-H_2SO_4 (A), a pair of 1 mM Q-HCl and 1 mM brucine (B) and a pair of 1 mM Q-HCl and 1 mM picric acid (c). Correlation coefficients were 0.89 in A, 0.32

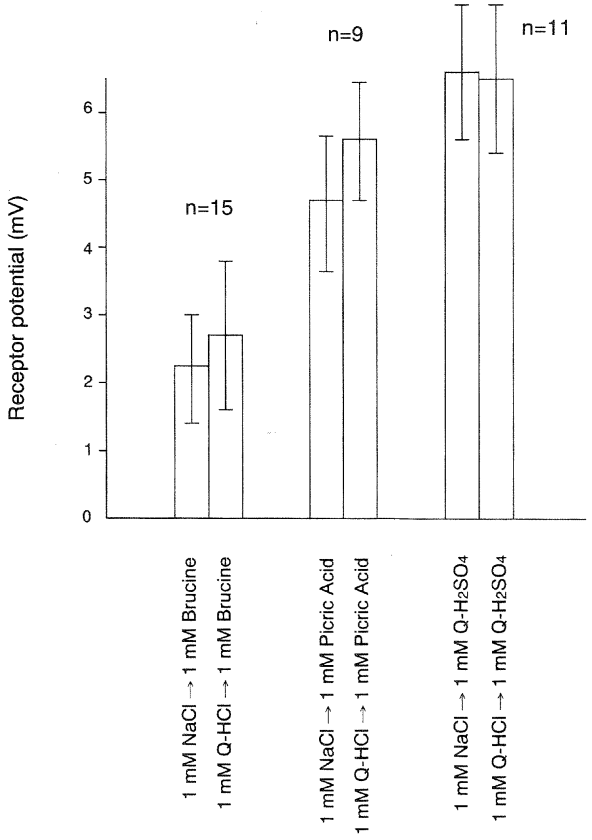

FIG. 12. The mean amplitude of receptor potentials in response to test bitter solutions after adaptation of taste cells to 1 mM NaCl and 1 mM Q-HCl. Test solutions were 1 mM Q-H_2SO_4, brucine and picric acid.

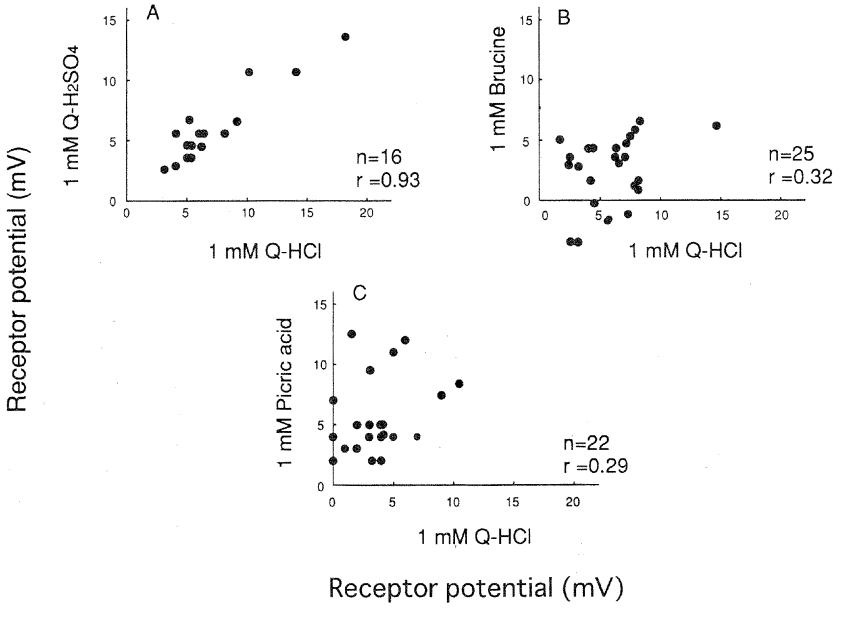

FIG. 13. Relationships between amplitudes of receptor potentials in taste cells induced by paired bitter stimuli. Test bitter solutions were 1 mM Q-HCl, Q-H_2SO_4, brucine and picric acid, which were applied to the taste cells adapted to 1 mM NaCl. Paired stimuli were 1 mM Q-HCl and 1 mM Q-H_2SO_4 (A), 1 mM Q-HCl and 1 mM brucine (B) and 1 mM Q-HCl and 1 mM picric acid (C).

in B and 0.29 in C. The correlation was significant only between Q-HCl and Q-H_2SO_4 ($P<0.01$). This indicates that receptor site for Q-HCl is the same as that for Q-H_2SO_4, but receptor site for brucine or picric acid differs from that for Q-HCl.

DISCUSSION

It is known that quinine and related alkaloids have dual actions on various kinds of cells [3, 13]. In general, a short-term application of quinine to a cell causes an enhancement of the function, but the long-term application causes an inhibition of the function [3, 13]. The experiments of an action of Q-HCl on gustatory neural and cellular responses indicate that a short period of application of Q-HCl to the tongue enhances the gustatory responses [5, 6, 8–10, 14, 17, 18], but the long period of application depresses them [1, 9]. It has been suggested that the enhancing action of Q-HCl on frog taste cells is due to a conformational change in receptor molecules existing at the taste receptive membrane [6, 8, 9, 14] and that the depressing action of Q-HCl is due to the penetration of Q-HCl molecules into the gustatory receptive membrane [1]. In this study, we investigated the effect of a short-term adaptation of frog taste cells to Q-HCl on the taste cell responses induced by basic stimuli. In the present experiments, the amplitude of initial phasic responses of frog taste cells to salt stimuli is larger under Q-HCl adaptation than under water adaptation. Also, the rate of rise of the phasic receptor potentials induced by salt stimuli is rapider under Q-HCl adaptation than under water adaptation. Therefore, the significant increase of gustatory neural responses in the frog to salt stimuli after Q-HCl [5, 6, 8–10] originates from the higher amplitude and rapider rate of rise of the phasic receptor potentials for salt stimuli after Q-HCl. The amplitude of tonic receptor potentials for salts is almost the same under Ringer, Q-HCl and water adaptation (Figs. 3, 4, 6), indicating that the number of salt stimulus-binding receptor sites is not increased during Q-HCl adaptation. It is assumed that the enhancement of the amplitude and the rate of rise of initial phasic receptor potential to a salt after 10 sec adaptation to Q-HCl is due to an increase in interaction between conformationally activated receptor site and salt stimulus.

Salt response enhancement in the gustatory nerve after Q-HCl adaptation is induced by an action of Q-HCl dissolved in water, but is not by an action of Q-HCl dissolved in Ringer and salt solution [6, 9]. The same phenomena are observed in taste cell responses (Fig. 2). It is assumed that the conformational change of salt stimulus-binding receptors is most effectively induced by quinine molecules in water and that the existence of salt ions in a Q-HCl solution depresses the stimulant action of quinine molecules.

Our previous study shows that gustatory neural responses to acids after 10 sec adaption to Q-HCl are much larger than those after 10 sec water adaptation [14]. In the present experiment on frog taste cells the same result is found (Figs. 7 and 8). Similarly it is presumed that enhancement of acid responses after Q-HCl adaptation is due to a conformational change of acid receptor sites by quinine molecules. We have proposed that the proton-gated cation channels and proton-transporters existing in the receptive membrane of frog taste cells are related to the generation of acid responses [11]. These channels and transporters may not be blocked during a short period of application of Q-HCl which is known as K^+ channel blocker.

Gustatory neural responses for sucrose are larger after 10 sec Q-HCl adaptation than after 10 sec water adaptation [14]. However, taste cell responses to sucrose after Q-HCl are the same as those after water (Fig. 10). The discrepancy between gustatory nerve and cell in the sucrose responses after Q-HCl is obscure. However, there are two possible explanations. One is that taste cells located at the proximal region of the tongue have higher sensitivity to sucrose after Q-HCl. We used apical and middle regions of the tongue when intracellular recordings were made from taste cells. The other possibility is a difference in the flow rate of stimulus solutions. We used a flow rate of 0.36–0.78 ml/sec in the experiments on the gustatory nerves [6, 9, 14] and a flow rate of 0.13 ml/sec in the present experiments on the gustatory cells. The amplitude and peak time of gustatory responses are dependent on the flow rate of taste solutions [7].

Gustatory neural responses to Q-H_2SO_4 and brucine after 10 sec Q-HCl adaptation are greatly reduced, but that to picric acid after the Q-HCl adaptation is not changed [14]. This fact reflects similarity and dissimilarity of receptor sites. The taste cell responses to the bitter stimuli after 10 sec Q-HCl adaptation are the same as those after 1 mM NaCl adaptation (Figs. 11 and 12). Neither enhancement nor depression occur in the receptor potentials to the bitter stimuli following Q-HCl. These findings indicate that receptor sites for Q-H_2SO_4, brucine and picric acid may not be changed conformationally by a short period of Q-HCl adaptation.

Correlation between receptor potentials in taste cells induced by a pair of Q-HCl and Q-H_2SO_4 is high, but those between receptor potentials induced by a pair of Q-HCl and brucine and by a pair of Q-HCl and picric acid are low, indicating that Q-HCl and Q-H_2SO_4 bind to the same receptor, but brucine and picric acid do not bind to the receptor for Q-HCl.

ACKNOWLEDGMENTS

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